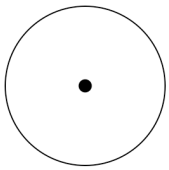
FUNDAMENTALS OF

GENERATIVE MEDICINE



VOLUME I:

CONCEPTS, SYSTEMS AND PATHWAYS

PETER J. D'ADAMO, ND MIFHI

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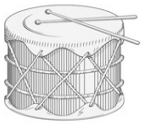

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"You will seek not a near, but a distant, objective,
and you will not be satisfied with what you have done.
All that you may achieve or discover you will regard as a fragment of a larger pattern,
which from his separate approach every true scholar is striving to descry."

Aims of the Harvard Society of Fellows (1938)

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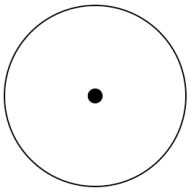
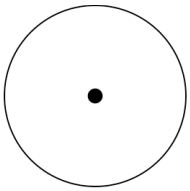


VI: Downstream

"Everything is a miracle. It is a miracle that one does not dissolve in one's bath like a lump of sugar."

—Pablo Picasso

The four major classes of compounds essential to life are nucleic acids, proteins, lipids, and carbohydrates. Over the past 30 years the first three classes have received much attention from chemists and biologists, whereas during most of that time the carbohydrates were largely neglected, partly in the belief that their chemistry and biology had been fully worked out. In the past decade, however, research on carbohydrates has been revived, and it is now expanding rapidly. Owing to many new developments, carbohydrate research is today broad and diverse.



Practical glycomics

Carbohydrates comprise only about 1 percent of the human body; proteins comprise 15 percent, fatty substances 15 percent and inorganic substances 5 percent (the rest being water). Nevertheless, carbohydrates are important constituents of the human diet, accounting for a high percentage of the calories consumed. Thus some 40 percent of the calorie intake of Americans (and some 50 percent of that of Britons and Israelis) is in the form of carbohydrates: glucose, fructose, lactose (milk sugar, a disaccharide of glucose and galactose), sucrose, and starch.

Carbohydrates are the fuel of life, being the main source of energy for living organisms and the central pathway of energy storage and supply for most cells. They are the major products through which the energy of the sun is harnessed and converted into a form that can be utilized by living organisms. According to rough estimates, more than 100 billion tons of carbohydrates are formed each year on the earth from carbon dioxide and water by the process of photosynthesis. Polymers of glucose, such as the starches and the glycogens, are the mediums for the storage of energy in plants and animals respectively. Coal, peat, and petroleum were probably formed from carbohydrates by microbiological and chemical processes.

Carbohydrates are the most abundant group of biological compounds on the earth, and the most abundant carbohydrate is cellulose, a polymer of glucose; it is the major structural material of plants. Another abundant carbohydrate is chitin, a polymer of N-acetylglucosamine; it is the major organic component of the exoskeleton of arthropods, such as insects, crabs, and lobsters, which make up the largest class of organisms, comprising some 900,000 species (more than are found in all other families and classes together). It has been estimated that millions of tons of chitin are formed yearly by a single species of crab! (1)

The name *carbohydrate* was originally assigned to compounds thought to be hydrates of carbon, that is, to consist of carbon, hydrogen, and oxygen. They are typical hexose monosaccharides, meaning that they have six carbon atoms. However, carbohydrates now include polyhydroxy aldehydes, ketones, alcohols, acids and amines, their simple derivatives and the products formed by the condensation of these different compounds through glycosidic linkages (essentially oxygen bridges) into oligomers (*oligosaccharides*) and polymers (*polysaccharides*).

The biological roles of carbohydrates are particularly important in the assembly of complex multicellular organs and organisms, which requires interactions between cells and the surrounding matrix. All cells and numerous macromolecules in nature carry an array of covalently attached sugars (*monosaccharides*) or sugar chains (*oligosaccharides and polysaccharides*), the latter that are generically referred to as "glycans." (2)

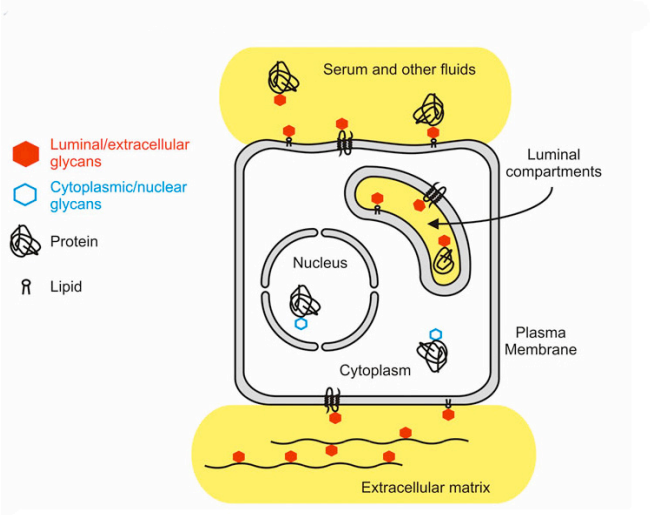


Figure 6.1.1 Localization of glycoconjugates in the intracellular and extracellular compartments.

Because many carbohydrates are on the outer surface of cellular and secreted macromolecules, and are often freestanding entities, they are in a position to modulate or mediate a wide variety of events in cell–cell, cell–matrix, and cell–molecule interactions critical to the development and function of a complex multicellular organism. Much of the current interest in carbohydrates is focused on such substances as *glycoproteins* and *glycolipids*, complex carbohydrates in which sugars are linked respectively to proteins and lipids. They are termed *glycoconjugates*. They can also act as mediators in the interactions between different organisms (for example, between host and a parasite). In addition, simple, rapidly turning over, protein-bound glycans are abundant within the nucleus and

cytoplasm, where they can serve as regulatory switches. A more complete paradigm of molecular biology must therefore include glycans, often in covalent combination with other macromolecules, (glycoconjugates) such as glycoproteins and glycolipids. (3) The term *glycan* may also be used to refer to the carbohydrate portion of a glycoconjugate, such as a glycoprotein, glycolipid, or a proteoglycan.

During the initial phase of the molecular biology revolution of the 1960s and 1970s, studies of glycans lagged far behind those of other major classes of molecules. This was in large part due to their inherent structural complexity and the great difficulty in determining their sequences. Also inhibiting interest was the fact that their biosynthesis could not be directly predicted from a DNA template. In addition, unlike genome products, glycans are highly dynamic and have extraordinarily complex biosynthetic pathways. The development of many new technologies for exploring the structures and functions of glycans has since opened a new frontier of molecular biology. The coming together of the traditional disciplines of carbohydrate chemistry and biochemistry with a modern understanding of the cell and molecular biology of glycans, and in particular, their conjugates with proteins and lipids, is called "glycobiology." (4)

Analogous to genomics and proteomics, *glycomics* represents the systematic methodological elucidation of the "glycome" (the totality of glycan structures) of a given cell type or organism. The *glycome*, a subset of glycobiology, is immense and far more complex than the genome or proteome. In the past decade, over 30 genetic diseases have been identified that alter glycan synthesis and structure, and ultimately the function of nearly all organ systems. Many of the causal mutations affect key biosynthetic enzymes, but more recent discoveries point to defects in chaperones and Golgi-trafficking complexes that impair several glycosylation pathways. As more glycosylation disorders and patients with these disorders are identified, the functions of the glycome are starting to be revealed. (5)

1. Sharon N. Carbohydrates Sci. Am. 245: (5) 90-116. 1980
2. Varki A and Sharon N. Historical Background and Overview: Varki A, Cummings R, Esko J, Freeze H, Stanley P, Bertozzi C, Hart G, and Etzler M. *Essentials of Glycobiology*, 2nd edition, Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2009.
3. Ibid.
4. Rademacher TW, Parekh RB, Dwek RA. Glycobiology. Annu Rev Biochem. 1988;57:785-838
5. Freeze HH. Genetic defects in the human glycome. Nat Rev Genet. 2006 Jul; 7(7):537-51.
6. Taylor ME and Drickamer. *Introduction to Glycobiology*. Oxford University Press 2nd Edition 2006

MONOSACCHARIDES AND GLYCANS

The study of carbohydrates and their derivatives has greatly enriched chemistry, particularly with respect to the role of molecular shape and conformation in chemical reactions. Recent carbohydrate investigations have played a decisive role in the characterization of various antibiotics and anti-tumor agents. Such studies have led to the discovery of new biosynthetic reactions and enzymatic control mechanisms and are contributing significantly to the understanding of many fundamental biological processes, for example the interaction of cells with their environment and with other cells.

Monosaccharides

A carbohydrate that cannot be hydrolyzed into a simpler form is known as a *monosaccharide*. A monosaccharide is a carbohydrate that cannot be hydrolyzed into a simpler form. Monosaccharides are the most basic units of biologically important carbohydrates. Examples of monosaccharides include glucose, fructose, galactose, xylose, and ribose. All simple monosaccharides have the general empirical formula $C_x(H2O)_n$, where n is an integer ranging from 3 to 9. Monosaccharides are the building blocks of disaccharides such as sucrose and the polysaccharides.

Monosaccharides are classified by the type of *carbonyl group* (a carbon atom double-bonded to an oxygen atom) they contain. A carbonyl group at the end of the carbon chain signifies an aldehyde group, whereas a carbonyl group at an inner carbon denotes a ketone group. These two classes of monosaccharides are therefore named *aldoses* and *ketoses*. Each carbon atom that supports a *hydroxyl group* (a compound containing an oxygen atom bound covalently with a hydrogen atom) has a non-superimposable mirror image (*chirality*) giving rise to a number of isomeric forms all with the same chemical formula. For instance, galactose and glucose both are hexose sugars with an aldehyde group on one end (aldohexoses) but have different chemical and physical properties.

Glucose is the best-known monosaccharide; indeed, it has probably been investigated more thoroughly than any other organic compound. References to grape sugar, which is glucose, are to be found in Moorish writings of the 12th century. In 1747, the German pharmacist Andreas Marggraf (1709-1782) whose isolation of pure sucrose from sugar beets is an example of the chemical art of the time at its best, wrote of isolating from raisins “eine Art Zucker” different from cane sugar; it was what is now called glucose. Later workers established that the sugar in grapes is identical with the sugar found in honey, in the urine of diabetics and in acid hydrolysates of starch and cellulose. The French chemist Jean Baptiste Andre Dumas (1800-1884) gave it the name glucose in 1838. The structure of glucose and of several other monosaccharides, including fructose, galactose, and mannose, was established by about 1900, mainly by the work of the German chemist Franz Emil Fischer (1887-1947) who thereby laid the foundations of carbohydrate chemistry. The classification of monosaccharide structures began with Fischer.

A monosaccharide and an alcohol reacting together (with a molecule of water being eliminated) create *glycosides*. The bond formed is called a *glycosidic linkage*. There are two forms of glycosidic linkage: the alpha (α) glycosidic linkage is below the plane of the rings and the beta (β) glycosidic linkages are above the plane of the rings. α linkages or β linkages confer very different structural properties and biological functions upon sequences that are otherwise identical in composition, as classically illustrated by the marked differences between starch and cellulose (both homopolymers of glucose), the former largely α1-4 linked and the latter β1-4 linked throughout.

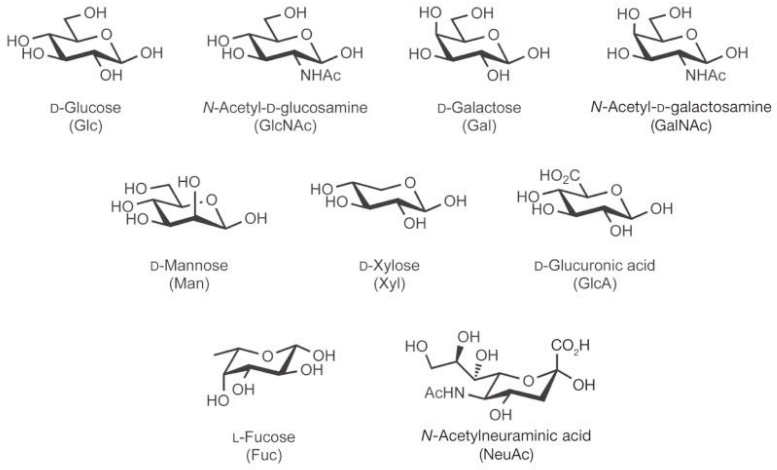


Figure 6.1.2 Common monosaccharides found in vertebrates (2)

Monosaccharides exist in solution as an equilibrium mixture of open "open-chain" (*acyclic*) or "ring" (*cyclic*) forms. The ring form of a monosaccharide generates a chiral anomeric center at C-1 for aldo sugars or at C-2 for keto sugars. The percentage of each form depends on the sugar structure. Ring forms of the monosaccharides are the rule in *oligosaccharides*, which are linear or branched chains of monosaccharides attached to one another via glycosidic linkages (the term *polysaccharide* is typically reserved for large glycans composed of repeating oligosaccharide motifs).

Although several hundred distinct monosaccharides are known to occur in nature, only a small number of these are commonly found in animal glycans:

- *Pentoses:* Five-carbon neutral sugars (D-xylose)
- *Hexoses:* Six-carbon neutral sugars (D-glucose, D-galactose, D-mannose)
- *Hexosamines:* Hexoses with an amino group that are either free or N-acetylated (N-acetyl-D-glucosamine, N-acetyl-D-galactosamine)
- Deoxyhexoses: Six-carbon neutral sugars without the hydroxyl group at the 6-position (L-fucose)
- Uronic acids: Hexoses with a negatively charged carboxylate at the 6-position (D-glucuronic acid, L-iduronic acid)
- Sialic acids: Nine-carbon acidic sugars (N-acetylneuraminic acid)

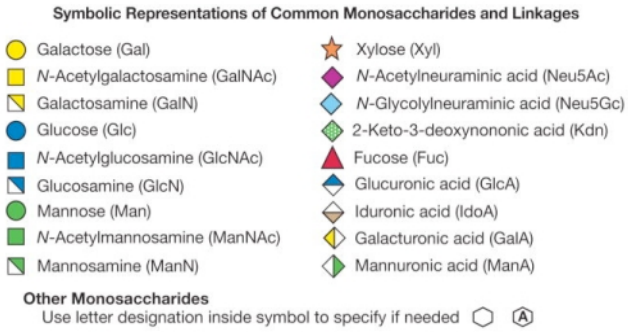


Figure 6.1.3 The monosaccharide symbol set and abbreviation nomenclature. (2)

The creation of a disaccharide (such as lactose), by the formation of a glycosidic linkage between the two monosaccharides glucose and galactose, is an energy-unfavorable process, and as with many such processes, the free energy needed to form the bond is produced by coupling to the energy favorable hydrolysis of phosphate anhydride bonds. This takes place in two stages. The energy of hydrolysis of two high-energy phosphate bonds in adenosine triphosphate (ATP) is first used to drive the formation of a nucleotide sugar donor, uridine diphosphate (UDP)-galactose. UDP-galactose is then used to make the glycosidic linkage with glucose. A *glycotransferase* catalyzes the transfer of sugar from a nucleotide donor and is thus responsible for the formation of the glycosidic linkage. Each glycotransferase has specificity for a nucleotide sugar "donor" and an "acceptor.'

Name that glycotransferase

The full name of the glycotransferase indicates the nature of the nucleotide sugar donor, the nature of the acceptor and the nature of the bond thus formed. For example, the formation of lactose would be catalyzed by

UDP-galactose: glucose β-1,4-galactosyltransferase

By convention, and for convenience, shorthand is often used: in this case the glycotransferase would be denoted

β 1,4-galactosyltransferase

A glycosidic linkage has been formed between the C1 of the galactose and the 4-hydroxy group of the glucose, and the C1 is in the β configuration.

Breaking glycosidic linkages also requires specific enzymes but does not require any input of energy. Glycosidic linkages are broken by *glycosidases*. Like glycotransferases, glycosidases are highly specific, with each glycosidase catalyzing the hydrolysis of linkages involving a specific sugar. For example, the enzyme sialidase, more commonly known as *neuraminidase*, catalyzes the release of a sialic acid (NeuAc) residue from the non-reducing terminus of many oligosaccharides known as neuraminic acids. Neuraminidase enzymes are a large family, found in a range of organisms. The most commonly known neuraminidase is the viral neuraminidase, a drug target for the prevention of influenza infection. The viral neuraminidases are frequently used as antigenic determinants of the influenza virus --the "N" in the viral classification H_xN_x is the particular neuraminidase of the influenza strain in question. Some variants of the influenza neuraminidase confer more virulence to the virus than others do.

Name	Emblem	Word Symbol
N-acetylgalactosamine		GalNAc
N-acetylglucosamine		GlcNAc
N-acetylneuraminic acid(1)		Neu5Ac or NeuAc
5,9-N,O-diacetylneuraminic acid(1)		Neu5,9Ac_2
fucose (6-deoxygalactose)		Fuc
galactosamine		GalN
galactopyranose 3-sulfate		Galp3S
galactose		Gal
galacturonic acid		GalA
glucosamine		GlcN
glucose		Glc
glucose 6-phosphate		Glcp6P
glucuronic acid		GlcA
N-glycoloylneuraminic acid(1)		Neu5Gc or NeuGc
myo-inositol (2)		Ins
mannose		Man
4-O-methylgalactose		Gal4Me
rhamnose		Rha
xylose		Xyl

Figure 6.1.4 Recommended abbreviations for some monosaccharides, derivatives and related compounds. 1. Acylated neuraminic acids and other derivatives of neuraminic acid may also be called sialic acids (abbreviated Sia) when the nature of the *N*-acyl substituent(s) is not relevant, or is unknown. 2. *myo*-Inositol with the numbering of the 1D configuration.

Glycans with different covalent structures take up different shapes in the same way that proteins with different amino acid sequences have distinct three-dimensional structures and folds. Because oligosaccharides can be branched, it is customary to employ the term *conformation* to describe their arrangement in three-dimensional space. Conformations of monosaccharides and oligosaccharides heavily influence their reactivity and recognition by other molecules, which are essential to mammals and other organisms. Monosaccharides have a rather limited number of conformations: "chair" confirmations are generally favored over "boat" conformations since the cause less atomic crowding in the ring. In different chair positions, each group attached to the ring either projects sideways (equatorial position) or points upwards or downwards (axial position).

Steric effects arise from the fact that each atom within a molecule occupies a certain amount of space. If atoms are brought too close together, there is an associated cost in energy due to overlapping electron clouds and this may affect the molecule's preferred conformation and reactivity. In biochemistry, steric effects are often exploited in naturally occurring molecules such as enzymes, where the catalytic site may be buried within a large protein structure. In pharmacology, steric effects determine how and at what rate a drug will interact with its target bio-molecules. Steric and stereoelectronic effects are common interactions that dictate the three-dimensional shape of a carbohydrate. Atoms in the axial position are more likely to clash sterically. *Lectins* are an example of sugar binding proteins that are highly specific to the conformation of a particular carbohydrate.

Pyranose is a collective term for carbohydrates that have a chemical structure consisting of a six-membered ring consisting of five carbon atoms and one oxygen atom. Hexoses residues predominate in biological glycans and one of their chair conformations, the $^4C_1$, is energetically favored because the arrangement places the bulky C6-O6 groups (and most of the hydroxyl groups) in the cucumber-cool equatorial position.

Conformation plays a huge role in the characteristics of the cell wall of plants. The polysaccharide in cell walls is *cellulose*, a repeating polymer of β-1,4-linked glucose residues that form microfibrils due to the steric effects of van der Waals packing. Alignment of the microfibrils into cell wall fibers involves additional types of polysaccharides known as hemicelluloses. Difference in the linkages of between cellulose and hemicellulose prevent the formation of *hemicellulose* microfibrils, but still allows the hemicellulose to coat the cellulose microfibrils, bridge them and organize the microfibrils in three dimensional space. Aligned microfibrils resist push-pull forces and layering them in crisscrossed arrays provides tensile strength in multiple dimensions. Other acidic polysaccharides, known as *pectins*, fill the spaces between the microfibrils, help hold them together, and may serve as signaling molecules.

GLYCANS

The term *glycan* refers to any polysaccharide or oligosaccharide. A true structure-function understanding of the relationship for the glycans can be difficult. Proteins, despite their diverse biological roles, share two basic features that unify the study of their properties: first, each protein is synthesized as an identical copy by translation of mRNA; second, the resulting protein gains its specific activity from the precisely folded three-dimensional structure. In contrast, glycans can be assembled without any sort of template through a series of individually catalyzed reactions. The resulting products, because many different proteins are modified with a relatively common set of glycans structures and different copies of a single polypeptide backbone, can be embellished and modified with scores of differing glycans.

Glycans may resist classification into a set of "simple rules" for some very good reasons; one being that the functions of the protein and glycan portions of the glycoprotein may be independent of each other. In other words, for the same glycoprotein, all copies of a particular protein perform the same role regardless of what particular glycans that they contain *and* all copies of a particular glycans perform the same function although they are attached to different proteins. This "fellow traveler" arrangement can be particularly useful when glycans function as "tags" and help direct protein trafficking. Many glycans participate in various "quality-control" checks. The many common glycans serve to hold secretory glycoproteins in various luminal compartments during this QC checking. Since one set of glycans can serve this role for a wide number of proteins, it is assumed that although the proteins will serve a wide variety of functions once outside the cell, the glycans will have no future function when the glycoprotein reaches the outside of the cell.

Conversely, in other instances, glycans may mediate extracellular adhesion (or anti-adhesion) on the surface of the plasma membrane completely independent of the particular protein to which it is attached. This provides a way of producing high densities without requiring a correspondingly high density of one particular type of membrane protein or lipid.

A *glycoconjugate* is a compound in which one or more monosaccharide or oligosaccharide units (*the glycone*) are covalently linked to a noncarbohydrate moiety (*the aglycone*). An oligosaccharide that is not attached to an aglycone possesses the reducing power of the aldehyde or ketone in its terminal monosaccharide component. This end of a sugar chain is therefore often called the reducing terminus or *reducing end*, terms that tend to be used even when the sugar chain is attached to an aglycone, and has thereby lost its reducing power. Correspondingly, the outer end of the chain tends to be called the *nonreducing end.*

Glycans can be found attached to proteins as in *glycoproteins* and *proteoglycans*. These are generally found on the exterior surface of cells attached either to an oxygen molecule (*O-linked glycans)* or to a nitrogen molecule *(N-linked glycans).* Glycans can also be attached to lipids forming *glycolipids.* In addition, glycoconjugates are secreted into biological fluids, such as serum, and they make up the insoluble extracellular matrix that surrounds cells.

Glycoconjugate structures are encoded "indirectly" into the genome. Compared with proteins, there is an extra step in the process. The glycans proteins are not encoded directly into the DNA, but rather arise from transcription and translation of the particular genes needed to produce the glycosyltransferases that in turn control the production of the glycans portion of the glycoconjugates.

N-glycans

All classes of glycoconjugates have been extensively studied, but the N-linked glycans attached to soluble, secreted proteins are perhaps understood best. This reflects the historical availability of serum glycoproteins for investigation. N-Linked glycans ("N-glycan") are found attached to the R-group nitrogen (N) of asparagine in the sequence of three consecutive amino acids in a protein that can serve as the attachment site (often called a *sequon*.) A sequon is either Asparagine-*X*-Serine or Asparagine-*X*-Threonine, where *X* is any amino acid except proline and usually involving an N-acetyl glucosamine (GlcNAc) residue.

N-glycans share a common pentasaccharide core region and can be generally divided into three main classes: the *oligomannose* type (in which only mannose residues are attached to the core), the *complex* type (in which "antennae" initiated by N-Acetylglucosaminyltransferases (GlcNAcT's) are attached to the core), and *hybrid* type (in which only mannose residues are attached to the Manα1–6 arm of the core and one or two antennae are on the Manα1–3 arm).

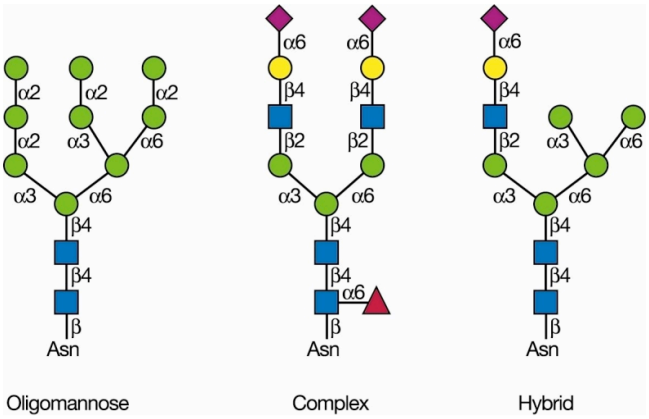


Figure 6.1.5 Classes of N-glycans. Mannose sugars are green circles. N-acetyl glucosamine sugars are the blue squares. (3)

A fascinating aspect of N-glycans is their complicated biosynthesis. N-linked glycans are derived from a "core" 14-sugar unit assembled in the cytoplasm and endoplasmic reticulum. Two N-acetyl glucosamine residues are first attached to a lipid (dolichol phosphate) on the external side of the endoplasmic reticulum membrane. Then five mannose residues are added to this structure. The partially finished core glycan is then flipped across the endoplasmic reticulum membrane, so that it is now located within the reticular lumen via an incompletely understood "flippase." This is followed by the addition of four more mannose residues. Finally, three glucose residues are added to this structure. Following full assembly, the glycan is transferred to a peptide chain, within the reticular lumen. This core structure of N-linked glycans thus consists of 14 residues (3 glucose, 9 mannose, and 2 N-acetylglucosamine).

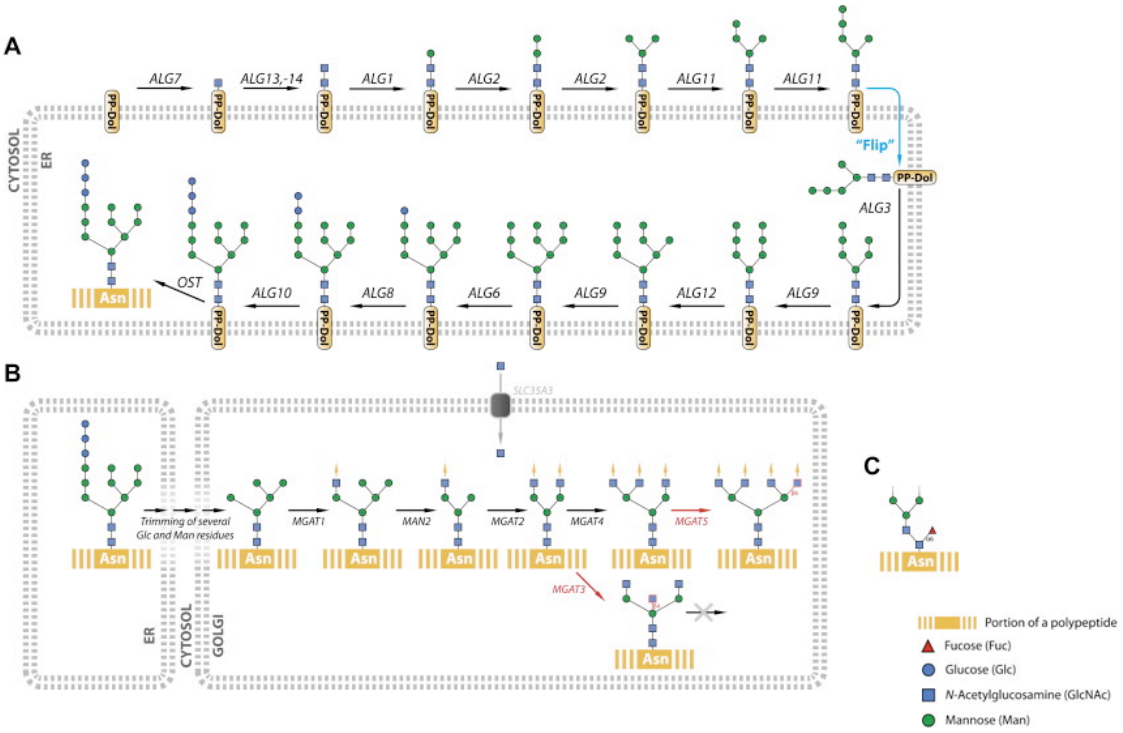


Figure 6.1.6 Processing of an initial high-mannose N-linked glycan to generate complex glycans. First, two GlcNAc's are added to the Dol-PP anchor in the outer leaflet of ER's lipid membrane. Further, five mannose saccharides are attached to the structure. This precursor located in cytosol, is now flipped by a yet not fully elucidated mechanism to the inner leaflet. For the rest of the synthesis process, this glycan structure is situated inside the ER lumen. Here, four more mannoses as well as three glucoses are added to create the mature N-glycan precursor. Genes encoding transferases that are responsible for these reactions are designated ALG (asparagine-linked glycosylation). The mature precursor is then detached from its dolichol anchor and transferred to a target polypeptide sequence co-translationally by the large enzyme complex, OST (modified from Varki et al., 2009). B) N-glycan branching. After being transferred to a protein, the N-glycan goes through glucose and mannose trimming, the former being involved in polypeptide folding quality control. The resulting Man5GlcNAc2 structure may be branched – a process mediated by the MGAT family of GlcNAc-transferases. Up to four branches can be added by MGAT1, 2, 4, and 5 respectively and further elongated (orange arrows). Of these, MGAT5 appears to be the most interesting in carcinogenesis; the branch it initiates is preferentially elongated by polylactosamine. In addition to the four previously mentioned branches, a so-called bisecting β-3 branch may be added by MGAT3. This bisecting GlcNAc terminates all further branching, including that mediated by MGAT5. Thus, activity of MGAT3 might inhibit polylactosamine synthesis. The two key reactions, performed by MGAT3 and MGAT5, are highlighted in red. The MGAT transferases require UDP-GlcNAc, which is imported through a transporter (SLC35A3). Genes encoding relevant transferases are displayed in black italic font. C) Core fucosylation. This is one of the possible modifications made to N-glycans' core structure. Modified from Potapenko IO, Haakensen VD, Lüders T, Helland A, Bukholm I, Sørlie T, Kristensen VN, Lingjaerde OC, Børresen-Dale AL. Glycan gene expression signatures in normal and malignant breast tissue; possible role in diagnosis and progression. Mol Oncol. 2010 Apr; 4(2):98-118.

Once transferred to the peptide chain, N-linked glycans generally undergo extensive processing reactions, whereby the three glucose residues are removed, as well as several mannose residues, depending on the N-linked glycan in question. The first process involves the removal of some of the remaining sugar residues by processing exoglycosidases (glucosidase I and glucosidase II). The removal of the glucose residues is dependent on

proper protein folding. The remaining structure is subject to the actions of a series of mannosidases that remove some or all of the four remaining mannose residues. These processing reactions occur in the Golgi apparatus. Modification reactions may involve the addition of a phosphate or acetyl group onto the sugars, or the addition of new sugars, such as neuraminic acid. Processing and modification of N-linked glycans within the Golgi does not follow a linear pathway. As a result, many different variations of N-linked glycan structure are possible, depending on enzyme activity in the Golgi.

In vertebrate N-glycans, the main core modification is the addition of fucose in an α1,6-linkage to the N-acetylglucosamine adjacent to asparagine in the core. The most important "capping" or "decorating" reactions involve the addition of sialic acid, fucose, galactose, N-acetylgalactosamine, and sulfate to the branches described in the preceding paragraph. Capping sugars are most commonly α-linked and therefore protrude away from the β-linked ribbon-like poly-N-acetyllactosamine branches, thus facilitating the presentation of terminal sugars to lectins and antibodies.

N-linked glycans are extremely important in proper protein folding in eukaryotic cells. *Chaperone proteins* in the endoplasmic reticulum, such as the lectin Calnexin and Calreticulin bind to the three glucose residues present on the core N-linked glycan. These chaperone proteins then serve to aid in the folding of the protein to which the glycan is attached. Following proper folding, the three glucose residues are removed, and the glycan moves on to further processing reactions. If the protein fails to fold properly, the three glucose residues are reattached, allowing the protein to re-associate with the chaperones. The different glycoforms are recognized by specialized lectins. The folding sensor UGGT acts as an unusual molecular chaperone and covalently modifies the Man9 N-glycan of a misfolded protein by adding a glucose moiety and converts it to Glc1Man9 that rebinds the lectin Calnexin. (6) This cycle may repeat several times until a protein reaches its proper conformation. N-linked glycans play an important role in cell-cell interactions.

N-linked glycans also contribute to protein folding by steric effects. For example, cysteine residues in the peptide may be temporarily blocked from forming disulfide bonds with other cysteine residues, due to the size of a nearby glycan. The presence of an N-linked glycan therefore allows the cell to control what cysteine residues will form disulfide bonds. If a protein repeatedly fails to fold properly, it is excreted from the endoplasmic reticulum and degraded by cytoplasmic proteases. Perhaps not altogether surprisingly (considering their role in the maintenance of protein structure) N-glycans are substantially decreased in Alzheimer's disease patients but not in controls. (4) Inhibition of the epidermal growth factor receptor is a well-recognized avenue of therapeutic approach to a variety of cancers. Blocking N-glycan precursor biosynthesis appears to be a novel therapeutic strategy for targeting EGFR and RTK signaling in both gliomas and other malignant tumors. (5)

The complex N-glycans that fail to be synthesized in knockout mice are important in retaining growth factor and cytokine receptors at the cell surface, probably through interactions with glycan-binding proteins such as galectins or cytokines such as transforming growth factor β. Cell-surface receptors and a glucose transporter lacking branches of a complex N-glycan have a shorter residence time on the cell surface, and their signaling is attenuated. Deletion of genes encoding sialyltransferases, fucosyltransferases, or branching N-Acetylglucosaminyltransferases has generally produced viable mice with defects in immunity or neuronal cell migration, emphysema of the lung, or inflammation.

Abnormal N-linked glycans are a hallmark of disrupted cellular external architecture due to inflammation, cancer, or infection. (8) They are recognized by the NCR3 receptor on Natural Killer cells as a sign that the cell in question is compromised. N-glycans carried on CD45 modulate galectin-1 binding, CD45 signaling, and T cell death. (7) N-glycans may carry the sugar determinants recognized by selectins that mediate cell–cell interactions

important for leukocyte extravasation from the blood stream and regulate lymphocyte homing to lymph nodes. N-Glycans are more highly branched when cells become cancerous, and this change facilitates cancer progression. Thus, certain glycosyltransferases may be appropriate targets for the design of cancer therapeutics.

O-glycans

Whereas the N-linked glycans are bound to the nitrogen atom of asparagine side chains, the *O-linked glycans* are bound to the oxygen atom of serine or threonine side chains. In eukaryotes, O-linked glycans are assembled one sugar at a time on a serine or threonine residue of a peptide chain in the Golgi apparatus. Unlike with N-linked glycans, there is no yet known "consensus sequence;" although, the placement of a proline residue at either -1 or +3 relative to the serine or threonine is favorable for O-linked glycosylation.

The most common O-linked glycoproteins are often termed *mucins*. In mucins, O-glycans are covalently α-linked via an N-acetylgalactosamine (GalNAc) moiety to the -OH of serine or threonine by an O-glycosidic bond, and the structures are named *mucin O-glycans* or *O-GalNAc glycans.* Mucin glycoproteins are ubiquitous in mucous secretions on cell surfaces and in body fluids. (9)

There are four common O-GalNAc glycan core structures, designated cores 1 through 4 and an additional four designated cores 5 through 8. (9) Mucin O-glycans can be branched, and many sugars or groups of sugars on mucin O-glycans are antigenic. Important modifications of mucin O-glycans include O-acetylation of sialic acid and O-sulfation of galactose and N-acetylglucosamine. Thus, mucin O-glycans are often very heterogeneous, with hundreds of different chains being present in some mucins.

Mucins

Mucus is the slimy and viscoelastic secretion that covers the epithelial surface of tubular organs such as tracheobronchial, gastrointestinal, reproductive tracts, and other specialized organs. In the body, specialized epithelial cells known as goblet cells secrete mucus, and these cells are abundant in the epithelium of the gastrointestinal, respiratory, and reproductive tracts, and the secretory epithelial surfaces of the liver, pancreas, gall bladder, kidney, salivary, and lacrimal glands. (14) Mucus secretions adhere to the epithelial surface, where they serve as a protective diffusion barrier against harmful substances and act as a lubricant between the lumen and the cell surface. (15,16) The composition of mucus varies with its location and pathophysiological conditions, but normally mucus is composed of water, inorganic salts, immunoglobulins, secreted proteins, and mucins. (13)

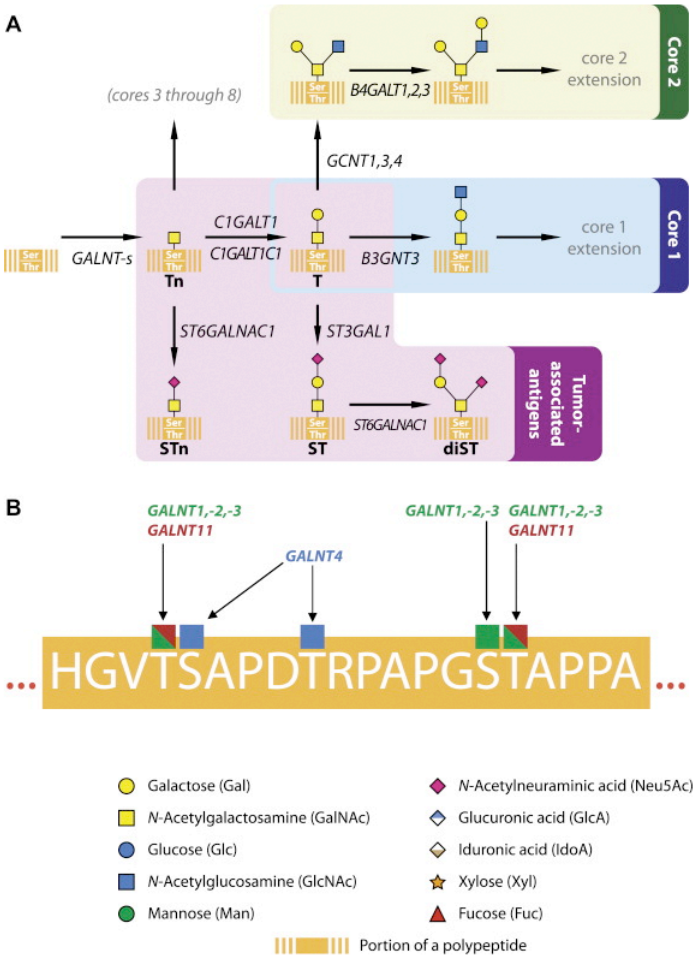


Figure 6.1.7 O-linked glycosylation. A) Synthesis of O-linked glycans. Several different core O-glycan structures exist, but some are very rare. The initial synthesis of cores 1 and 2 as well as tumor-associated antigens (T antigens) are illustrated. Each of the cores can be extended and then further modified by, for example, fucosylation. Genes encoding relevant transferases are displayed in black italic font. B) Initiation of O-glycosylation on MUC1. This illustration shows a single VNTR (orange) of MUC1 with its amino acid sequence, and specificities of the well-studied GalNAcT's. The enzymes involved are sometimes overlapping in specificity, although it should be noted that some transferases require preceding activity of other GalNAcT's (not shown). Modified from Potapenko IO, Haakensen VD, Lüders T, Helland A, Bukholm I, Sørlie T, Kristensen VN, Lingjaerde OC, Børresen-Dale AL. Glycan gene expression signatures in normal and malignant breast tissue; possible role in diagnosis and progression. Mol Oncol. 2010 Apr; 4(2):98-118.

Mucins are present at many epithelial surfaces of the body, including the gastrointestinal, genitourinary, and respiratory tracts, where they shield the epithelial surfaces against physical and chemical damage and protect against infection by pathogens. Mucin O-glycans begin with an α-linked N-acetylgalactosamine residue linked to serine or threonine. The key characteristic of mucins is their ability to form gels; therefore, they are a key component in most gel-like secretions, serving functions from lubrication, to cell signaling, to forming chemical barriers. Mucins are the most abundant macromolecules in mucus and are responsible for its biochemical and biophysical properties due to their nature and

extent of glycosylation. (17) The mucins are a closely related family of O-glycoproteins that play an important role in the renewal and differentiation of the epithelium, cell adhesions, immune response, and cell signaling.

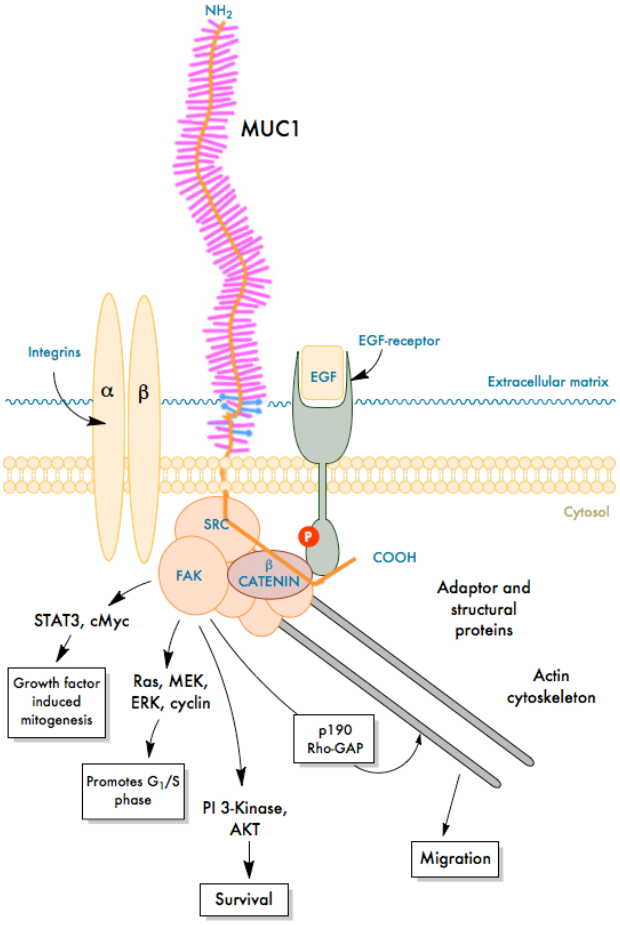


Figure 6.1.7 MUC1 structure and relationship to signaling cascades.

The hallmark of mucins is the presence of repeated peptide stretches called "variable number of tandem repeat" (VNTR) regions that are rich in serine or threonine O-glycan acceptor sites and have an abundance of clustered mucin O-glycans that may comprise 80% of the molecule by weight. The tandem repeats are usually rich in proline residues that appear to facilitate O-GalNAc glycosylation. Mucins may have hundreds of O-GalNAc glycans attached to serine or threonine residues in the VNTR regions. The clustering of O-GalNAc glycans causes mucin glycoproteins to adopt an extended "bottle brush" conformation. (9)

Mucins are very large glycoproteins (well over 10^6 Daltons) composed of carbohydrate and amino acids in a roughly 3:1 ratio. At least 19 human mucin genes have been distinguished: *MUC1, MUC2, MUC3A, MUC3B, MUC4, MUC5AC, MUC5B, MUC6, MUC7, MUC8, MUC12, MUC13, MUC15, MUC16, MUC17, MUC19*, and *MUC20.*

Mucins are classified into three categories: Strictly secreted, gel-forming mucins, mucins either tethered at the cell surface or secreted in the mucus and exclusively secreted non-gel forming mucins.

From a prognosis point of view, their expression and alterations in glycosylation are associated with the development and progression of malignant diseases. (13) Their aberrant expression is well documented in a variety of inflammatory or malignant diseases. Therefore, mucins can be used as valuable markers to distinguish between normal and disease conditions. Indeed, this alteration in glycosylation patterns generates several epitopes in the oligosaccharide side chains that can be used as diagnostic and/or prognostic markers. Many of the mucins are oncofetal antigens and increased mucin production occurs in many adenocarcinomas, including cancer of the pancreas, lung, breast, ovary, colon, etc.

Major classes of mucins:

- Secreted/gel-forming mucins (MUC2, MUC5AC, MUC5B, MUC6, and MUC19)
- Membrane-bound mucins (MUC1, MUC3A, MUC3B, MUC4, MUC11, MUC12, MUC13, MUC15, MUC16, MUC17, MUC20, and MUC21)
- Soluble mucins (MUC7, MUC8, and MUC9)

About 20 different mucin genes have been cloned, and they are expressed in a tissue-specific fashion. For example, different mucin genes are expressed in different regions of the gastrointestinal tract, suggesting that they serve specific functions. The expression of mucin genes is regulated by a large number of cytokines and growth factors, differentiation factors, and bacterial products. Mucins hydrate and protect the underlying epithelial cells, but they have also been shown to have roles in fertilization, blastocyst implantation, and the immune response.

Because the O-glycans are hydrophilic and usually negatively charged, they promote binding of water and salts and are major contributors to the viscosity and adhesiveness of mucus, which forms a physical barrier between lumen and epithelium. The removal of microbes and particles trapped in mucus is an important physiological process. However, in diseases such as cystic fibrosis, the abnormally high viscosity of the mucus leads to obstruction and life-threatening tissue malfunction.

O-linked glycans, particularly mucin, have been found to be important in developing normal intestinal microflora. Certain strains of intestinal bacteria specifically bind to mucin, allowing them to colonize the intestine. (18) A novel mucin-degrading bacterium designated *Akkermansia muciniphila* is a common member of the human intestinal tract and that its colonization starts in early life and develops within a year to a level close to that observed in adults but decreases in the elderly. (19)

T and Tn antigens

The simplest mucin O-glycan is a single N-acetylgalactosamine residue linked to serine or threonine. Named the *Tn antigen*, this glycan is often antigenic. The most common O-GalNAc glycan is Galβ1-3GalNAc-, and it is found in many glycoproteins and mucins. It is termed a core 1 O-GalNAc glycan because it forms the core of many longer, more complex structures. It is antigenic and is named the T (Thomsen-Friedenreich) antigen. Gerhard Uhlenbruck (b. 1929) established the chemical structure of the T antigen in 1969 at the University of Cologne. (25) Both Tn and T antigens may be modified by sialic acid to form sialylated-*Tn or -T antigens*, respectively.

Mucins also trap bacteria via specific receptor sites within the O-glycans of the mucin. Some sugar residues or their modifications can serve as "decoys," thus masking underlying antigens or receptors. For example, O-acetyl groups on the sialic acid residue of the sialyl-Tn antigen prevent recognition by anti-sialyl-Tn antibodies. Gut bacteria often actively remove this decoy. Bacteria can cleave sulfate with sulfatases or terminal sugars with glycosidases.

Other O-linked glycans

- *Glycophorins* are sialoglycoproteins found on the membrane of a red blood cell. They are membrane-spanning proteins and carry sugar molecules. It is heavily glycosylated (60%). Glycophorins are rich in sialic acid and heavily glycosylated, which gives the red cells a very hydrophilic-charged coat, enabling them to circulate without adhering to other cells or vessel walls. *Leukosialin*, also called CD43 or sialophorin, is a major sialoglycoprotein expressed widely in various leukocytes including granulocytes, monocytes, macrophages, and T-lymphocytes. (21)

- *Notch transmembrane receptors* are important regulators of cell fate determination in numerous cell types. Notch signaling in mammals are covalently modulated with O-fucose on many epidermal growth factor-like (EGF) repeats of the extracellular domain. Removal of O-fucose affects Notch signaling in myelopoiesis and lymphopoiesis, and the O-fucose glycan in the Notch1 ligand-binding domain is required for optimal T-cell development. (20) The recent discovery that O-glucose modification of Notch EGF repeats is also required for Notch function has further expanded the range of glycosylation events capable of modulating Notch signaling. (22) This places glycosylation alongside phosphorylation as a means to modulate protein-protein interactions and their resultant downstream signals.

- *Sialyl Lewis^x* (sialyl Le^X) is one of the most important blood group antigens and is displayed on the terminus of glycolipids that are present on the cell surface. SLe^X is a tetrasaccharide carbohydrate that is usually attached to O-glycans on the surface of cells. It is known to play a vital role in cell-to-cell recognition processes. The Sialyl Lewis^X determinant, E-selectin ligand carbohydrate structure, is constitutively expressed on granulocytes and monocytes, and it mediates inflammatory extravasation of these cells.

- *Thrombospondins*, a family of anti-angiogenic proteins, are O-linked. Similar to the EGF repeat in Notch signaling, a second type of cysteine-rich motif, known as a thrombospondin type 1 repeat (TSR), is also modified with O-fucose glycans. (5) The ADAMTS (A Disintegrin and Metalloproteinase with Thrombospondin motifs) proteins are a family of metalloproteinases with sequence similarity to the ADAM proteases, which contain the thrombospondin type 1 sequence repeat motifs (TSR's)

common to extracellular matrix proteins. ADAMTS proteins have recently gained attention with the discovery of their role in a variety of diseases, including tissue and blood disorders, cancer, osteoarthritis, Alzheimer's and the genetic syndromes Weill-Marchesani syndrome (ADAMTS10), thrombotic thrombocytopenic purpura (ADAMTS13), and Ehlers-Danlos syndrome type VIIC (ADAMTS2). (26)

Glycosaminoglycans and proteoglycans

Another class of cellular glycans is the *glycosaminoglycans* (GAG's), the most abundant heteropolysaccharides in the body. These comprise 2-aminosugars linked in an alternating fashion with uronic acids, and include polymers such as heparin, heparan sulfate, chondroitin, keratin, and dermatan. Some glycosaminoglycans are found attached to the cell surface, where they are linked through a single xylosyl residue to a protein. *Chondroitin sulfate* is a sulfated glycosaminoglycan composed of a chain of alternating sugars (N-acetylgalactosamine and glucuronic acid). It is usually found attached to proteins as part of a proteoglycan. A chondroitin chain can have over 100 individual sugars, each of which can be sulfated in variable positions and quantities. GAG's are highly negatively charged molecules, with extended conformation that imparts high viscosity to the solution.

GAG's are located primarily on the surface of cells or in the extracellular matrix (ECM). Along with the high viscosity of GAG's comes low compressibility, which makes these molecules ideal for a lubricating fluid in the joints. Chondroitin sulfate is an important structural component of cartilage and provides much of its resistance to compression. At the same time, their rigidity provides structural integrity to cells and provides passageways between cells, allowing for cell migration. The majority of GAG's in the body are linked to core proteins, forming proteoglycans (also called *mucopolysaccharides*). (23)

One well-defined function of the GAG heparin is its role in preventing coagulation of the blood. Heparin is abundant in granules of mast cells that line blood vessels. The release of heparin from these granules, in response to injury, and its subsequent entry into the serum leads to an inhibition of blood clotting. Several genetically inherited diseases, for example the lysosomal storage diseases, result from defects in the lysosomal enzymes responsible for the metabolism of complex membrane-associated GAG's. These specific diseases are termed *mucopolysaccharidoses (MPS).* The inactivity of specific lysosomal enzymes that normally degrade glycosaminoglycans leads to the accumulation of proteoglycans within cells. This leads to a variety of disease symptoms, depending upon the type of proteoglycan that is not degraded.

Any protein with one or more covalently attached glycosaminoglycan chains is termed a *proteoglycan.* Proteoglycans and GAG's perform numerous vital functions within the body, some of which remain to be studied. Virtually all mammalian cells produce proteoglycans and secrete them into the ECM, insert them into the plasma membrane, or store them in secretory granules. (24)

Proteoglycans are a major component of the animal extracellular matrix, the "filler" substance existing between cells in an organism. Here they form large complexes to other proteoglycans, to hyaluronan, and to fibrous matrix proteins (such as collagen). Evidence also shows they can affect the activity and stability of proteins and signaling molecules within the matrix. The protein component of proteoglycans is synthesized by ribosomes and translocated into the lumen of the rough endoplasmic reticulum. Glycosylation of the proteoglycan occurs in the Golgi apparatus in multiple enzymatic steps.

During development, secreted morphogens such as Wnt protein, Hedgehog (Hh), and bone morphogenic protein (BMP) are emitted from their producing cells in a morphogenetic field, where they specify different cell fates in a direct concentration-dependent manner. Understanding how morphogens form their concentration gradients to pattern tissues has been a central issue in developmental biology. Over the past decade, one of the main findings in this field is the characterization of heparan sulfate proteoglycan (HSPG) as an essential regulator for morphogen gradient formation. HSPG's can directly influence morphogen gradient formation at various levels, including morphogen movement, signaling, and trafficking. HSPG's can also interact with other molecules such as lipoprotein, which is required for morphogen movement and distribution. (26)

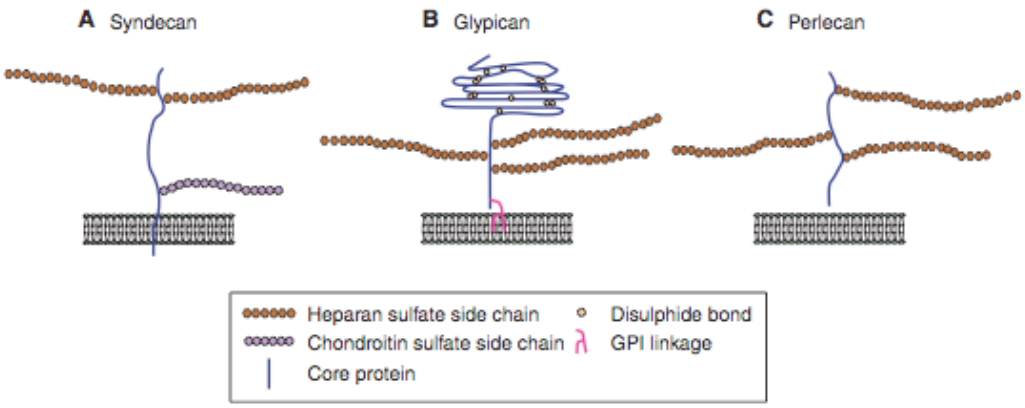


Figure 6.1.8 Three main classes of cell-surface heparan sulfate proteoglycans. (A) Syndecan core proteins are transmembrane proteins that contain a highly conserved carboxy-terminal cytoplasmic domain. Heparan sulfate (HS) chains attach to serine residues distal from the plasma membrane. Some syndecans also contain chondroitin sulfate (CS) chain(s) that attaches to serine residue(s) near the membrane. (B) The glypican core proteins are disulfide-stabilized globular core proteins that are linked to the plasma membrane by a glycosylphosphatidylinositol (GPI) anchor. HS chains link to serine residues adjacent to the plasma membrane. (C) Perlecans are secreted HSPG's that carry HS chains.

1. Sharon N. Carbohydrates. Sci Am. 1980; 245: (5) 90-116.
2. Varki A and Sharon N. Historical Background and Overview. In: Varki A, Cummings R, Esko J, Freeze H, Stanley P, Bertozzi C, Hart G, and Etzler M. *Essentials of Glycobiology*. 2nd edition, Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2009.
3. Stanley P. Schachter H. Taniguchi N. N-Glycans. In: Varki A, Cummings R, Esko J, Freeze H, Stanley P, Bertozzi C, Hart G, and Etzler M. *Essentials of Glycobiology*. 2nd edition, Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2009.
4. Chen CC, Engelborghs S, Dewaele S, Le Bastard N, Martin JJ, Vanhooren V, Libert C, De Deyn PP. Altered Serum Glycomics in Alzheimer Disease: A Potential Blood Biomarker? Rejuvenation Res. 2010 Apr 28.
5. Contessa JN, Bhojani M, Freeze HH, Ross BD, Rehemtulla A, Lawrence TS. Molecular Imaging of N-linked Glycosylation Suggests Glycan Biosynthesis is a Novel Target for Cancer Therapy. Clin Cancer Res. 2010 Apr 22.
6. Määttänen P, Gehring K, Bergeron JJ, Thomas DY. Protein quality control in the ER: The recognition of misfolded proteins. Semin Cell Dev Biol. 2010 Mar 25.
7. Earl LA, Bi S, Baum LG. N- and O-glycans modulate galectin-1 binding, CD45 signaling, and T cell death. J Biol Chem. 2010 Jan 22;285(4):2232-44
8. Mulcahy H, O'Rourke KP, Adams C, Molloy MG, O'Gara F.LST1 and NCR3 expression in autoimmune inflammation and in response to IFN-gamma, LPS and microbial infection. Immunogenetics. 2006 Jan; 57(12):893-903.

9. Brockhausen I, Schacter H, Stanley P. O-GalNAc Glycans. In: Varki A, Cummings R, Esko J, Freeze H, Stanley P, Bertozzi C, Hart G, and Etzler M. *Essentials of Glycobiology*, 2nd edition, Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2009
10. Singh AP, Moniaux N, Chauhan SC, Meza JL, Batra SK. Inhibition of MUC4 expression suppresses pancreatic tumor cell growth and metastasis. Cancer Res. 2004 Jan 15;64(2):622-30
11. Singh AP, Chauhan SC, Bafna S, Johansson SL, Smith LM, Moniaux N, Lin MF, Batra SK. Aberrant expression of transmembrane mucins, MUC1 and MUC4, in human prostate carcinomas. Prostate. 2006 Mar 1; 66(4):421-9.
12. Rubin BK. Mucus and mucins. Otolaryngol Clin North Am. 2010 Feb; 43(1):27-34, vii-viii.
13. Rachagani S, Torres MP, Moniaux N, Batra SK. Current status of mucins in the diagnosis and therapy of cancer. Biofactors. 2009 Nov-Dec; 35(6):509-27.
14. Forstner JF. Intestinal mucins in health and disease. Digestion. 1978; 17:234–263.
15. Hollingsworth MA, Swanson BJ. Mucins in cancer: protection and control of the cell surface. Nat Rev. 2004; 4:45–60.
16. Moniaux N, Escande F, Porchet N, Aubert JP, Batra SK. Structural organization and classification of the human mucin genes. Front Biosci. 2001;6:D1192–D1206
17. Carraway KL, Hull SR. O-glycosylation pathway for mucin-type glycoproteins. Bioessays. 1989 Apr; 10(4):117-21.
18. Patsos G, Corfield A. Management of the human mucosal defensive barrier: evidence for glycan legislation. Biol Chem. 2009 Jul; 390(7):581-90.
19. Collado MC, Derrien M, Isolauri E, de Vos WM, Salminen S. Intestinal integrity and Akkermansia muciniphila, a mucin-degrading member of the intestinal microbiota present in infants, adults, and the elderly. Appl Environ Microbiol. 2007 Dec; 73(23):7767-70.
20. Stanley P, Guidos CJ. Regulation of Notch signaling during T- and B-cell development by O-fucose glycans. Immunol Rev. 2009 Jul; 230(1):201-15.
21. Fukuda M. Leukosialin, a major O-glycan-containing sialoglycoprotein defining leukocyte differentiation and malignancy. Glycobiology. 1991 Sep; 1(4):347-56.
22. Luther KB, Haltiwanger RS. Role of unusual O-glycans in intercellular signaling. Int J Biochem Cell Biol. 2009 May;41(5):1011-2
23. Esko JD, Kimata K, and Lindahl U. Proteoglycans and Sulfated Glycosaminoglycans. In: Varki A, Cummings R, Esko J, Freeze H, Stanley P, Bertozzi C, Hart G, and Etzler M. *Essentials of Glycobiology*, 2nd edition, Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2009
24. Kolset SO, Prydz K, Pejler G. Intracellular proteoglycans. Biochem J. 2004; 379: 217–227
25. Sharon N, Lis H. *Lectins*, 2nd Edition. pp. 15-16. Kluwer Academic Publishers. Nicholson AC, Malik SB, Logsdon JM Jr, Van Meir EG. Functional evolution of ADAMTS genes: evidence from analyses of phylogeny and gene organization. BMC Evol Biol. 2005 Feb 4; 5(1):11.
26. Yan D, Lin X. Shaping morphogen gradients by proteoglycans. Cold Spring Harb Perspect Biol. 2009 Sep; 1(3):a002493.

CELL MEMBRANE AND GLYCOLIPIDS

The cell membrane (also called the plasma membrane) is the biological membrane separating the interior of a cell from the outside environment. It surrounds all cells and it is selectively-permeable, controlling the movement of substances in and out of cells, and contains a wide variety of biological molecules, primarily proteins and lipids, which are involved in a variety of cellular processes such as cell adhesion, ion channel conductance and cell signaling. The plasma membrane also serves as the attachment point for the intracellular cytoskeleton and, if present, the extracellular cell wall.

The plasma membrane of cells is made of a combination of glycosphingolipids and protein receptors organized in glycolipoprotein microdomains termed *lipid rafts*. (1-3) These specialized membrane microdomains compartmentalize cellular processes by serving as organizing centers for the assembly of signaling molecules, influencing membrane fluidity and membrane protein trafficking, and regulating neurotransmission and receptor trafficking. (3) Lipid rafts are more ordered and tightly packed than the surrounding bilayer, but float freely in the membrane bilayer. (4)

The cell membrane consists primarily of a thin layer of amphipathic phospholipids that spontaneously arrange so the hydrophobic “tail” regions are shielded from the surrounding polar fluid, causing the more hydrophilic “head” regions to associate with the cytosolic and extracellular faces of the resulting bilayer. This forms a continuous, spherical lipid bilayer. In animal cells, cholesterol is normally found dispersed in varying degrees throughout cell membranes, in the irregular spaces between the hydrophobic tails of the membrane lipids, where it confers stiffening and strengthening effects on the membrane. Because of the hydrophobic interior of the lipid bilayer, polar molecules cannot enter the cell. However, cells devised means of transferring small polar molecules. Transport proteins, each specialized for a certain molecule, can transport polar molecules across the membrane.

Membrane transport of small molecules

There are several types of membrane transport proteins. Transport proteins, each specialized for a certain molecule, can transport polar molecules across the membrane. There are several types of membrane transport proteins. Uniports simply move solutes from one side to another. Cotransport systems work by simultaneously sending two solutes across the lipid bilayer. There are two types of cotransport systems: symport, in which the solutes are sent in the same direction, and antiport, in which they are sent in opposite directions. These transport proteins work passively, meaning that the cell doesn't have to expend energy sending the solute in or out. This effect is dependent on the solute moving in its natural direction, i.e., moving from solution that is more concentrated to less concentrated or from positive to negative.

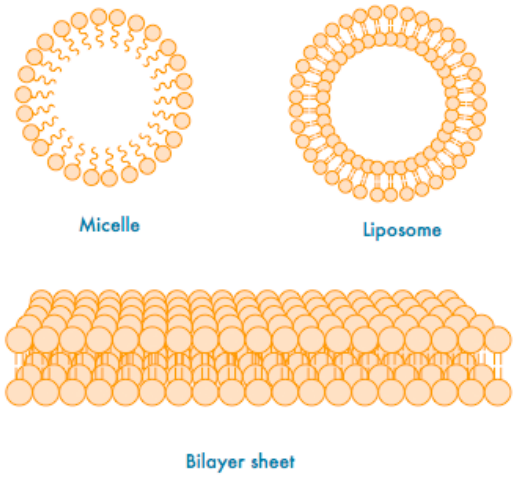


Figure 6.1.9 Self-organization of phospholipids: a spherical liposome, a micelle, and a lipid bilayer

Some specific examples of transport membranes are channel proteins, which allow solutes to cross if they are the correct size and charge. Carrier proteins bind to the solute and lead it through the bilayer. These are examples of passive transport. To move a solute against their natural direction - for example higher concentration to lower concentration, energy (ATP) is needed to pump the solute in or out.

The arrangement of hydrophilic heads and hydrophobic tails of the lipid bilayer prevent polar solutes (e.g. amino acids, nucleic acids, carbohydrates, proteins, and ions) from diffusing across the membrane, but generally allows for the passive diffusion of hydrophobic molecules. This affords the cell the ability to control the movement of these substances via transmembrane protein complexes such as pores and gates.

The *apical membrane* of a polarized cell is the surface of the plasma membrane that faces the lumen. This is particularly evident in epithelial and endothelial cells, but also describes other polarized cells, such as neurons. The *basolateral membrane* of a polarized cell is the surface of the plasma membrane that forms its basal and lateral surfaces. It faces towards the interstitium and away from the lumen. *Tight junctions* that join epithelial cells near their apical surface prevent the migration of proteins from the basolateral membrane to the apical membrane. The basal and lateral surfaces thus remain roughly equivalent to one another, yet distinct from the apical surface.

Membrane transport of macromolecules

Most cells use exocytosis and endocytosis to secrete and ingest macromolecules, respectively. In exocytosis, the contents of special vesicles are released when the vesicle fuses with the cell membrane. In endocytosis, the membrane depresses and pinches off, enclosing the molecule. Two different sizes are formed, pinocytotic (small) and phagocytic (large). In receptor-mediated endocytosis, coated pits and vesicles bind to specific receptors on the cell surface, allowing the cell to select which molecules to take and which to reject.

Two main categories of molecule transport exist in cells, active transport, and passive transport. For small molecules such as oxygen, ethanol, and carbon dioxide, the molecules could easily cross the membrane via passive transport, in the form of simple diffusion through a concentration gradient. However, the means that cells use to transfer small molecules are not sufficient for transporting macromolecules, which include proteins, polynucleotides, and polysaccharides. To transport these macromolecules, cells rely on active transport. There are two basic means of active transport - by *exocytosis* and by *endocytosis*. Exocytosis involves sending macromolecules out of the cell, while the opposite applies to endocytosis.

Exocytosis

Macromolecules that are released don't simply drift towards the cell's membrane and squeeze their way out. They are actually packaged in a vesicle that separates them out from the rest of the cell. The vesicle fuses with its specific membrane structure and its contents are released without the vesicle, which is incorporated back into the cell's membrane.

Proteins, for example, that are to be secreted from the cell are made on the rough endoplasmic reticulum (ER). They are then transported to the Golgi complex by ER induced vesicles. The Golgi complex sorts and packages the proteins into vesicles that separate themselves off the Golgi complex and eventually fuse with the cellular membrane.

Some molecules are secreted continually from the cell, but others are selectively secreted. To control secretion, specific substances are stored in secretory vesicles, which are released when triggered by an extracellular signal. The signal, hormones being an example, binds to its specific cell surface receptor. Then the concentration of free Ca2+ is increased in the cell. The increased concentration of Ca2+ triggers exocytosis, causing the secretory vesicles to fuse with the cellular membrane, thereby releasing the substances outside the cell.

Endocytosis

There are two types of endocytosis. *Pinocytosis* involves ingesting small molecules and/or fluids surrounding the cell in a process known as fluid-phase endocytosis. *Phagocytosis* involves the ingestion of large molecules, such as microorganisms or cell debris using large vesicles, or vacuoles.

The endocytic route by which a molecule, virus, or drug complex enters the cell is highly dependent on its size, charge, and composition, as well as on the cell type into which it is entering. For example, although specialized immune cells may nonspecifically take up molecules such as the remnants of other cells that have undergone apoptosis via phagocytosis, specific complementary sequences are needed by hormones to enter the target epithelial cells via receptor-mediated endocytosis.

Most of the ingested molecules, which are surrounded by small vesicles called the primary vesicle, are fused with secondary lysosomes. The molecules that have been ingested are then deposited at the cytosol, where the cell uses them and most of the vesicle is reintegrated into the cellular membrane. In some cases, the vesicle bypasses the secondary lysosome and goes directly to its target.

Often, a cell needs to ingest a molecule selectively. For this case, it uses a special process, called receptor-mediated endocytosis. The molecules ingested bind to specific cell surface receptors and are internalized at a greater rate than fluid is by fluid-phase endocytosis. An example receptor-mediated endocytosis is the intake of low-density lipoproteins, or LDL. When a cell needs cholesterol to make more membrane, it makes receptors for LDL and places them in the cellular membrane. The LDL then binds to the receptors, where it is taken in rapidly by the cell. Certain individuals contain defective genes for making the cell surface receptor for LDL. These people are unable to ingest LDL and thus have a higher concentration of LDL.

> Keeping it all in balance
>
> Eukaryotic cells are nearly continuously ingesting the surrounding fluids and molecules. In so doing, they are also ingesting their own cellular membrane at a rapid rate. Macrophages, for example ingest 3% of its cellular membrane each minute, or 100% each half-hour, so obviously the membrane is being added to by exocytosis at about the same rate it is being removed by endocytosis.

Various medications enter the cell and are processed via these endocytic pathways, an increased understanding of endocytosis and the cellular trafficking that occurs thereafter has a great deal of relevance to current molecular medicine. Indeed, gene therapy, which is presently being investigated for its therapeutic potential in treating immunodeficiency and metabolic diseases, cancer and heart disease, employs a variety of viral and nonviral vectors that can be delivered to the target cells of the body and are subsequently endocytosed and dissembled. The mechanisms by which vectors such as adenoviruses, adeno-associated viruses, retroviruses, and liposomes enter the cell are being increasingly focused on as the effort to increase the efficiency and safety of gene therapy continues. (5)

Receptor endocytosis

Ligand-induced endocytosis of signaling receptors is thought to be an important mechanism for negatively regulating signaling from the cell surface. Receptor endocytosis can attenuate the strength or duration of many plasma membrane-regulated signaling processes by physically reducing the concentration of cell surface receptors accessible to the ligand.

Receptor endocytosis involves the capture of transmembrane proteins and their extracellular ligands into cytoplasmic vesicles that are pinched off from the plasma membrane. Clathrin-coated pits mediate the best-studied pathway of receptor internalization. These are small areas of the plasma membrane that are covered from the cytoplasmic surface with clathrin triskelions, which consist of three clathrin heavy chains and three clathrin light chains assembled into the polyhedral clathrin lattice. Receptors are recruited to clathrin-coated pits by directly interacting with the clathrin coat adaptor complex AP2 or by binding to other adaptor proteins, which in turn interacts with the clathrin heavy chain and/or AP2. Clathrin-coated pits invaginate with the help of several accessory proteins and pinch off to form a clathrin-coated vesicle in a process that requires the GTPase dynamin. Several clathrin-independent pathways of endocytosis also exist, although the

precise mechanisms and structural components involved in these pathways are not well understood. (25)

Glycocalyx

The cell membrane contains many integral membrane proteins, which pepper the entire surface. These structures, which can be visualized by electron microscopy or fluorescence microscopy, can be found on the inside of the membrane, the outside, or membrane spanning. These may include integrins, cadherins, desmosomes, clathrin-coated pits, caveolae, and different structures involved in cell adhesion. The *glycocalyx* is an important feature in all cells, especially epithelia with microvilli. Recent data suggest the glycocalyx participates in cell adhesion, lymphocyte homing, and many other boundary functions.

The glycocalyx is chemically unique in everyone but identical twins, and acts like an identification tag that enables the body to distinguish its own healthy cells from transplanted tissues, invading organisms and diseased cells. Human blood groups and transfusion compatibility are determined by glycoproteins.

The glycocalyx, the "sugar-rich" covering that coats plasma membranes, may be an extraneous coat and/or an integral part of the plasma membrane. (16) It is considered to form a hydrophilic polyanionic gel coat on the enterocyte surface and is thought to maintain cell surface charge, protect against physical trauma, regulate ionic and macromolecular access, and form a cationic store. (17)

Thus, absence of the glycocalyx would have profound consequences on normal cell function and may result in an inability to maintain cellular homeostasis. This could explain why the vast majority of cases of microvillus atrophy do not exhibit hydramnios, as the amniotic fluid, which is swallowed by the fetus and bathes the luminal surface of the gut, is comparable in osmolality to serum,(18,19) resulting in no osmotic pressure across the gut in utero. This changes dramatically following birth and could explain the rapid onset of symptoms.

In the intestine, there are many glycosylated proteins associated with the apical epithelial membrane and it is uncertain which ones provide the physical characteristics of the glycocalyx listed above, or whether a particular component is responsible. Components include brush border enzymes, mucins, and a filamentous matrix (termed the filamentous brush border glycocalyx -FBBG), the major component of which is an approximately 400 kDa molecular weight transmembrane mucin-type glycoprotein (20) containing O-acetylated sialic acid. (22) The 400 kDa FBBG in the rabbit has the same morphological appearance as the fine filamentous material on the microvilli of humans and various other species, including bats, rodents, and amphibians. (23) Brush border enzymes are normally inserted into the apical membrane in microvillous atrophy. Many of these brush border hydroxylases are under the influence of ABO polymorphism. (24)

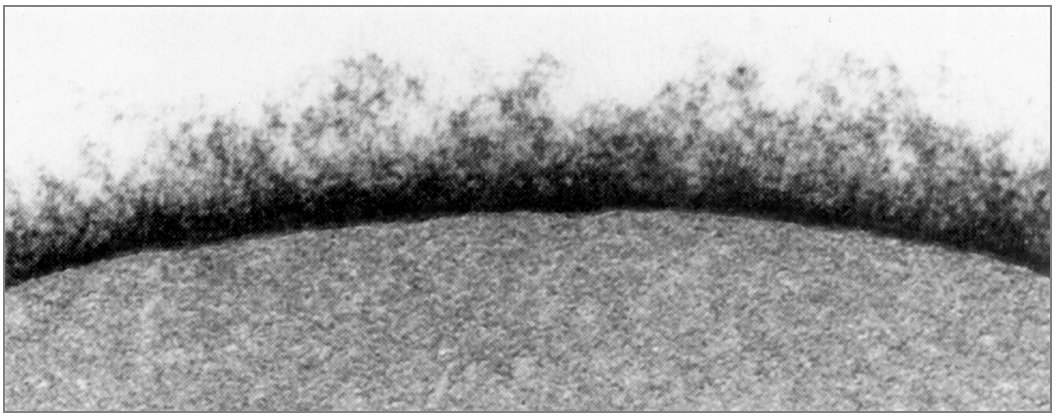


Figure 6.1.10 Erythrocyte glycocalyx. Taken from Voet and Voet, *Biochemistry 3rd Edition, Wiley 2004*.

Types of glycolipids

The cell membrane consists of three classes of amphipathic lipids: phospholipids, glycolipids, and steroids. The amount of each depends upon the type of cell, but in the majority of cases, phospholipids are the most abundant. In RBC studies, 30% of the plasma membrane is lipid. Plasma membranes also contain carbohydrates, predominantly glycoproteins, but with some glycolipids (cerebrosides and gangliosides). For the most part, no glycosylation occurs on membranes within the cell; rather glycosylation generally occurs on the extracellular surface of the plasma membrane.

Glycolipids are lipids with a carbohydrate attached. Their role is to provide energy and to serve as markers for cellular recognition. The term *glycolipid* designates any compound containing one or more monosaccharide residues bound by a glycosidic linkage to a hydrophobic moiety such as an acylglycerol, a sphingoid, a ceramide (*N*-acylsphingoid) or a prenyl phosphate. One type of glycolipid found in human red blood cells is involved in the ABO blood group antigens. They occur where a carbohydrate chain is associated with phospholipids on the exoplasmic surface of the *cell membrane*. The carbohydrates are found on the outer surface of all eukaryotic cell membranes. The carbohydrate structure of the glycolipid is controlled by the glycosyltransferases that add the lipids and glycosyl hydrolases that modify the glycan after addition.

Glycolipids extend from the phospholipid bilayer into the aqueous environment outside the cell where it acts as a recognition site for specific chemicals as well as helping to maintain the stability of the membrane and attaching cells to one another to form tissues.

Glycosphingolipids

Membrane lipids other than phospholipids include the glycolipids glycosphingolipids (GSL) in animals. GSL are a large family of glycan structures covalently associated with lipids. These glycans are often subdivided into several series, named the lacto-, neolacto-, ganglio- and globo-series. They contain a hydrophobic ceramide anchor N-acylsphingosine and a hydrophilic headgroup composed of saccharides. They are normally found at the outer surface of cell membranes. The composition of the saccharide moiety is cell type specific,

depends on the developmental stage of the organism, or can change with the oncogenic state of a cell.

Gangliosides

In 1943, the German scientist Ernst Klenk (1896-1971) first applied the name *ganglioside* to lipids newly isolated from ganglion cells of the brain. Gangliosides are molecules composed of a glycosphingolipid (ceramide and oligosaccharide) with one or more sialic acids (AKA n-acetylneuraminic acid, NANA) linked on the sugar chain. The 60+ known gangliosides differ mainly in the position and number of NANA residues. They are the component of the cell plasma membrane that modulates cell signal-transduction events. It appears that they concentrate in lipid rafts.

Gangliosides are more complex glycosphingolipids in which oligosaccharide chains containing N-acetylneuraminic acid (NeuNAc) are attached to a ceramide. NeuNAc, an acetylated derivative of the carbohydrate sialic acid, makes the head groups of gangliosides anionic. In all gangliosides, the ceramide is linked through its C-1 to a β-glucosyl residue, which, in turn, is bound to a β-galactosyl residue.

Gangliosides, glycosphingolipids, and glycoproteins found on the surface of oligosaccharides provide cells with distinguishing surface markers that can serve in cellular recognition and cell-to-cell communication. Structures similar to the ABO blood group antigens on the surface of human cells can be oligosaccharide components of glycosphingolipids in addition to being linked to proteins to form glycoproteins. Gangliosides are highly important in immunology. Natural and semisynthetic gangliosides are considered possible therapeutics for neurodegenerative disorders.

The carbohydrate portion of the ganglioside, GM1, present on the surface of intestinal epithelial cells, is the site of attachment of cholera toxin, the protein secreted by *Vibrio cholerae*. It is widely reported that cholera toxin remains a significant cause of gastrointestinal disease globally, particularly in developing countries where access to clean drinking water is at a premium. Vaccines are prohibitively expensive and provide only short-term protection. Consequently, there is scope for continued development of novel treatment strategies. One example is the use of galactooligosaccharides (GOS) as functional mimics for the cell-surface toxin receptor GM1. (9)

Cerebrosides

The discovery of the *glycosphingolipids* is generally attributed to Johan L. W. Thudichum, who in 1884 published on the chemical composition of the brain. In his studies he isolated several compounds from ethanolic brain extracts which he coined *cerebrosides*. (6)

The fundamental structure of a cerebroside is ceramide. *Ceramides* are a family of lipid molecules. A ceramide is composed of sphingosine and a fatty acid and they are found in high concentrations within the cell membrane of cells. They are one of the component lipids that make up sphingomyelin, one of the major lipids in the lipid bilayer. For years, it was assumed that ceramides and other sphingolipids found in the bilayer cell membrane were purely structural elements. This is now known to be not completely true. Perhaps one of the most fascinating aspects of ceramide is that it can act as a signaling molecule. The most well-known functions of ceramides as cellular signals include regulating the differentiation, proliferation, programmed cell

death, and apoptosis of cells. (10) Sphingosine is the main long-chain base present in ceramide. Monoglycosyl and oligoglycosylceramides having a mono or polysaccharide bonded glycosidically to the terminal OH group of ceramide are defined as *cerebrosides.* Cerebrosides are important components in animal muscle and nerve cell membranes.

Glycosphingolipids are a class of biomacromolecules assembled through stepwise action of glycosyltransferases. Composed of a hydrophobic ceramide part and a hydrophilic complex carbohydrate part, glycosphingolipids are synthesized in the endoplasmic reticulum and Golgi complex, where they traffic to the outside layer of plasma membrane. The cell surface glycosphingolipids are constantly recycled to the lysosome where they are degraded by glycosidases with the assistance of sphingolipid activator proteins. (14)

The sugar residue can be either glucose or galactose; the two major types are therefore called glucocerebrosides and galactocerebrosides. Galactocerebrosides are typically found in neural tissue, while glucocerebrosides are found in other tissues. A defect in the degradation of glucocerebrosides is Gaucher's disease. Galactosylceramide is the principal glycosphingolipid in brain tissue. Galactosylceramides are present in all nervous tissues, and can compose up to 2% dry weight of grey matter and 12% of white matter.

Glucosylceramide is found at low levels in animal cells such as the spleen, erythrocytes, and nervous tissues, especially neurons. Glucosylceramide is a major constituent of skin lipids, where it is essential for lamellar body formation in the stratum corneum and for the maintenance of the barrier to water permeability of the skin. Glucosylceramide is the only glycosphingolipid common to plants, fungi, and animals. It is usually considered the principal glycosphingolipid in plants. It is a major component of the outer layer of the plasma membrane.

Some of the most devastating inborn errors in metabolism are those associated with defects in the enzymes responsible for the lysosomal degradation of membrane glycosphingolipids that are particularly abundant in the membranes of neural cells. Many of these disorders lead to severe psychomotor retardation and early lethality.

Today, sphingolipids and their glycosylated derivatives are the subjects of intense study aimed at elucidating their role in the structural integrity of the cell membrane, their participation in recognition and signaling events, and in particular their involvement in pathological processes that are at the basis of human disease such as the sphingolipidoses and Type 2 diabetes.

Glycosphingolipids serve as ligands for receptors involved in signal transduction and immune recognition. Stimulation of invariant natural killer T (iNKT) cells with SGL-S23, a novel synthetic glycolipid analog of alpha-galactosylceramide with an elongated sphingosine chain, has been shown to suppress K/BxN serum transfer arthritis strongly, indicating that glycolipid ligands holds promise with regard to the treatment of autoimmune diseases such as rheumatoid arthritis. (7) Plant sphingoid bases prepared from wheat-flour cerebroside possess apoptotic effects on human colorectal cancer DLD-1. Morphological changes such as condensed chromatin fragments were found, so those sphingoid bases reduced cell viability through causing apoptosis in these cells. (8) New immunotherapy approaches might be explored by interfering with glycolipid metabolism or by directly supplementing rationally designed glycolipids.

One of the most clinically important classes of glycosphingolipids is those that confer antigenic determinants on the surfaces of cells, particularly erythrocytes. The ABO blood group antigens are the carbohydrate moieties of glycolipids on the surface of cells as well as the carbohydrate portion of serum glycoproteins. When present on the surface of cells the ABO carbohydrates are linked to sphingolipid and are therefore of the glycosphingolipid class. When the ABO carbohydrates are associated with protein in the form of glycoproteins they are found in the serum and are referred to as the secreted forms. Some individuals produce the glycoprotein forms of the ABO antigens ("secretors") while others ("non-secretors") do not.

Ceramide accumulation has been found following exposure of cells to a variety of apoptotic agents:

- TNF-α
- Fas ligand
- Endotoxin/ Lipopolysaccharide
- UV Light
- Chemotherapeutic agents
- 1,25-dihydroxy-vitamin D
- Gamma interferon
- Heat
- Ionizing radiation
- Ceramidase Inhibitors
- Retinoic acid
- Hypoxia

Galactolipids

Galactolipids are the main part of plant membrane lipids where they substitute phospholipids to conserve phosphate for other essential processes. Galactolipids play a crucial role in photosynthesis and are important for the adaptation of membrane-lipid composition in plants to phosphate-limiting conditions. (11)

The galactolipid galactocerebroside (GalC) and its sulfated derivative sulfatide is also in abundance present (together with a small group of proteins) in myelin, the membrane around the axons in the nervous system of vertebrates. Myelination is a developmentally regulated process whereby myelinating glial cells elaborate large quantities of a specialized plasma membrane that ensheathes axons. The myelin sheath contains an unusual lipid composition in that the glycolipid galactosylceramide (GalC) and its sulfated form sulfatide constitute a large proportion of the total lipid mass. These glycolipids have been implicated in a range

of developmental processes such as cell differentiation and myelination initiation. (12)

Ureaplasma urealyticum, a species of Mycoplasma, can bind to germ cell sulfogalactosylglycerolipids (SGG) and degrade sperm resulting in infertility. SGG the major mammalian male germ cell glycolipid, synthesized via sulfation of galactosylglycerolipid in early primary spermatocytes. (13)

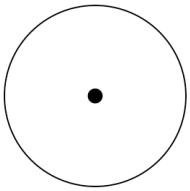
Globosides

A globoside is a type of glycosphingolipid with GalNAc as the side chain. The side chain can be cleaved by beta-hexosaminidase. If the enzyme is not functioning correctly, then globosides can accumulate, leading to Sandhoff disease.

The mechanistic studies on immune recognition of carbohydrates have led to advances in the identification of the precise sugar structures recognized by the immune system. Invariant NKT cells are a hybrid cell type of Natural Killer cells and T cells, whose development is dependent on thymic positive selection mediated by double positive thymocytes through their recognition of natural ligands presented by the CD1d antigen. Genetic evidence suggests that beta-glucosylceramide-derived glycosphingolipids are natural ligands for NKT cells. One in particular, isoglobotriaosylceramide (iGb3), is a stimulatory NKT ligand. (14) Based on genetic data and cellular immunological assays iGb3 is one of the candidates likely recognized by NKT cells under pathophysiological conditions such as cancer and autoimmune disease. (15)

1. Thomas S, Pais AP, Casares S, Brumeanu TD. . Analysis of lipid rafts in T cells. Molecular Immunology 2004; 41: 399-409.
2. Thomas S, Kumar RS, Brumeanu TD. Role of lipid rafts in T cells. AITE. 2004; 52: 215-224.
3. Korade Z. Lipid rafts, cholesterol, and the brain. Neuropharmacology. 2008; 55:1265-273.
4. Simons K and Ehehalt R. Cholesterol, lipid rafts, and disease. The Journal of Clinical Investigation. 2002; 110:597-603.
5. Ziello JE, Huang Y, Jovin IS. Cellular endocytosis and gene delivery. Mol Med. 2010 May-Jun; 16(5-6):222-9.
6. Wennekes T, van den Berg RJ, Boot RG, van der Marel GA, Overkleeft HS, Aerts JM. Glycosphingolipids—nature, function, and pharmacological modulation. Angew Chem Int Ed Engl. 2009; 48(47):8848-69.
7. Kaieda S, Tomi C, Oki S, Yamamura T, Miyake S. Activation of invariant natural killer T cells by synthetic glycolipid ligands suppresses autoantibody-induced arthritis. Arthritis Rheum. 2007 Jun; 56(6):1836-45.
8. Sugawara T, Kinoshita M, Ohnishi M, Miyazawa T. Apoptosis induction by wheat-flour sphingoid bases in DLD-1 human colon cancer cells. Biosci Biotechnol Biochem. 2002 Oct; 66(10):2228-31.
9. Sinclair HR, de Slegte J, Gibson GR, Rastall RA. Galactooligosaccharides (GOS) inhibit Vibrio cholerae toxin binding to its GM1 receptor. J Agric Food Chem. 2009 Apr 22; 57(8):3113-9.
10. Taha TA, Mullen TD, Obeid LM.A house divided: ceramide, sphingosine, and sphingosine-1-phosphate in programmed cell death. Biochim Biophys Acta. 2006 Dec; 1758(12):2027-36.
11. Kelly AA, Dörmann P. Green light for galactolipid trafficking. Curr Opin Plant Biol. 2004 Jun; 7(3):262-9.
12. Marcus J, Popko B. Galactolipids are molecular determinants of myelin development and axo-glial organization. Biochim Biophys Acta. 2002 Dec 19; 1573(3):406-13.
13. Ma J, Xu C. Relationship between mycoplasma infection and germ cell sulfogalactosylglycerolipid. Zhonghua Nan Ke Xue. 2004 Mar; 10(3):215-7, 221.
14. Li Y, Thapa P, Hawke D, Kondo Y, Furukawa K, Furukawa K, Hsu FF, Adlercreutz D, Weadge J, Palcic MM, Wang PG, Levery SB, Zhou D. Immunologic glycosphingolipidomics and NKT cell development in mouse thymus. J Proteome Res. 2009 Jun; 8(6):2740-51.
15. Zhou D. The immunological function of iGb3. Curr Protein Pept Sci. 2006 Aug; 7(4):325-33.
16. Ito S. Structure and function of the glycocalyx. Fedn Proc 1969; 28:12–25.
17. Gupta BL. The relationship of mucoid substances and ion and water transport, with new data on intestinal goblet cells and a model for gastric secretion. Symp Soc Exp Biol 1989;43:81–110

18. Gillibrand PN. Changes in the electrolytes, urea, and osmolality of the amniotic fluid with advancing pregnancy. J Obstet Gynaec Brit Cwlth. 1969; 76:898–905.
19. Sinha RS, Carlton M. The volume and composition of amniotic fluid in early pregnancy. J Obstet Gynaec Brit Cwlth 1970; 77:211–14.
20. Maury J, Nicoletti C, Guzzo-Chambraud L, et al. The filamentous brush border glycocalyx, a mucin-like marker of enterocyte hyper-polarization. Eur J Biochem 1995; 228:323–31.
21. Maury J, Bernadac A, Rigal A, et al. Expression and glycosylation of the filamentous brush border glycocalyx (FBBG) during rabbit enterocyte differentiation along the crypt-villus axis. J Cell Sci 1995; 108:2705–13.
22. Ito S. The enteric surface coat on cat intestinal microvilli. J Cell Biol 1965; 27:475–91.
23. Phillips AD, Fransen JAM, Hauri HP, et al. The constitutive exocytotic pathway in microvillous atrophy. J Pediatr Gastroenterol Nutr 1993; 17:239–46.
24. Green FR, Greenwell P, Dickson L, Griffiths B, Noades J, Swallow DM. Expression of the ABH, Lewis, and related antigens on the glycoproteins of the human jejunal brush border. Subcell Biochem. 1988; 12:119-53.
25. Sorkin A, von Zastrow M. Endocytosis and signaling: intertwining molecular networks. Nat Rev Mol Cell Biol. 2009 Sep; 10(9):609-22.



Glycan signaling and expression

"Traffic signals in New York are just rough guidelines."

—David Letterman

A common theme behind many of the recent findings, which is also a powerful driving force in carbohydrate research, is the realization that monosaccharides (the basic units of carbohydrates) can serve, as nucleotides and amino acids do, as code words in the molecular language of life, so that the specificity of many natural compounds is written in monosaccharides. It should also be noted that in the excitement about nucleic acids a simple fact is being forgotten: they too are complex carbohydrates, since monosaccharides are among their major constituents (ribose in RNA and deoxyribose in DNA). Until recently, it was not recognized that nature can employ sugars for the synthesis of highly specific compounds that can act as carriers of biological information. This capability arises from the fact that a large number of structures can be formed from a small number of monomers.

In other words, monosaccharides can serve as letters in a vocabulary of biological specificity, where the words are formed by variations in the nature of the sugars present, the type of linkage and the presence or absence of branch points. It is now known that the specificity of many natural polymers is written in terms of sugars, not amino acids or nucleotides. This idea is not entirely novel, but it has only recently become well established. (6)

GLYCANS AS SIGNALING AND ADHESION DEVICES

The central dogma of molecular biology limits the downstream flow of genetic information to proteins. Progress from the last two decades of research on cellular glycoconjugates justifies adding the enzymatic production of glycan antennae with information-bearing determinants to this famous and basic pathway. In the 1920's, it was still believed that only proteins carried the specific information in biological polymers. Between 1925 and 1937, Oswald T. Avery (1877-1955) of the Rockefeller Institute demonstrated that pure polysaccharides could carry specific immunological messages as antigens: substances that stimulate the production of an antibody specific to themselves. Thus, the highly purified Type III Pneumococcus "specific soluble substance" was an antigen even though it did not have any of the properties of a protein. This substance was shown to be polysaccharide, consisting of repeating units of cellobiuronic acid (a disaccharide of glucose and glucuronic acid). (4-5) It was Avery, by the way, who also discovered that DNA was the carrier of genetic information in cells.

Recent work suggests that metabolite availability to the hexosamine and Golgi N-glycosylation pathways exerts control over the assembly of macromolecular complexes on the cell surface and, in this capacity, acts upstream of signaling and gene expression. The structure and number of N-glycans per protein molecule cooperate to regulate lectin binding and thereby the distribution of glycoproteins at the cell surface. Congenital disorders of glycosylation provide insight as extreme hypomorphisms, whereas milder deficiencies may encompass many common chronic conditions, including autoimmunity, metabolic syndrome, and aging. (10)

It is well established that glycans are ideally suited for the formation of specificity determinants that can be recognized by complementary structures, which presumably are carbohydrate-binding proteins, on other cells or molecules. An impressive variety of regulatory processes including cell growth and apoptosis, folding and routing of glycoproteins and cell adhesion/migration have been unraveled and mediated or modulated by specific protein (lectin)-carbohydrate interactions. Currently, the potential for medical applications in anti-adhesion therapy or drug targeting is one of the major driving forces fueling progress in glycoscience.

The first indication that sugars serve as specificity determinants came from the discovery in 1941 by George K. Hirst (1906-1971) and by Ronald Hare (1899-1986) that the influenza virus caused red blood cells to agglutinate, or clump. (2) The molecular basis of this phenomenon was for a time obscure. Mainly because of the efforts of Alfred Gottschalk (1894-1973) in Australia, it was shown that the influenza virus binds to the red blood cell through sialic acid units on the cell surface. If the sialic acid is removed from the cell surface by the enzyme neuraminidase, the influenza virus will no longer bind to the cell.

The role of glycans in recognition has been best demonstrated in the control of the lifetime of glycoproteins in the circulatory system and their uptake into the liver and of the uptake of lysosomal enzymes by cells. In the course of an effort to understand the biological role of ceruloplasmin, a copper-transport protein found in the blood serum of man and other animals; when sialic acid was removed from ceruloplasmin and reinjected, the modified ceruloplasmin almost completely disappeared from the circulatory system within 15 minutes. This was in striking contrast to the native glycoprotein, almost all of which remained in circulation after the same length of time. (8) The exposure of terminal, nonreducing galactosyl residues by removal of sialic acid provides a means by which the liver recognized and removed the defective molecule from the circulation. (7)

Galactose serves as a recognition marker that determines the survival time of many serum glycoproteins in the circulatory system of man, the rabbit, and the mouse. In bird and reptile species, the recognition marker appears to be primarily acetylglucosamine. Clearance systems in which fucose and mannose are the markers have also been found.

By the covalent (electron-sharing) attachment of glycans to proteins or by a modification of the sugars in glycoproteins it may thereby be possible to control the protein's lifetime in the circulation and to direct them to the liver and perhaps also to other organs, as well as into lysosomes. Such techniques will have far-reaching uses for enzyme replacement therapy in cases of genetic disease and for delivering drugs accurately into target organs and cells. Sugars on cell surfaces also appear to determine the life span of circulating cells and their distribution in the body. Bertram M. Gesner and Victor Ginsburg originally demonstrated this role in 1964. They found that radioactively labeled rat lymphocytes migrated to the spleen when they were re-injected into the animal. If before re-injection the sugar fucose was removed from the surface of the cells by treatment with a specific glycosidase, the lymphocytes migrated to the liver instead, as if the fucose on the lymphocytes served as a "ZIP" code directing them where to go. (1)

Old red blood cells have less sialic acid on their surface than young ones, and so it has been postulated that the decrease of sialic acid is the sign responsible for the removal of the older red blood cells from the circulatory system. This hypothesis seemed to be further substantiated by the finding that when red blood cells are taken out of the circulation, and when the sialic acid is removed from their surface and they are re-injected into the blood, their life span is extremely short; only a couple of days out of the normal lifetime of 120. In spite of these striking correlations, there is considerable doubt whether the removal of sialic acid and the exposure of galactose units on the surface of the red blood cell are responsible for the removal of senescent red cells from the blood under physiological conditions in vivo. (3)

Certain relatively specific changes in expression of glycans are also often found in the course of transformation and progression to malignancy as well as other pathological situations such as inflammation. These spatially and temporally controlled patterns of glycan expression imply the involvement of glycans in many normal and pathological processes, the precise mechanisms of which are understood in only a few cases. (9)

No doubt, the molecular events underlying embryogenesis, especially of the nervous system, will be the major goal of biology well into the near future. Experiments in a number of laboratories are now in progress to elucidate the roles of glycans in these processes. (11) Gangliosides and glycoproteins of the N-glycan type are essential for the survival of the embryo and/or its normal development in the mouse. (12)

Notch receptors are highly conserved intercellular signaling pathways that direct embryonic cell-fate decisions. These receptors are regulated by "Fringe" proteins. Recent evidence shows that Fringe is a fucose-specific GlcNAc-transferase. (13,14)

Glycosaminoglycans can be considered signaling glycans because they interact with receptor tyrosine kinases and/or their ligands and facilitate changes in cell behavior. For example, hyaluronan oligosaccharides can bind to specific membrane proteins, such as CD44. In some cells, binding results in clustering of CD44, which activates kinases such as c-Src and focal adhesion kinase (FAK). Phosphorylation changes the interaction of the cytoplasmic tail of CD44 with regulatory and adaptor molecules that modulate cytoskeletal assembly/disassembly and cell survival and proliferation (15)

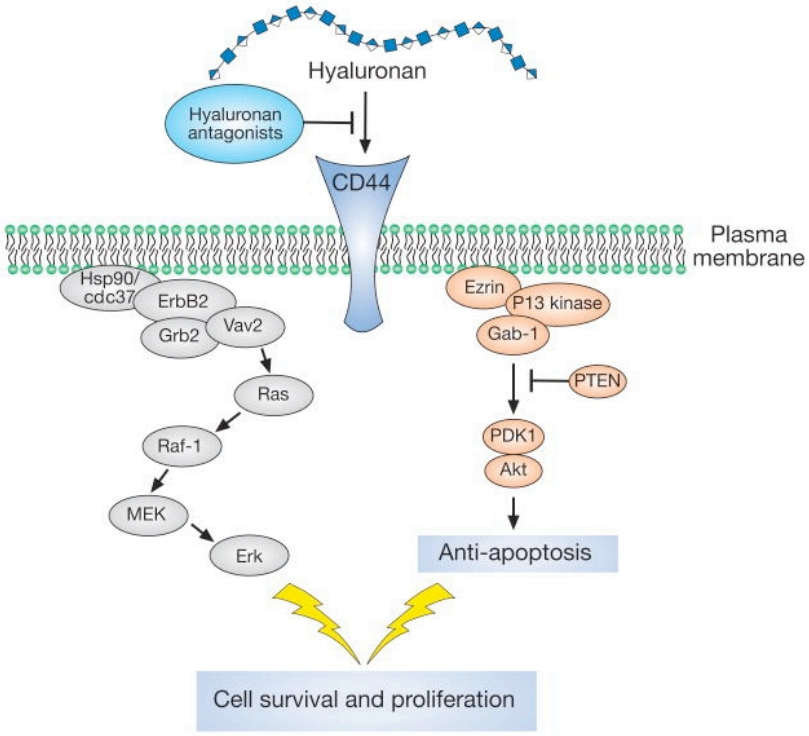


Figure 6.2.1 Schematic diagram of signaling pathways activated by binding of hyaluronan to CD44. In tumor cells, activation results in proliferation and invasion. In embryonic stem cells, it can result in epithelial to mesenchymal transition. (15)

In contrast to hyaluronan-dependent signal transduction, signaling via the sulfated GAG's heparan sulfate (HS) and chondroitin sulfate (GS) occurs by a more indirect mechanism. Sulfated GAG's bind to many ligand/receptor pairs, thereby lowering the effective concentration of ligand required to engage the receptor or increasing the duration of the receptor response. An example of this is the ability of exogenous heparin or endogenous heparan sulfate proteoglycans to activate fibroblast growth factor (FGF) receptors by FGF. Pluripotent embryonic stem cells (ESCs) must select between alternative fates of self-renewal and lineage commitment at each division during continuous proliferation. (17) Sulfated GAG's also facilitate the formation of morphogen gradients in tissues during early development. (16) Because the gradient determines cell specification during development, the glycan indirectly affects signaling responses in receptive cells.

Glycosynaptic microdomains

At least some of the biological effects glycosphingolipids (GSL's) convey are mediated through a somewhat unusual mechanism, emergent importance of which deserves a separate mention. It has been known for some time that GSL's have a tendency to aggregate in cell membranes forming clusters without recruitment of cholesterol. Such aggregates are necessary for glycosphingolipids to exert some of their biological activities. The term "glycosynapse" was introduced to describe such functional GSL aggregates. The aggregates have been subdivided into several subtypes depending on function and contents. Their importance is thought to relate to their ability to convey adhesion —

between cells, as well as between cells and extracellular matrix— and potential to associate to and modulate a number of key receptors such as growth factor receptors and integrins. (18-20)

1. Gesner BM, Ginsburg V. Effect of glycosidase on the fate of transfused lymphocytes. Proc Natl Acad Sci U S A. 1964 Sep; 52:750-5.
2. Meenan PJ. The laboratory diagnosis of epidemic influenza. Irish Journal of Medical Science. 1948; 23(4):169-175.
3. Lutz HU, Fehr J. Total sialic acid content of glycophorins during senescence of human red blood cells. J Biol Chem. 1979 Nov 25; 254(22):11177-80.
4. Heidelberger M, Avery OT. The soluble specific substance of Pneumococcus: Second Paper. J Exp Med. 1924 Aug 31; 40(3):301-317.
5. Heidelberger M, Goebel WF, Avery OT. The soluble specific substance of Pneumococcus: Third Paper. J Exp Med. 1925 Oct 31; 42(5):727-745.
6. Sharon N and Lis H. Carbohydrates in Cell Recognition. Sci Am. 1993 Jan; 94-102.
7. Morell AG, Gregoriadis G, Scheinberg IH, Hickman J, Ashwell G. The role of sialic acid in determining the survival of glycoproteins in the circulation. J Biol Chem. 1971 Mar 10; 246(5):1461-7.
8. Morell AG, Irvine RA, Sternlieb I, Scheinberg IH, Ashwell G. Physical and chemical studies on ceruloplasmin. V. Metabolic studies on sialic acid-free ceruloplasmin in vivo. J Biol Chem. 1968 Jan 10; 243(1):155-9.
9. Varki A and Sharon N. Historical Background and Overview: Varki A, Cummings R, Esko J, Freeze H, Stanley P, Bertozzi C, Hart G, and Etzler M. *Essentials of Glycobiology*, 2nd edition, Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2009.
10. Dennis JW, Nabi IR, Demetriou M. Metabolism, cell surface organization, and disease. Cell. 2009 Dec 24; 139(7):1229-41.
11. Roseman S. Reflections on glycobiology. J Biol Chem. 2001 Nov 9; 276(45):41527-42.
12. Kawai H, Allende ML, Wada R, Kono M, Sango K, Deng C, Miyakawa T, Crawley JN, Werth N, Bierfreund U, Sandhoff K, Proia RL. Mice expressing only monosialoganglioside GM3 exhibit lethal audiogenic seizures. J Biol Chem. 2001 Mar 9; 276(10):6885-8.
13. Brückner K, Perez L, Clausen H, Cohen S. Glycosyltransferase activity of Fringe modulates Notch-Delta interactions. Nature. 2000 Jul 27; 406(6794):411-5.
14. Moloney DJ, Panin VM, Johnston SH, Chen J, Shao L, Wilson R, Wang Y, Stanley P, Irvine KD, Haltiwanger RS, Vogt TF. Fringe is a glycosyltransferase that modifies Notch. Nature. 2000 Jul 27; 406(6794):369-75.
15. Etzler M and Esko JD. Free Glycans as Signaling Molecules. In: Varki A, Cummings R, Esko J, Freeze H, Stanley P, Bertozzi C, Hart G, and Etzler M. *Essentials of Glycobiology*, 2nd edition, Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press. 2009.
16. Mulloy B, Rider CC. Cytokines and proteoglycans: an introductory overview. Biochem Soc Trans. 2006 Jun; 34(Pt 3):409-13.
17. Kraushaar DC, Yamaguchi Y, Wang L. Heparan sulfate is required for embryonic stem cells to exit from self-renewal. J Biol Chem. 2010; Feb 19; 285(8):5907-16.
18. Tillack TW, et al. Localization of globoside and Forssman glycolipids on erythrocyte membranes, Biochim Biophys Acta. 1983; 733:15–24.
19. Todeschini AR, Hakomori SI. Functional role of glycosphingolipids and gangliosides in control of cell adhesion, motility, and growth, through glycosynaptic microdomains. Biochim Biophys Acta. 2008; 1780.
20. Hakomori SI. Inaugural article: the glycosynapse. Proc. Natl. Acad. Sci. U.S.A. 2002; 99:225–232.

Cellular adhesion

Cell adhesion molecules (CAMs) are cell surface proteins involved in the binding of cells, usually leukocytes, to each other, to endothelial cells, or to extracellular matrix. Specific signals produced in response to wounding and infection control the expression and activation of certain of these adhesion molecules. CAMS play a major role in morphogenesis and organogenesis. In vertebrates, a significant fraction of genes encodes cell-adhesion molecules. Multiple signal-transduction pathways have been described that modulate the adhesion process. These pathways have been studied in detail for cadherins and integrins —two major adhesion systems controlling cell–cell and cell–substrate interactions. Recent findings confirm that a given cell-adhesion molecule can be implicated

at different stages of development in processes as diverse as cell positioning, tissue patterning and compartmentalization, axon guidance and synaptogenesis. (6)

The interactions and responses then initiated by the binding of these CAMs to their receptors/ligands play important roles in the mediation of the inflammatory and immune reactions that constitute one line of the body's defense against these insults. Most of the CAMs characterized so far fall into three general families of proteins: the immunoglobulin (Ig) superfamily, cadherins, the integrin family, or the selectin family.

Cadherins

Central to the process of embryonic cellular adhesion is a class of transmembrane proteins known as *cadherins*. Cadherins require calcium ions for their binding, hence the name. Quite a number of different classes of cadherin molecules exist, each designated with a prefix (E-cadherin, N-cadherin, etc.) that notes the type of tissue with which it is associated. It has been observed that cells containing a specific cadherin subtype tend to cluster together: cells containing E-cadherin stick best to other cells with E-cadherin expressing cells, etc. and they will sort out from cells containing N-cadherin in their membranes. This pattern is called *homophilic binding*.

Different members of the cadherin family are found in different locations:

- *E-cadherins* are found in epithelial tissue
- *N-cadherins* are found in neurons
- *P-cadherins* are found in the placenta
- *T-cadherins* are tethered to the plasma membrane

The embryo must adhere to the uterus and embed itself in the uterine wall if development is to continue, which is why the first differentiation event in mammalian development distinguishes the outer cells that bind to the uterus (*trophoblast cells*) from the *inner cell mass* (those cells that will generate the adult organism). The trophoblast cells are endowed with both E-cadherins and P-cadherins and these cadherins recognize similar cadherins on the uterine cells. Second, they have receptors (the integrin proteins) for the collagen and the heparan sulfate glycoproteins of the uterine wall that work with the cadherins to maximize the adherence of the embryo to the uterus.

Of the cadherins, the epithelial form (E-cadherin) is the best understood and most studied. Various signaling molecules and transcription factors regulate the expression of E-cadherin. Loss of E-cadherin function or expression has been implicated in cancer progression and metastasis. E-cadherin down-regulation decreases the strength of cellular adhesion within a tissue, resulting in an increase in cellular motility and subsequent metastasis. This in turn may allow cancer cells to cross the basement membrane and invade surrounding tissues. E-cadherin deregulation is a hallmark of certain types of breast cancer. (1) E-cadherin expression is markedly reduced or absent in the great majority of invasive lobular carcinomas versus invasive duct carcinoma. (2)

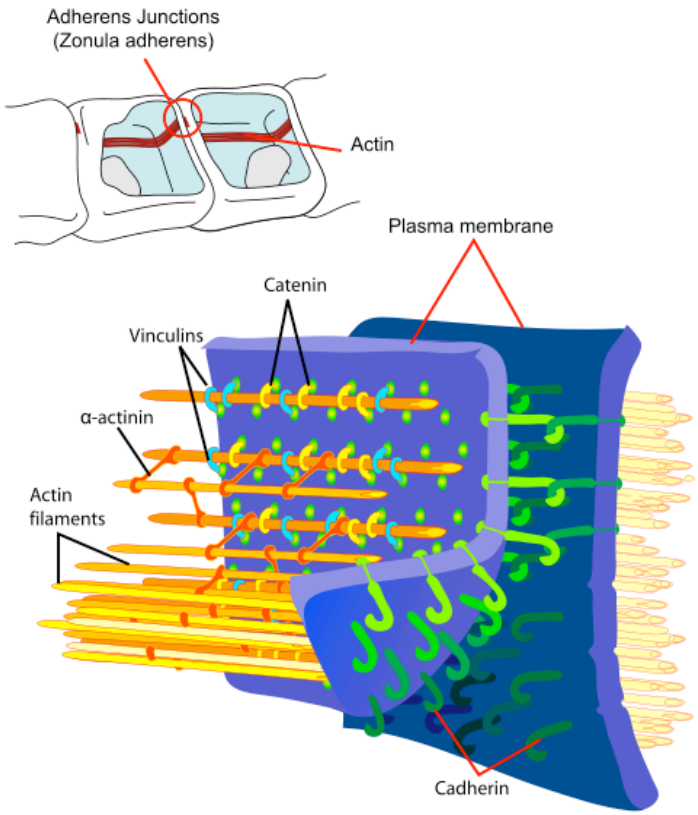


Figure 6.2.2 The cell-cell junction, called adherens or zonula adherens, and the main proteins involved in it.

As an intercellular adhesive, it binds to catenins (β-catenin, p-120 catenin) as the E-cadherin complex part of the protein complexes that occur at cell-cell junctions in epithelial tissues called *adherens complexes.* Adherens complexes are necessary for the creation and maintenance of epithelial cell layers by regulating cell growth and adhesion between cells. Because cellular adhesion (or rather lack of it) is associated with tumor metastasis, genomic or environmental disruptions of cadherin or catenin structure or function are associated with a wide variety of neoplasms. Under certain circumstances, β-catenin can build up in the cytosol and act as an oncogene. Disruption of β-catenin catabolism has been linked to colorectal cancer via its interactions with the product of the *APC* gene, which is mutated in adenomatous polyposis of the colon. (3)

During development, the cadherins often work with other adhesion systems, such as the *integrins*. Integrins play an important, if not critical, role in morphogenesis via their adhesive and signaling effects on the extra-cellular matrix (ECM) and components of the ECM such as fibronectin, vitronectin, collagen, and laminin. Connection with ECM molecules can cause a signal to be relayed into the cell through protein kinases (integrin-linked kinases; ILK) that are connected indirectly and temporarily with the intracellular end of the integrin molecule.

Evidence now points to ILK as having crucial, multifaceted roles in the normal development and function of several tissues including growth, proliferation, survival, differentiation, migration, invasion, and angiogenesis. Many of precise cellular processes regulated by ILK are dependent on contextual cues.

The tight regulation of these cellular functions is essential for tissue homeostasis, and their dysregulation is important for the development and progression of cancer. The over-expression of ILK is often a prominent feature of human malignancies and its increased abundance in tumor tissues correlates with poor patient outcome. Thus, ILK is an attractive therapeutic target in human cancer. (4)

N-cadherin is known to be involved in a wide spectrum of functions in the establishment of asymmetry, in somitogenesis, in heart morphogenesis and in synaptic contact. The mechanisms driving the repositioning of cells involve the orchestration of different adhesion and signaling molecules. Additional mechanisms either controlling or maintaining tissue compartments and cell or axon guidance rely heavily on other families of receptors and their cognate ligands such as ephrins, semaphorins, and netrins. (6)

Cadherins are important in the establishment of cell polarity and cell sorting during embryonic development. The term *cadherin switching* usually refers to a switch from expression of E-cadherin to expression of N-cadherin, but also includes situations in which E-cadherin expression levels do not change significantly but the cells turn on (or increase) expression of N-cadherin. One role of cadherin switching is to allow a select population of cells to separate from their neighbors – for example, during processes such as gastrulation, epiblast cell ingression through the primitive streak and neural crest emigration from the neural tube. Switching from E-cadherin to a different cadherin promotes a motile phenotype, which is also essential for processes like gastrulation and tumor invasion/metastasis.

Cadherin-mediated cell-cell contact can activate Rac1 and/or Cdc42, depending on the experimental system, whereas RhoA activity is decreased as cells become confluent. RhoA, but not Rac1 or Cdc42, is activated upon N-cadherin-mediated cell-cell contact. Rho GTPases and cadherins cooperate not only in cell adhesion but also in cell motility. In addition, they suggest that cadherin switching during tumorigenesis can influence cell behavior by activating the small GTPases that promote motility and invasion. (7)

A class of cellular adhesion molecules involved in early development, which are a bit are tangential to morphogenesis, but are worthy of mention, with regards to infertility, are the *selectins*. A type of *lectin,* as they bind to sugars (sialylated carbohydrates), selectins are involved in many of the common forms of inflammation, leukocyte extravasation, and cellular "homing." L-selectin plays an important role in leukocyte-endothelial cell interactions and may play a critical role in the success of human trophoblast implantation. Fetal trophoblasts have been shown to express L-selectin whilst human uterine epithelial cells also upregulate L-selectin oligosaccharide-based ligands during the implantation window of receptivity. These results suggest that trophoblast L-selectin mediates interactions with the uterus and that this adhesion mechanism may be critical to establishing human pregnancy. (5)

1. Zagouri F, Sergentanis TN, Zografos GC. Precursors and preinvasive lesions of the breast: the role of molecular prognostic markers in the diagnostic and therapeutic dilemma. World J Surg Oncol. 2007 May 31; 5:57.
2. Rosen, P. *Rosen's Breast Pathology*, 3rd ed., 2009, p. 704. Lippincott Williams & Wilkins.
3. Kennell J, Cadigan KM. APC and beta-catenin degradation. Adv Exp Med Biol. 2009; 656:1-12.

4. McDonald PC, Fielding AB, Dedhar S. Integrin-linked kinase—essential roles in physiology and cancer biology. J Cell Sci. 2008 Oct 1; 121(Pt 19):3121-32.
5. Genbacev OD, Prakobphol A, Foulk RA, Krtolica AR, Ilic D, Singer MS, Yang ZQ, Kiessling LL, Rosen SD, Fisher SJ. Trophoblast L-selectin-mediated adhesion at the maternal-fetal interface. Science. 2003 Jan 17; 299(5605):405-8.
6. Thiery JP. Cell adhesion in development: a complex signaling network. Curr Opin Genet Dev. 2003 Aug; 13(4):365-71.
7. Wheelock MJ, Shintani Y, Maeda M, Fukumoto Y, Johnson KR. Cadherin switching. J Cell Sci. 2008 Mar 15; 121(Pt 6):727-35.

The immunoglobulin superfamily

The immunoglobulin superfamily (IgSF) is a large group of cell surface and soluble proteins that are involved in the recognition, binding, or adhesion processes of cells. Molecules are categorized as members of this superfamily based on shared structural features with immunoglobulins (also known as antibodies) they all possess a domain known as an immunoglobulin domain or fold. Members of the IgSF include cell surface antigen receptors, co-receptors and co-stimulatory molecules of the immune system, molecules involved in antigen presentation to lymphocytes, cell adhesion molecules, certain cytokine receptors, and intracellular muscle proteins. They are commonly associated with roles in the immune system. A conservative estimate is that 4–5% of the human genome is dedicated to adhesion including 865 members in the Ig superfamily. (1)

IgSF of molecules includes ICAM-1, ICAM-2, ICAM-3, VCAM-1, and MadCAM-1, all of which bind to integrins on leukocytes and mediate their flattening onto the blood vessel wall with their subsequent extravasation into the surrounding tissue. Chemokines such as MCP-1 and IL-8 cause a conformational change in integrins so that they can bind to their ligands.

The integrin family of cellular adhesion molecules (CAMS) serves as receptors for the ICAMs and VCAMs. The integrins are heterodimeric proteins consisting of an alpha and a beta chain that mediate leukocyte adherence to the vascular endothelium or other cell-cell interactions. Different sets of integrins are expressed by different populations of leukocytes to provide specificity for binding to different types of CAMs expressed along the vascular endothelium.

Intercellular adhesion molecule 1 (ICAM-1)

Intercellular adhesion molecule (ICAM)-1 is an immunoglobulin (Ig)-like cell adhesion molecule expressed by several cell types including leukocytes and endothelial cells. ICAM-1 plays an important role in both innate and adaptive immune responses. It is involved in the trans-endothelial migration of leukocytes to sites of inflammation, as well as interactions between antigen presenting cells (APC) and T cells (immunological synapse formation). ICAM-1 is present in atherosclerotic lesions and is involved in their progression. A soluble form of ICAM-1 (sICAM-1) has been found in plasma. ICAM-1 levels are elevated in the serum of patients with cardiovascular disease, autoimmune disorders, as well as cancer, and several studies have correlated serum levels of ICAM-1 with the severity of these diseases. (13)

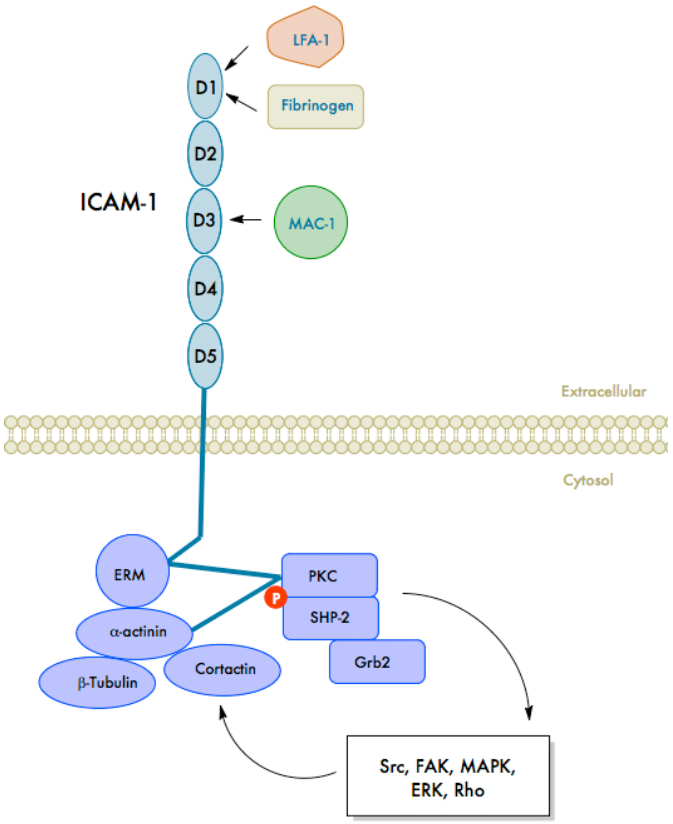


Figure 6.2.3 Model of ICAM-1 showing dimmer-specific binding and downstream signaling

ICAM-1 is a type I transmembrane protein with a molecular weight of 80–114 kDa depending on its level of glycosylation (unglycosylated ICAM-1 has a molecular weight of 60 kDa). The extracellular portion of ICAM-1 consists of 453 mainly hydrophobic amino acids, which form five immunoglobulin (Ig)-like domains. The extracellular region is attached to a single hydrophobic transmembrane region (24 residues) and a short cytoplasmic tail (28 residues). Each Ig domain has a β-sheet structure, which is stabilized by disulfide bonds. The cytoplasmic tail lacks classical signaling motifs, but has one tyrosine residue, which may be important for signaling. (13) The gene sequence of ICAM-1 consists of seven exons, which are separated by six introns. Exon 1 encodes the signal sequence, exons 2–6 each encode one of the five extracellular domains, and exon 7 encodes for the transmembrane region and cytoplasmic tail. (12)

The primary receptors for ICAM-1 are integrins, which mediate cell-cell interactions and allow for signal transduction. ICAM-1 interacts specifically with its receptors to induce a reversible adhesion interaction. Since normal immune function relies on ICAM-1 for processes like T cell activation and leukocyte recruitment, it is understandable that alterations in ICAM-1 structure or expression are associated with immune disorders. Several ligands for ICAM-1 have been identified; including the membrane bound b2 integrins LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18) on leukocytes, fibrinogen, rhinoviruses, and *Plasmodium falciparum*-infected erythrocytes. (13)

Trans-endothelial migration can be divided into four sequential, but overlapping steps summarized in the figure below:

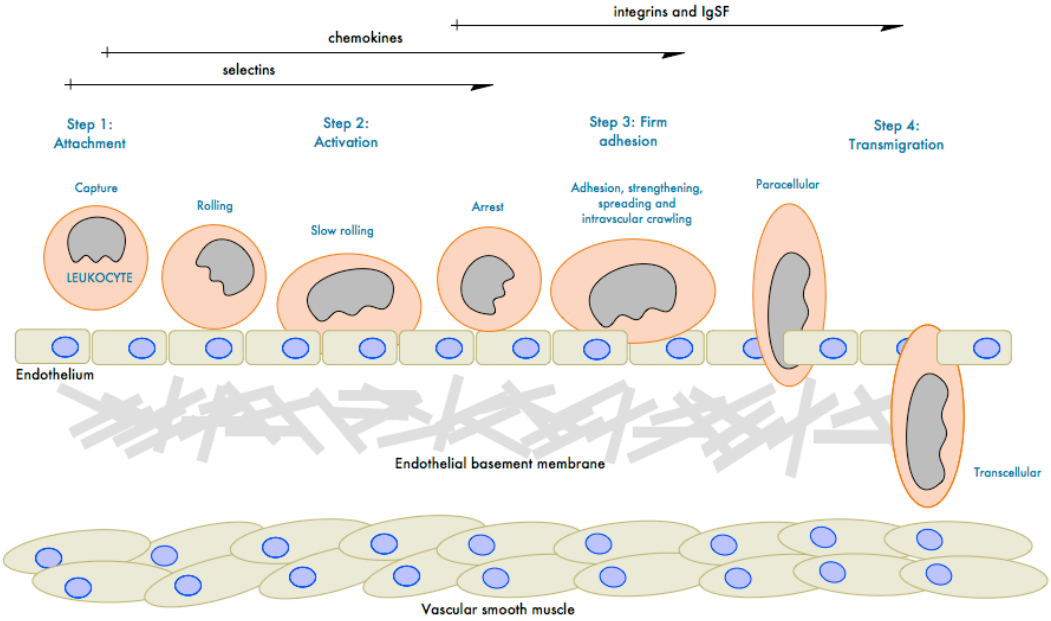


Figure 6.2.4 Cartoon depicting the transendothelial migration of leukocytes into sub-endothelial space

- *Step 1:* involves the rolling and tethering of leukocytes, facilitated by interactions between selectins and the sialylated carbohydrate portion of E- and P-selectin, both of which are present on the endothelium, and bind to carbohydrate structures closely related to sialyl Lewis^X on leukocytes. P-selectin also binds to P-selectin-glycoprotein ligand (PSGL)-1. L-Selectin, which is present on all circulating leukocytes, binds to CD34, PSGL-1 and sialyl Lewis X present on endothelial cells. This step prolongs contact with the blood vessel wall and enhances exposure to chemokines.

- *Step 2:* Chemokines are necessary for the activation of integrins on the leukocyte cell surface and for the direction of leukocyte migration. Integrins, which are present on leukocytes in an inactive form, undergo a conformational change upon cell activation, which leads to increased adhesion to their ligands.

- *Step 3:* Once firmly attached, leukocytes spread and slowly migrate over the endothelium. Arrested, activated leukocytes adhere firmly to the endothelium via LFA-1/ICAM-1, VLA-4/VCAM-1 and a4b7/MAD-CAM-1. A number of studies have demonstrated the importance of ICAM-1 for the initial steps of the process of transendothelial cell migration by using antiICAM-1 antibodies or an ICAM-1-deficient endothelium.

- *Step 4:* Leukocytes migrate through the endothelial cell barrier into the sub-endothelial space [81]. Several junctional proteins are located on the endothelial cell. Cell junction proteins include platelet endothelial cell adhesion molecule (PECAM)-1, VE-cadherin, junctional adhesion molecules

(JAM's), and CD99. The firm adhesion of leukocytes to the endothelium via ICAM-1 triggers increased intracellular $Ca^{2+,}$ the activation of p38 and *Rho*, while VCAM-1 binding leads to *rac1* activation. The activation of these signaling molecules is thought to facilitate transmigration by triggering endothelial cell contraction and by weakening the bonds of the junctional adhesion molecules.

A soluble ICAM-1 (sICAM-1) molecule has been identified in serum that consists of the five extracellular Ig-domains of the membrane-bound ICAM-1-molecule, but lacks the transmembrane and cytoplasmic domains. SICAM-1 is produced by a variety of different cells including HUVEC, human saphenous vein endothelial cells, human aortic endothelial cells, melanoma cells, and hematopoietic cell lines. SICAM-1 is present in normal human serum at concentrations between 100–450 ng/ml. (18) Increased levels of sICAM-1 have been found in serum from patients with cardiovascular disease, cancer, and autoimmune diseases. Several studies have correlated serum levels of sICAM-1 with severity of these diseases. (13)

A number of studies have investigated the use of sICAM-1 as a biomarker for cardiovascular disease prognosis. A significant correlation between sICAM-1 concentrations and future coronary artery disease has been demonstrated by several groups (14-16) but refuted by others. (17)

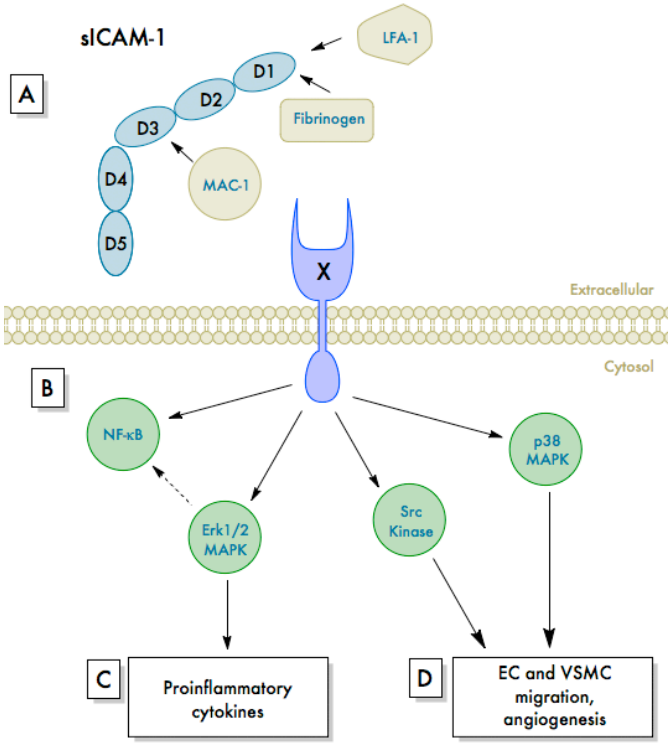


Figure 6.2.5 The signaling cascades downstream of sICAM-1's binding to target cells that have been partially elucidated. (A) The binding of sICAM-1 to an unidentified receptor X on the target cells" surface leads to (B) the activation of signaling pathways including NF-κB, Erk-1/-2 and p38 MAPK, as well as Src kinases. By mechanisms that are not fully elucidated, sICAM-1 induces (C) the secretion of a number of pro-inflammatory cytokines and chemokines, possibly via Erk and MAPK, and (D) induces EC and VSMC

migration and angiogenesis via p38 MAPK and Src but not Erk (no inhibition via PD98059). Modified from Lawson C, Wolf S. ICAM-1 signaling in endothelial cells. Pharmacol Rep. 2009 Jan-Feb; 61(1):22-32.

RAGE and advanced glycation

Numerous findings, ranging from epidemiological studies to molecular analyses of mouse models, have highlighted a strong contribution of chronic inflammation to tumor development. (3) Whether driven by genetic alterations, tissue damage or arising from preceding infection, the generation of an inflammatory microenvironment supports tumorigenesis by promoting cancer cell survival, proliferation, migration, and invasion. (4)

The receptor for advanced glycation end products (RAGE) is a multiligand receptor of the immunoglobulin superfamily of cell surface molecules that acts as a pattern recognition receptor. Besides binding ligands actively participating in inflammation and immune responses, RAGE serves as an endothelial adhesion receptor for leukocyte integrins and promotes leukocyte recruitment and extravasation of infiltrating cells. Engagement of RAGE subsequently converts transient cellular stimulation into sustained cellular dysfunction driven by long-term activation of the pro-inflammatory nuclear factor-kappa-B. (6) These transcription factors regulate the expression of important cytokines, such as tumor necrosis factor α (TNF-α), interleukin-1 and -6 (IL-1, IL-6) that are critically involved in the crosstalk between cancer cells and cells of the tumor stroma.

RAGE is a single transmembrane receptor of the immunoglobulin superfamily that is mainly expressed on immune cells, neurons, activated endothelial and vascular smooth muscle cells, bone forming cells, and a variety of cancer cells. RAGE is a multifunctional receptor that binds a broad repertoire of ligands and mediates responses to cell damage and stress conditions. These include not only advanced glycation end products and β-sheet fibrils, but also several members of the S100 protein family (S100B, S100P, S100A4, S100A6, S100A8/9, S100A11–13), high mobility group box-1 (HMGB1), and prions. (7) RAGE activates programs responsible for acute and chronic inflammation, and it is implicated in a number of pathological diseases, including diabetic complications, stroke, arteriosclerosis, arthritis, and neurodegenerative disorders. (2)

Increased expression of RAGE has been documented in a variety of acute and chronic inflammatory diseases, such as septicemia, rheumatoid arthritis, osteoarthritis, arteriosclerosis, chronic renal disease, inflammatory bowel disease, vasculitis, and late diabetic complications. (6) RAGE expression has been detected in a variety of human tumors, including brain, breast, colon, colorectal, lung, prostate, oral squamous cell, and ovarian cancer, as well as lymphoma and melanoma. (8)

Targeting RAGE is a novel strategy for clinical interventions and thereby hitting two important compartments during carcinogenesis: the transformed tumor cells as well as the pro-tumorigenic microenvironment. Several approaches have been considered to pharmacologically antagonize RAGE or RAGE ligand-induced pathologies, including cancer. (5) None of the long-term studies necessary to appraise potential side effects of using blocking antibodies or small molecule inhibitors are yet available. This is of particular interest, as RAGE has been shown to be important for cellular repair and tissue regeneration/homeostasis. (9)

Integrins

Integrins are receptors that mediate attachment between a cell and the tissues surrounding it, which may be other cells or the extracellular matrix (ECM). They also play a

role in cell signaling and thereby define cellular shape, mobility, and regulate the cell cycle. Typically, receptors inform a cell of the molecules in its environment and the cell evokes a response. Not only do integrins perform this outside-in signaling, but they also operate an inside-out mode. Thus, they transduce information from the ECM to the cell as well as reveal the status of the cell to the outside, allowing rapid and flexible responses to changes in the environment, for example to allow blood coagulation by platelets. Integrins work alongside other proteins such as cadherins, cell adhesion molecules, and selectins to mediate cell-cell and cell-matrix interaction and communication. Integrins bind cell surface and ECM components such as fibronectin, vitronectin, collagen, and laminin.

Integrins couple the ECM outside a cell to the cytoskeleton inside the cell. Which ligand in the ECM the integrin can bind to is mainly decided by which α and β subunits the integrin is made of. Among the ligands of integrins are fibronectin, vitronectin, collagen, and laminin. The connection between the cell and the ECM may help the cell to endure pulling forces without being ripped out of the ECM. The ability of a cell to create this kind of bond is also of vital importance in ontogeny.

Cell attachment to the ECM is a basic requirement to build a multicellular organism. Integrins are not simply hooks, but give the cell critical signals about the nature of its surroundings. Together with signals arising from receptors for soluble growth factors like VEGF, EGF, and many others, they enforce a cellular decision on what biological action to take, be it attachment, movement, death, or differentiation. Thus, integrins lie at the heart of many cellular biological processes.

There are many types of integrin, and many cells have multiple types on their surface. One reason for the difficulties encountered when trying to characterize the integrin family is that many of their ligands are large multi-adhesive extracellular matrix (ECM) molecules that, in addition to binding integrins, bind other proteins including ECM molecules, growth factors, cytokines, and matrix-degrading proteases. Integrins are of vital importance to all animals and have been found in all animals investigated, from sponges to mammals. Integrins have been extensively studied in humans. (18)

Cancer progression is a multi-step process that enables tumor cells to invade through extracellular tissues and metastasize to distal organs. (21) Compelling recent evidence demonstrates that cooperation between signals from the extracellular matrix (ECM) and growth factors enhances malignant behaviors of aggressive cancer cells, such as proliferation, migration, survival, and invasion. (22) Intracellular signals generated by growth factors and their receptor tyrosine kinases (RTKs) are generated independently from those produced by interaction between the ECM and integrin, and the synergy between these signals plays an important role in tumor growth and metastasis. (23)

Gravel root, RAGE and β2 integrin

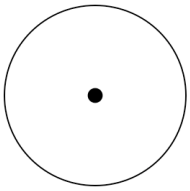
RAGE is expressed on endothelial cells and acts as an adhesion receptor for leukocytes by physical interaction with β2 integrin Mac-1. Crude ethanolic extract of the anti-rheumatic herbal drug gravel root (rhizome of *Eupatorium purpureum*) was identified as a potent inhibitor of some beta 1 and beta 2 integrin-mediated cell adhesions. The active principle of gravel root has now been isolated and identified cistifolin (as 5-acetyl-6-hydroxy-2,3-dihydro-cis-2-isopropenyl-3- tiglinoyloxybenzofuran). Cistifolin inhibits the Mac-1 (CD11b/CD18)-dependent monocyte adhesion to fibrinogen in a concentration-dependent manner. (10,11)

There is considerable cross-talk between integrins and receptor tyrosine kinases and collaborative activity between integrins and RTKs provide a mechanism for signaling systems important to carcinoma progression. (19) Major integrins expressed on breast epithelial cells include α2β1, α3β1, αvβ3, αvβ5, αvβ6, α5β1, α6β1, and α6β4. (20)

Insulin-like growth factor (IGF) axis and cell-surface integrin receptors have considerable cross-talk that controls many aspects in the regulation of cell physiology. Signals transmitted between integrins and the IGF-1 receptor (IGF-1R) have important conditioning effects upon IGF during growth and morphogenesis. (24) Three cell surface proteins, alpha V beta 3 integrin, integrin associated protein, and SHPS-1, have been shown to modulate both IGF-1 receptor-linked signaling and cellular growth and migration responses that are stimulated by IGF-1. (25)

1. Brummendorf T, Lemmon V: Immunoglobulin superfamily receptors: cis-interactions, intracellular adapters, and alternative splicing regulate adhesion. Curr Opin Cell Biol 2001, 13:611-618.
2. Riehl A, Németh J, Angel P, Hess J. The receptor RAGE: Bridging inflammation and cancer. Cell Commun Signal. 2009 May 8; 7:12.
3. Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002 Dec 19-26; 420(6917):860-7.
4. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature. 2008; 454:436–444.
5. Bierhaus A, Stern DM, Nawroth PP. RAGE in inflammation: a new therapeutic target? Curr Opin Investig Drugs. 2006; 7:985–991.
6. Bierhaus A, Stern DM, Nawroth PP. RAGE in inflammation: a new therapeutic target? Curr Opin Investig Drugs. 2006 Nov; 7(11):985-91.
7. Schmidt AM, Yan SD, Yan SF, Stern DM. The multiligand receptor RAGE as a progression factor amplifying immune and inflammatory responses. J Clin Invest. 2001; 108:949–955.
8. Logsdon CD, Fuentes MK, Huang EH, Arumugam T. RAGE and RAGE ligands in cancer. Curr Mol Med. 2007; 7:777–789.
9. Clynes R, Moser B, Yan SF, Ramasamy R, Herold K, Schmidt AM. Receptor for AGE (RAGE): weaving tangled webs within the inflammatory response. Curr Mol Med. 2007; 7:743–751.
10. Habtemariam S. Cistifolin, an integrin-dependent cell adhesion blocker from the anti-rheumatic herbal drug, gravel root (rhizome of Eupatorium purpureum). Planta Med. 1998 Dec; 64(8):683-5.
11. Habtemariam S. Anti-inflammatory activity of the antirheumatic herbal drug, gravel root (Eupatorium purpureum): further biological activities and constituents. Phytother Res. 2001 Dec; 15(8):687-90.
12. van de Stolpe A, van der Saag PT: Intercellular adhesion molecule-1. J Mol Med, 1996, 74, 13–33.
13. Lawson C, Wolf S.ICAM-1 signaling in endothelial cells. Pharmacol Rep. 2009 Jan-Feb; 61(1):22-32.
14. Hwang SJ, Ballantyne CM, Sharrett AR, Smith LC, Davis CE, Gotto AM Jr, Boerwinkle E: Circulating adhesion molecules VCAM-1, ICAM-1, and E-selectin in carotid atherosclerosis and incident coronary heart disease cases: the Atherosclerosis Risk In Communities (ARIC) study. Circulation, 1997, 96, 4219–4225
15. Malik I, Danesh J, Whincup P, Bhatia V, Papacosta O, Walker M, Lennon L et al.: Soluble adhesion molecules and prediction of coronary heart disease: a prospective study and meta-analysis. Lancet, 2001, 358, 971–976.
16. Ridker PM, Hennekens CH, Roitman-Johnson B, Stampfer MJ, Allen J: Plasma concentration of soluble intercellular adhesion molecule 1 and risks of future myocardial infarction in apparently healthy men. Lancet, 1998, 351, 88–92.
17. Ridker PM, Buring JE, Rifai N: Soluble P-selectin and the risk of future cardiovascular events. Circulation, 2001, 103, 491–495.
18. Barczyk M, Carracedo S, Gullberg D. Integrins. Cell Tissue Res. 2010 Jan; 339(1):269-80.
19. Soung YH, Clifford JL, Chung J. Crosstalk between integrin and receptor tyrosine kinase signaling in breast carcinoma progression. BMB Rep. 2010 May; 43(5):311-8.
20. Shaw LM. Integrin function in breast carcinoma progression. J. Mammary. Gland. Biol. Neoplasia. 4, 367-376.(1999)
21. Hanahan D. and Weinberg RA. The hallmarks of cancer. Cell 100, 57-70.(2000)
22. Larsen M, Artym VV, Green JA, Yamada KM. The matrix reorganized: extracellular matrix re-modeling and integrin signaling. Curr. Opin. Cell Biol. 18,463-471. (2006)
23. Giancotti FG, Ruoslahti E. Integrin signaling. Science 285, 1028-1032.(1999)
24. Beattie J, McIntosh L, van der Walle CF. Cross-talk between the insulin-like growth factor (IGF) axis and membrane integrins to regulate cell physiology. J Cell Physiol. 2010 Apr 28;224(3):605-611

25. Clemmons DR, Maile LA. Minireview: Integral membrane proteins that function coordinately with the insulin-like growth factor I receptor to regulate intracellular signaling. Endocrinology. 2003 May; 144(5):1664-70.



Blood group polymorphisms

> *"It is difficult to understand how agglutinins are produced in individuals who do not have the respective antigenic substances in their red blood cells. However, group A and B antigens are believed to enter the body in the food, in bacteria, or by other means, and these substances presumably initiate the development of anti-A or anti-B agglutinins."*
>
> —Arthur Guyton, *Textbook of Medical Physiology*

Although the use of blood groups in anthropology has largely been supplanted by the molecular technologies, they remain a critically important determinant of many phenotypic characteristics, and are of great interest to physicians and scientists who are interested in polymorphic influences on physiology and pathology. In addition, many of the genetic aspects of blood group expression interact with other gene system through linkage, pleiotropism, and epistasis, often affording insight into functions well upstream of the erythrocyte. Many blood group glycans are expressed in the digestive tract, where they serve to condition the gut microbiome.

ABO, SECRETOR AND LEWIS BLOOD GROUP POLYMORPHISMS

Perhaps the best-known glycans structures are the ABO substances and ABO typings are the best-known blood group system. Karl Landsteiner of the Rockefeller Institute first described the ABO blood-group system in 1900, (3) but it was not until 1953 that Walter Morgan and Winifred Watkins of the Lister Institute demonstrated that the specificity of the major blood groups is determined by sugars. (4) For example, the difference between the blood groups A and B lies in a single sugar unit at the end of a carbohydrate chain of a glycoprotein or glycolipid on the surface of the red blood cell.

Although evidence points to the fact that the individual genetic mutations that produced the ABO genes are quite ancient, this is trivial importance with regard to the actual demographics of the individual ABO blood groups in ancient populations. In genetics, it is not the actual age of the gene that always matters; the frequency or degree of drift of the gene also carries a lot of weight.

Blood groups were one of the first truly unique and objective ways to help categorize the locations, characteristics and migration patterns of human population groups; and although many new genomic technologies have been developed, they still play an important role, especially when combined with other genetic markers. Because of the ubiquitous nature and the relative stability of the ABO antigens in tissue, at least if kept dry, geneticists and anthropologists quickly seized upon the ABO system as a welcome relief form the tedious and largely arbitrary nature of bone anthropometrics.

Individuals of different blood groups have different susceptibilities to many types of infection, such as plague, smallpox, syphilis, *E. coli* infections and malaria. In the pre-medical, pre-antibiotic era, these would have sweeping and profound effects on populations, sculpting affected gene pools towards the more resistant blood group.

The fact that there are no lethal mutations and individuals have been described lacking ABO, H, and Lewis antigens has led to their being dismissed by some researchers as merely "icing on the cake" of glycoprotein structures. (1) Research suggests, however, that these antigens do indeed have a function far beyond "screwing up a transfusion." In fact, blood group antigens play important roles in the modulation of protein activity, infection, and cancer.

Although there are probably over a thousand publications on the associations of blood groups and disease, many are based totally on statistical analyses. Most of the earlier studies have been controversial, because they were small studies or had inadequate controls and/or had been analyzed incorrectly. Nevertheless, it is difficult to argue with the general pattern that emerges from the large body of statistical data on malignancy, coagulation, and infection. Recent findings in membrane chemistry, tumor immunology and infectious disease (especially relating to bacterial receptors), add a scientific rationale for some of these findings, and there is an increasing rationale for some of the earlier statistical findings. Blood group antigens do sometimes play an important biological role; this role may relate directly, or often be completely unrelated, to the red cell. (2)

Blood-group antigens have been developed as a self-declaration mechanism in higher organisms, as blood cells carry different DNA from that of germ-line cells, and their selfishness must be strictly limited. Differentiation and development including induction and inhibition also depend on the self-declaration--recognition mechanism. (9)

The majority of the oligosaccharides that form the A and B antigens are N-linked glycans on band 3, the erythrocyte anion-transport protein, although similar structures can be found on glycolipids as well. The antigenic glycans are built on the ends of polylactosamine chains by the action of a fucosyltransferase to generate H substance. In individuals with type O blood, this is the only modification. In blood type A, an N-acetylgalactosamine (GalNAc) residue is added to the terminal galactose residue, whereas in blood group B individuals a galactose (Gal) residue is substituted instead. The two monosaccharides differ by only a small group of atoms, but that little difference is sometimes a matter of life and death, since using the wrong type of blood in a transfusion can have fatal results.

Erythrocytes are typed as A, B, AB, or O(H), the latter indicating a lack of expression of either A or B. The H antigen is the precursor of A and B and is found on all red cell surfaces (up to ~1.7 x 10^6 antigens/RBC, or ~18,000/micron^2) except those of patients with the rare O_h Bombay or H null phenotype. Because H is a precursor of A and B, type O erythrocytes have more H antigen than A or B erythrocytes, which in turn have more H antigen than AB erythrocytes (which express both A and B antigens). The number of A and B antigens on the red cell surface ranges from 12 x 10^6 (~10,000-20,000/micron^2); in 75% of type A individuals, "double length" A antigens are also present (~500/micron^2). (5)

Reuben Ottenberg (1882-1959) suggested the fact that the ABO blood group system could be inherited in 1910. (6) The determination of ABO status is the result of two co-dominant alleles and one recessive allele. A and B blood groups are dominant over the O blood group, and the A and B group genes are themselves co-dominant. The ABH antigens are not primary gene products but instead the enzymatic reaction products catalyzed by the enzymes called glycosyltransferases. The two different glycosyltransferases responsible for the addition of the GalNAc or Gal residues are found on chromosome 9q band 34. In O-type individuals, null alleles are found at this locus in both copies of chromosome 9, so there is no functional transferase and these individuals express only H substance.

ABO genes consist of at least seven exons, and the coding sequence in the seven coding exons spans over 18kb of the genomic DNA. The single nucleotide deletion found in most (but not all) of the O alleles and responsible for the loss of the activity of the enzyme is located in exon 6. (7) The sugars that determine the specificity of substances in the ABO blood group are distributed in the biological world in forms similar to those found in human beings. The substances are therefore, also present in different mammals. The ABO blood-group substances are present in birds and amphibians and even in plants and bacteria.

The expression of blood group antigens was ubiquitously upregulated in the endothelial cells of fetal organs. In the process of their differentiation to endothelial naive embryonic mesenchymal cells expressed cytoplasmic ABH antigens. They were assumed as products of the activation of the respective genes. ABH antigen expression was considered as suggestive evidence for the assumption that blood group antigens could serve as early immunomorphologic markers of endothelial differentiation of mesenchymal cells, thus specifying the location of future blood vessels. Extending the conceptual framework of blood group antigens" significance we consider them as being possibly involved in the process of fetal morphogenesis. (8)

Intestinal alkaline phosphatase

Gene products, which may be expressed under plastic conditions, can contribute to further downstream gene expression by ecological elements. Beginning around 1965 researchers began to notice that people had different levels of an enzyme in their intestinal tract called

intestinal alkaline phosphatase (IAP) and that the levels of this enzyme varied according to ABO blood group and secretor status. (27) Type A non-secretors have the lowest levels, and type O secretors the highest, with type B's somewhere in the middle. The activity of intestinal alkaline phosphatase and serum alkaline phosphatase is strongly correlated with ABH secretor phenotypes. Independent of ABO blood group, ABH non-secretors have lower alkaline phosphatase activity than ABH secretors. It has been estimated that the serum alkaline phosphatase activity of non-secretors is only about 20% of the activity in the secretor groups. It appears likely that the ABO and secretor genes influence the rate at which the intestinal phosphatase enters the blood, or its catabolism, rather than its synthesis in the intestine. (28,29)

IAP has several important functions. During fetal development, IAP is the enzyme with the highest blood concentration during the critical period when the gut lining is developing. IAP also helps to split cholesterol and long chain fatty acids from food into smaller fatty acids. Finally, it also enhances the absorption of calcium from food. The concentration of the intestinal phosphatase is lowest in the serum during fasting and rises after ingestion of fat, reaching a peak at about seven to eight hours. The concentration of intestinal alkaline phosphatase in human thoracic-duct lymph rises after a fatty meal; and presumably, most of the intestinal phosphatase enters the blood by way of the lymphatic system.

Protein intake and bone loss

So, if type O secretors had higher levels of this enzyme, can assume they do a somewhat better job of digesting fats? Most fascinating, studies have shown that IAP likes a protein meal, as protein tends to switch IAP into overdrive; (30) which may be counterintuitive, as current nutritional wisdom holds that protein intake increases bone loss due to the use of bone calcium as a pH buffer. Paradoxically, for blood group A individuals not only are their levels of IAP lower, but evidence also suggests that the physical presence of the A antigen actually helps to inactivate what little IAP they do make. (31)

Dopamine-noradrenaline axis

There are strong indications that a gene regulating dopamine beta hydroxylase (DBH) activity is linked to the ABO blood group locus. (42) DBH is a key enzyme in the conversion of dopamine norepinephrine.

This linkage may help explain the continued significance of ABO group as a discreet and significant genetic marker for a variety of affective disorders, including type A behavior in men subsequent to myocardial infarction (43) and bipolar depression (44,45) each associated with blood group O. The ABO locus shows putative linkage with platelet monoamine oxidase activity, (46) reduced levels of which have been noted with group O healthy males. (47)

Additional evidence implies that a linkage exists between the ABO gene and the gene that regulates the activity of the enzyme argininosuccinate synthetase, which recycles arginine from citrulline in the production of nitric oxide. (48) A recent letter to the editor in the journal Lancet reported differences between ABO groups in their responsiveness to

inhaled nitric oxide (NO) therapy, with types with a B antigen (B and AB) having less success with this therapy. (49)

Elevated Factor VIII (FVIII) levels contribute to venous thrombotic risk. FVIII levels are determined largely by levels of von Willebrand factor (VWF), its carrier protein that protects FVIII against proteolysis. (50) ABO polymorphism is one of the best-characterized genetic modifiers of plasma FVIII; it accounts for approximately 30% of the total genetic effect. (51) Subjects with blood group non-O have higher VWF and FVIII levels than do individuals with blood group O. (52)

Gastric acidity, gastrin and pepsinogen

Since the prevalence of both pernicious anemia and gastric cancer is higher in individuals of blood group A and duodenal ulcer in those of group O, a hypothesis relating blood-group effects on acid secretion was inevitable. (53) Early work confirmed that acid output tended to be greater in group-O than in group-A subjects. (54,55)

In one study, serum pepsinogen A (pepsinogen I) levels were studied in relation to ABO blood group, age, and sex in 700 healthy blood donors. Serum pepsinogen A levels were higher in males than in females and rose with increasing age. Blood group O individuals showed higher serum pepsinogen A levels compared with blood group A. (56) There is also evidence that the type A antigen in gastric juice binds to pepsin and possibly inactivates it. (57) A recent study using serum pepsinogen levels as a marker for gastric atrophy showed a high association with blood groups A and B. (58) However, possibly owing to the polygenic nature of pepsinogen activity, one study failed to find any significant difference between ABO groups. (59)

Another study looking at ABO polymorphism and serum gastrin concentration after stimulation by a glycine drink could find no correlation with ABO blood group. (60) The study, however, used a simple pre- and post-prandial methodology. In a separate study, the concentrations of gastrin were measured in the blood of 121 fasting healthy Greek volunteers of both sexes and of different ABO blood groups, aged between 20 and 70 years. The testing took place after a test meal while the subjects were fasting, and again at 10 minutes and 40 minutes. The researchers found that gastrin levels took 40 minutes to increase after the meal in the blood groups A and B while in the blood group O significant increase had appeared already 10 minutes after the meal. (61)

Cholesterol

Although several studies on highly select populations have yielded conflicting results (62,63), the consensus is that blood group A has a significantly higher basal cholesterol level than the other blood groups. The relationship between ABO blood phenotype and total serum cholesterol level was examined in a Japanese population to determine whether elevated cholesterol levels are associated with blood group A. Their results showed that cholesterol levels were very significantly elevated in the blood group A group compared to non-A group ($P < 0.00001$). (64)

In a nationwide sample of more than 6000 black and white adolescents aged 12 to 17 years, ABO blood group and coronary risk factor levels were measured. Blood group A1 was associated with significantly higher serum total cholesterol levels in white females

independent of all other risk factors, in white males independent of age and weight, and in southern black females independent of age and weight. (65)

A separate study (the Bogalusa Heart Study) looked at 656 white and 371 black adolescents and found the same results with regard to cholesterol (A higher than others) and also showed higher levels of LDL lipoproteins in type A adolescents over the other blood groups. (66)

Whether the association between group A and elevated cholesterol levels is through linkage or environmental factors, such as diet, remains to be determined. The aforementioned ABO variations in intestinal alkaline phosphatase levels have been posited as a potential causative factor.

Stress chemistry

In addition to the previously mentioned associations between ABO group and affective disorders, several studies have identified difference between ABO group and possible chemical responses to stress. Interesting, individuals of blood group A appear to have a lower incidence of "Type A Personality."
(67)

A study that evaluated the influence of blood group (A versus O) coupled with a mirror drawing stressor on very low-density lipoprotein toxicity-preventing activity (TxPA) and plasma cortisol levels showed significant ABO variation. Exposure to the stressor significantly decreased TxPA and increased cortisol for the total group of 25 older adult males. However, the stress response patterns of the 15 blood group A males were different from those of the 10 type O subjects. The blood group A group had higher initial levels of TxPA and cortisol as well as quicker stress recovery rates than the type O group. (68)

Using the act of venipuncture as an inherently stressful event, researchers then proceeded to measure the cortisol and catecholamine response to venisection by humans with different blood groups. Blood group A individuals responded to the stressful situation with higher levels of cortisol and possibly, of adrenaline. (69)

Viscosity and rheological differences

Elevated Factor VIII (FVIII) levels contribute to venous thrombotic risk. FVIII levels are determined largely by levels of von Willebrand factor (VWF), its carrier protein that protects FVIII against proteolysis. (50) ABO polymorphism is one of the best-characterized genetic modifiers of plasma FVIII; it accounts for approximately 30% of the total genetic effect. (51) Subjects with blood group non-O have higher VWF and FVIII levels than do individuals with blood group O. (52)

Rheology is the science of deformation and flow. One common factor between solids, liquids, and all materials whose behavior is intermediate between solids and liquid is that if we apply a stress or load on any of them they will deform or strain. For our purposes, we will use the term to describe the dynamics between blood clotting (moving towards a solid state) and blood thinning (moving towards a liquid state). It might be tempting to substitute the word "viscosity" for rheology when talking about blood groups and clotting; but it does not cover the "dynamics" of how, when, and why blood can change texture; it only distinguishes one texture state form another.

There is evidence that the rheology of blood may play a role in a variety of chronic anxiety states. When compared to normal subjects, chronic depressive and schizoid patients had very significant differences in their blood rheology and in the ability of their red blood cells to aggregate. When patients having schizoid anxiety were compared to those having depressive anxiety, their ratio of albumin to globulin was increased. When patients were divided according to their ABO blood groups, significant differences were found in their albumin to fibrinogen ratio and their blood viscosity. This was particularly true for women who were type A and who suffered from depressive anxiety: their blood tended to be substantially "thicker" and have higher amounts of serum proteins in it than women with similar depression who were blood group O. (20)

Associations between the ABO phenotype and variations in blood rheology have been also reported in high blood pressure, (21) stress, (22) diabetes, (23) heart attack, cancer and thyroid disease, (24) renal failure (25) and malignant melanoma. (26)

ABO isohemagglutinins

Although it is well recognized that blood group antigens can serve as points of attachment for microbial adhesins, perhaps less appreciated (at least with regard to the ABO group) is the sheer destructive potential of the opposing blood group antibodies (*isohemagglutinins*.)

When an isohemagglutinin antibody encounters a foreign antigen, a reaction called agglutination occurs. This means that the antibody attaches to the antigen and makes it very sticky. When cells, viruses, parasites and bacteria are agglutinated, they stick together and "clump up," which makes the job of their disposal much easier. As microbes must rely on their slippery powers of evasion, this is a very powerful defense mechanism. It is rather like handcuffing criminals together; they become far less dangerous than when allowed to move around freely.

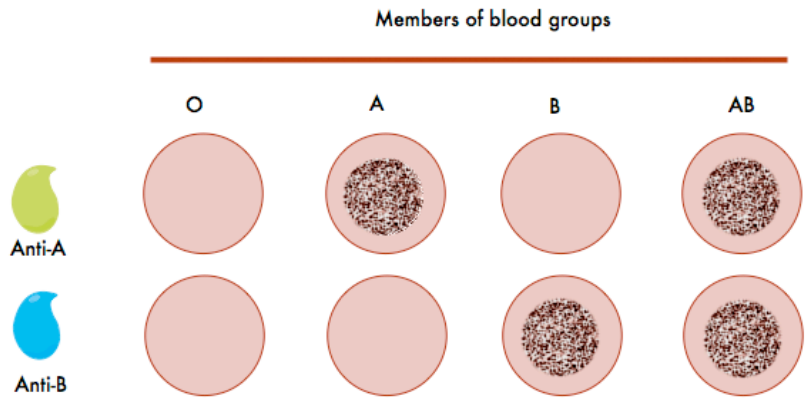


Figure 6.3.1 The agglutination of red blood cells by the appropriate testing serum is the mechanism of how blood group is determined. (After Cavalli-Sforza and Cavalli-Sforza, 1995)

Unlike IgG class, which require the assistance of the immune system to do their business, these isohemagglutinins kill things all by themselves, which is why getting the wrong blood group in a transfusion is so dangerous.

It is an interesting dichotomy that polymorphisms such as the blood groups that are capable of producing such a toxic reaction to the presence of an opposing blood group antigen but are themselves really just non-lethal mutations. Nobody dies just because they are one blood group or another, although there are many physiologic distinctions and consequences.

Although most scientists are aware of the relationship between the blood group antigens and antibodies that are the determining factor behind the classic transfusion relationships discovered by Landsteiner in 1900. However not very well known is the fact that foods and bacteria that possess many of these same opposing ABO blood group antigens are the source of induction for these IgM class antibodies.

Elevated levels of opposing blood group antibodies have been observed in a variety of conditions, including endometriosis, infertility, and autoimmune disease. (37)

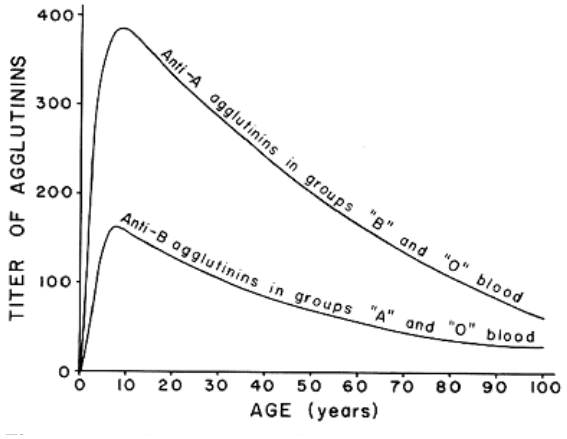


Figure 6.3.2 Average titers of anti-A and anti-B agglutinins in the blood of individuals who are blood groups A and B at different ages. (From Guyton A. Medical Physiology)

ABO blood group disease associations

ABO blood groups and peptic ulcer disease

Individuals of blood group O phenotype run an approximate 1.5 - 2 fold increase in developing acid peptic disease (11) although there is no direct correlation between ABO blood group phenotypes and the prevalence of *H. pylori* infection. In addition to H type 1, however, the Lewis b antigen is also a binding receptor for *H. pylori*; and in this capacity, it can best be described as a "virulence promoting factor." For virulent strains, Lewis b antigen binding activity targets the microbes to the epithelial cell surfaces and potentiates the effect of secretion of virulence factors such as the vacuolating cytotoxin and/or neutrophil activating/recruiting factors. (12)

ABO blood groups and stroke

A European study comparing 50 patients with stroke to the standard expected frequency of ABO blood groups in the surrounding population showed that the frequency of the blood group A in the patients with stroke was 120% greater than would normally be expected. In blood group B, the percentage was even higher (159% of expected rate of occurrence). O blood group patients were only 85% as likely to develop stroke when compared the percentage of O type blood in the surrounding population. (38)

A 1979 study of 220 stoke patients looked at the viscosity of their blood a few hours after the stroke event. About 80% of the patients had blood cells that easily aggregated. What was especially interesting was the discovery by the researchers that the clotting of blood patients with A and B blood groups was due to fibrinogen, whereas in blood groups O and AB they were caused by other clotting factors. (39)

The generalized tendency for individuals who are type A and AB is toward problems associated with blood clotting, whereas in blood groups B and O, the problems appear to be linked to excessive bleeding and poor clotting. This was verified in several studies, the largest being performed on over 1460 "stroke" patients, and reported in the journal Lancet. In 329 cases, the cause of death was certified as cerebral thrombosis (brain clot). In the thrombosis cases, there was an excess of patients of blood groups A and AB and a deficiency of blood groups O and B. In the 482 "strokes" that were the result of cranial bleeding, the reverse was found to be true: a significantly higher occurrence of patients who were blood groups O and B over those who were blood groups A and AB. (40)

ABO blood groups and peripheral artery disease, venous thromboembolism

It is estimated that peripheral artery disease (PAD) occurs in approximately 12% of the adult population, or approximately 22 million Americans. The prevalence of PAD increases with advancing age such that almost 20% of people over 70 years of age have the disease. E-selectin and thrombomodulin levels are always elevated in intermittent claudication, a disorder usually found with PAD, and carrying a distinct association with blood group A. (41)

The ABO blood groups were determined in 125 patients suffering from venous thrombosis in a Brazilian population. An excess of blood group A and a decrease of blood group O was observed among the patients. (42) This is consistent with the previously discussed influence of ABO on a third soluble endothelial product, von Willebrand Factor, and its role in thromboembolism.

ABO blood groups and heart disease

There is a clear-cut association with having A and AB phenotype and an increased risk for heart disease. This has been reported continuously in the scientific literature over the last 50 years. Individuals who are blood group A have higher rates of heart attack across all age groups, both the genders, and all ethnic and national groups.

In 1962, the Framingham Heart Study blood grouped the surviving 4125 members of the original study group of 5209 people first examined in 1948-51. The most striking observance was the lower rates of non-fatal heart disease in men ages 39-72 that were blood group O versus blood group A. (43) A 1994 Polish study on by-

pass surgery patients with highly advanced arteriosclerosis of the coronary arteries found a significantly higher number of cases with group AB and a deficiency in group O. (44) A 1981 German study of 13,175 patients showed a prevalence of A blood group in all types of heart disease examined. (45)

In a study of 191 coronary artery bypass candidates, investigators paradoxically found an excess of type O over type A. When they examined the data more closely, they concluded that that the tendency of type A to develop blood clots more readily ("thrombotic proneness") caused a poorer prognosis. In essence, the blood group A subjects were missing from the study because they had already died in greater numbers, leaving a disproportionate excess of type O among the long-term survivors. (46) In a study of male survivors of heart disease, researchers found that there were fewer type A patients before age 55 than otherwise would have been expected. (47)

An Italian study in 1975 of 746 patients with high blood pressure, 3258 with congenital heart disease, 4503 with a history of heart attack, found a significant lack of patients with type O blood, and a significant excess of blood group A in patients with myocardial infarction. The study also showed an excess of blood group A patients with high blood pressure, and a lack of patients who were blood group B. (48)

A study of 255 women published in the Journal of the American Medical Association originally to study the effects of smoking on the rates of heart attack in women also found several other factors significantly associated with heart attacks in this group, including hypertension, angina pectoris, family history, diabetes mellitus and blood group A. (49)

A 1985 study looked at blood group and heart attacks in two different age groups. The patients were divided into two groups: those who were 65 years old or older and younger patients. The predominance of blood group A in patients with cardiac infarction was “highly significant” in both age groups (P less than 0.005). This study was unique in that other risk factors, such as smoking, high blood pressure, diabetes, and high cholesterol levels, were excluded from the study. When the researchers looked specifically at the more elderly population, the predominance of blood group A in the older patients with cardiac infarction was even higher (P less than 0.001). The researchers concluded, “Our investigation strongly suggests the existence of a genetic factor associated with blood group A and independent of the other risk factors, which is also responsible for a greater incidence of cardiac infarction.” (50)

An eight-year study of 7662 men published in the British Medical Journal found blood group A is linked to the incidence of ischemic heart disease, as well as having higher total serum cholesterol concentrations. (51)

ABO blood groups and endometriosis

Women with endometriosis have a 2.9-fold increased risk in the A blood group distribution. The overall risk of women with endometriosis and A blood group was 2.89 (95%CI, 1.85-4.52). (71) Higher levels of opposing blood group antibodies were found in type A women with endometriosis. (149)

ABO blood groups and cancer

Perhaps the greatest focus of current research on the ABO blood group antigens is in the field of molecular oncology. Recent findings in membrane chemistry, tumor immunology, and infectious disease add a scientific rationale for several blood group associations and there is an increasing compelling rationale for some of the earlier statistical findings. The huge interest in blood group stems from the developing awareness that blood group antigens are important components in the process of cell maturation and control; for example, the appearance or disappearance of blood group antigens is a hallmark of malignancy in many common cancers.

Several "tumor antigens" or "tumor markers" are the known product of certain blood group precursors. Many of these tumor antigens are "A-like" which helps in part to explain the striking number of associations with blood group A and AB. On the contrary, autoimmune disorders tend to be associated with blood group O. The contrast with the cancer-type A association is an interesting one in view of the suggestion of earlier immunologists that there is a fundamental antithesis between the two classes of disease. In essence, the heightened surveillance and over-active immune activity, tends to result less malignancy, while overly tolerant immune activity tends to encourage it. These observations suggest a more general hypothesis that in the tissues of all people, both normal and cancerous, there are blood group A-like antigens present at a biochemical level that is usually inaccessible to the immune system. However, in the course of an autoimmune process or the immune response to a growing cancer, the antigen becomes accessible. At that point, a blood group A person, who cannot make anti-A antibodies, will be more likely than a blood group O person to tolerate the cancer, but less likely than an O person to attack his own tissues

The cancer-blood group A link is far from absolute; several tumors show consistent associations with O or B. This implies that cancer is a condition associated with derangement of blood group activity in general, and the expression of A-like antigens on the surface tumors is just simply the most common of theses derangements.

The presence of sialyl-Le(a) or sialyl-Le(x), which are ligands for selectins, promotes the metastatic process by facilitating interaction with the endothelium of distant organs. The loss of A and B antigens increases cellular motility, while the presence of H epitopes increases resistance to apoptosis by mechanisms that remain to be defined. (72)

ABO blood groups and gynecologic cancers

As a rule, gynecological tumors occur more frequently and are associated with worse prognosis in blood group A women. As examples, endometrial cancer occurs more frequently in type A, ovarian cancer occurs more frequently in A's and AB's. For both of these cancers blood group A is associated with worse 5- and 10-year survival. Conversely, the best survival rate is seen among blood group O women, followed by B women. Type B women are also the least likely to have an ovarian tumor that is malignant. With regard to cervical cancer, analysis also shows a strong trend towards higher frequency of cancer and poor outcomes among A women, a slight trend towards increased risk for B's, and a better 5-year survival among O blood phenotype. (80-86)

Breast cancer is the most common cancer among women. While many risk factors are associated with the development of breast cancer, it is seldom mentioned that blood group has an influence on susceptibility and outcomes. In fact, some researchers have even gone so far as to say that "blood groups were shown to possess a predictive value independent of other known prognostic factors" when discussing breast cancer. Other researchers have actually suggested that a degree of the susceptibility to breast cancer, from a gene perspective, might be a result of a breast cancer-susceptibility locus linked to the ABO locus located on band q34 of chromosome 9. (78,79)

A 1963 study reported that their results "unquestionably indicate that the increased cancer risk of individuals of blood group A is a consequence of a blood group A association with some pre-malignant process." An unexpected additional finding was the four- to six-fold excess of secondary ovarian carcinomas in women who were blood group A relative to that in women who were blood group O. (154) A 2010 study using data from 49,153 women in the Nurses' Health Study that examined the association between ABO blood group and incidence of epithelial ovarian cancer found no association with blood group A, and only a slight association with group B. (155) An earlier 1995 study, however, indicated that blood group A is associated with a poorer prognosis. (156) A 1993 study of 1261 women who had ovarian cancer between 1968 and 1986 showed that ovarian cancer was more common in women of blood group A than in others, with a relative incidence of 1.17. In particular, adenocarcinomas were the most common type of tumor and were associated with blood group A. The association was more striking in married women than in single women probably reflecting differences associated with parity. (157)

ABO blood groups and bladder cancer

Bladder cancer appears to be an exception to the generalized observation of A's and cancer aggressiveness. In a study by Llopis, et al., the researchers noticed that blood group O had a tendency to increased aggressiveness, higher tumor grade, and more relapses. (87) Surprisingly, blood group A individuals generally were less likely to have aggressive cancer and were somewhat protected against relapses of bladder cancer. Srinivas, et al. observed a similar trend. They found that among 141 patients with bladder cancer, individuals with blood group A had lower grade tumors and lower mortality rates. Blood group O's generally had higher-grade tumors and higher mortality rates. Other researchers have also observed similar trends, such as the blood group O tendency to higher-grade tumors, larger tumors, progression to advanced disease, and increased rates of mortality (especially after 8 years). (88-93)

ABO blood groups and gastric cancer

It has been consistently observed that blood group A is associated with an increased risk for stomach cancer and poorer survival. Blood group O on the other hand, appears to exert a protective effect by preventing the growth and spread of the tumor and being associated with longer survival times.

Because of this strong relationship between stomach cancer and blood group A, some researchers have hypothesized gastric cells produce an antigen immunologically related to blood group A. This appears to be the case to a degree, with stomach cancer cells expressing the A-like Thomsen-Friedenreich (T) antigen. Blood group A individuals have a tendency to a lower natural anti-Thomsen-

Friedenreich immune response. This tendency is quite strong in A's with stomach cancer who demonstrate the greatest and uniform suppression of the level of TFA agglutinins irrespective of age, cancer stage or tumor morphology. (97)

Stomach cancer is also often characterized by exuberant secretion of type A antigens. This characteristic is not limited to those who are type A blood. Large amounts of A antigen have also been observed in the less common tumors of types B and O. It appears that the progression of stomach cells to stomach cancer involves a necessary mutation at the ABO gene, the result of which is the production of A antigen, even if this is not the person's blood group. Of course, having a blood group such as O or B and capable of attacking A-like things, such as cancer cells, gives these blood groups a considerable advantage. Conversely, it appears that stomach and intestinal pre-cancerous and cancer cells tend to lose the H and B antigens, making immune detection in these blood groups more likely. (94-100)

The presence of p53 mutations is associated with stomach cancer and with blood group A. Because glucocorticoid receptors are found in high numbers on stomach cancer cells, high stress hormone levels might also contribute to poor outcomes in individuals with stomach cancer. (98)

ABO blood groups and pancreatic cancer

Compared with subjects having blood group O, a modestly higher risk of pancreatic cancer was observed among cases with blood group A or AB (73,75) and association that seems stronger if there is concurrent H. pylori infection. (74)

ABO blood groups and colorectal Cancer

Colorectal cancer is among the most frequent cancers in the United States, with an estimated 133,000 new cases predicted (94,000 for colon and 39,000 for rectum) and about 55,000 deaths from colorectal cancer are expected this year. Some of the most common risk factors include a family history of colorectal cancer, polyps, or inflammatory bowel disease. Other risk factors can include physical inactivity, exposure to certain chemicals and a high fat or low fiber diet. Early studies showed an association of cancers of the large intestine with blood group A. However, this association is weaker than that found with stomach cancer. Perhaps the largest link to blood group and colon cancer is found with respect to the appearance or disappearance of blood group antigens. It is commonly recognized that altered blood group antigen expression is a hallmark of malignancy in this form of cancer. During the progression to malignancy of colonic cancer cells, the blood group antigens A, B, H, and Le(b), which are normally expressed only in the proximal colon, can be re-expressed in distal colon cancers or deleted in proximal colon cancers. An individual can also actually even express an antigen that is incompatible with the individual's blood group (so a blood group B could express an A antigen). (115)

ABO blood groups and head and neck cancer

Cancer of the lip is significantly associated with type A. Cancers of the tongue, gum, and cheek have a blood group A association as well. Cancers of the salivary glands are strongly associated with A, and weakly with B. The gain in cancers of A's in this group is essentially at the expense of the O's, who have substantial protection against this type of cancer. The salivary glands also appear to have an association with being a secretor. Type A is over represented in esophageal cancer. Non-

secretors also have an association with this cancer. Blood group B also has a tendency to more cancers of the esophagus, while O's have a definite degree of protection.

As a rule, a higher intensity of oral disease is found among non-secretors. When it comes to pre-cancerous, or cancerous changes to tissue of the mouth and esophagus, non-secretors seem to fair worse than secretors. This oral disease susceptibility is reflected in the occurrence of epithelial dysplasia, for example, which is found almost exclusively in the non-secretor group. Barrett's esophagus, a condition often preceding the development of esophageal cancer, and esophageal cancer also has a positive association with Lewis (a+b-) non-secretor phenotypes. For cancer of the larynx and hypopharynx, we again find an A and B (and AB in his case) association. The A2 blood group (a less common variant of blood group A) was significantly more frequent in a group of patients with glottis cancer, while the A1B type was over represented in the group with hypopharynx cancer.

Structural changes to squamous cell cancers of the head and neck are quite common. In normal tissue of this region, ABO antigens are expressed. However, once squamous cell cancer develops, the A antigen disappears in about 1/3 of A's and AB's, and the H (or O) antigen is expressed in carcinoma cells not only from all blood group O individuals, but from virtually all individuals of blood groups A, B and AB as well. The T and Tn antigens we discussed earlier also become commonly expressed in these cancers. In essence, results suggest that the expression of the blood group-related H, T, and Tn carbohydrate chains is a common sequel of squamous cell cancer of the head and neck. As a rule, it appears that tumors expressing the H antigen (for blood groups A, B, and AB) have a poor prognosis. (109-113)

ABO blood groups and brain and nervous system cancer

A positive, consistent, and often very strong association has been found between blood group A and brain and nervous system tumors. A weaker association for these forms of cancer exists for blood group B. Conversely it has been a consistent finding that being a blood group O is a favorable prognostic factor for brain and nervous system cancers. (100-103)

An interesting occurrence has been noted with regard to blood group and malignant gliomas. Researchers investigating the use and efficacy of post-operative poly- and immuno-chemotherapy for this cancer decided to break results down by blood group. They found that when the efficacy of polychemotherapeutic and antibiotic intervention was analyzed by survival time, it was a promising intervention for blood group A and AB patients. However, it was ineffective in blood group O. Based on their results, the researchers concluded that an individual selection of the schedule of chemo- and immuno-chemotherapy should be selected by AB0 blood group. While this is currently an isolated finding, it does draw attention to the possibility that medical interventions for cancer and possibly many diseases could be made better if blood group was a component of the information looked at to judge efficacy of treatments. (104-108)

Only two studies have been conducted on skin cancer. In general, cancer of the skin appears to be strongly associated with blood group O. Blood group O has also been found to have the highest frequency of malignant melanoma. They also had the lowest average time of survival after diagnosis. Blood group A tended to have the longest survival times, with this trend particularly strong in blood group A women. (120)

ABO blood groups and bone and blood cancer

Bone cancers show the strongest association with blood group B, and a weaker association with blood group A. Evidence suggests that in general, blood group A individuals are more predisposed to leukemia. This trend is particularly strong for a more rare variety of blood group A (the A2 A's) and chronic lymphocytic leukemia associated. Similarly, blood group O appears to grant a degree of resistance especially in acute leukemia. This protection is most noted with female type O. Because of this effect, some researchers have suggested that there might be a "sex-responsive" gene near to the ABO gene locus on chromosome 9, which relatively protects group O women against acute leukemia. Hodgkin's disease has shown an association with blood group O. (121-123)

Leukemia and myelodysplastic syndromes are often characterized by a loss of blood group antigens. Moreover, after induction of complete remission, it is common for blood group to revert to normal and reappear on cells. (116-119)

ABO blood groups and infertility

ABO incompatible couples (a type A male fertilizing a type O female) are a frequent occurrence in miscarriages, especially very early in the gestational term. One study of 288 miscarriages showed that there was an excess of blood group A and type B in otherwise normal fetuses. It is concluded that the ABO incompatibility between mother and fetus is likely to be a cause of early miscarriages, but almost exclusively in chromosomally normal fetuses.

A study of 102 infertile couples showed that 87% were blood group incompatible. The same study also found that in seven couples with markedly delayed fertility, the nine children that did result were all blood group O, and hence would have been compatible with the mother. The authors suggested that the infertility was due to the presence of antibodies in the secretions of the mother's genital tract, or incompatible sperm from the father. (145)

In another study, 589 compatible mating couples were compared with 432 incompatible mating couples. The mean number of living children presented a significant difference. There was a 21% deficiency of type A children in the two groups. (146) Similarly, there was a 16% deficiency of type B children in the two groups. It appears that there is a 31.9% rate of miscarriage associated with incompatible matings, as compared with 17.15% in compatible matings. This has led some researchers to theorize that ABO incompatibility results in "cervical hostility" between the man's blood group antigen, which are present in his sperm, and the woman's opposing antibodies, present in her cervical mucus. (147)

In general, levels of isoagglutinins are higher in blacks than in whites. Whites had higher anti-A than anti-B levels, and those levels were higher in females than in males. In blacks, the anti-B levels were almost as high as were the levels of anti-A antibodies and little sex distinction was found. It has been my observation that blacks who are blood group B also appear to have a higher incidence of chronic health problems than do blacks of other blood groups, including diabetes and autoimmune disease. It may well be that the genesis of many future health problems originate in utero. (148)

Blood group incompatibility can lead to infertility. Opposing blood group antibodies can be induced by foods that contain opposing blood group antigens. It is reasonable to conclude that the many case histories of previously infertile women conceiving and producing healthy offspring by simply eating correctly for their blood group are the result of the lowering of these opposing blood group antibodies because the mothers were avoiding continued reinoculation with these problematic foods.

ABO blood groups and infectious disease

The previous section appears to imply a selection disadvantage for group A, and it has been argued that under present day civilized living conditions O carriers have a preservation advantage over blood group A. (52) This may be the result of the deletion of the selection factor "infectious disease" which may nevertheless regain importance if environmental changes occur. (53)

Infectious diseases, especially the worldwide epidemic diseases, have to great extent selective effects. This is demonstrated inter alia in the different "selection values" in the ABO blood group system. During the eons prior to anti-microbial intervention, selection variability via ABO polymorphism was the preeminent natural survival mechanism.

A morbidity and mortality variation among the ABO and secretor groups is presented in the following tables. A special examination of polymorphic differences in uropathic infectious disease is presented in the final table. For a more detailed examination of particular infectious scenarios, the reader is referred to a previous comprehensive survey by the author. (54)

Class	Mechanism	Description	Example
Selectivity	Adhesion kinetics	Adhesin or lectin specificities of the infectious agent based on particular ABH glycosylation	• Candida albicans (group O)
	Humoral dynamics	Inadequate isoagglutinin production or activity	• Neisseria gonorrhoeae (group B)
	Molecular mimicry	Infectious agent is antigenically similar to host's ABO group	• Giardia lamblia (group A)
Response variability	Host response	Variation in severity of disease through unique biologic response (examples: inappropriate inflammatory response; rosette formation)	• Cholera (group O) • Dengue Fever (group B) • Malaria (group A)
	Substrain susceptibility	Different ABO groups often show variations in susceptibility between individual bacterial, fungal, or parasitic species or viral strains.	• Influenza (all groups) • Malaria (group O versus group A)

Figure 6.3.6 Mechanisms of ABO influence on infectious pathology

Historically, some of the most catastrophic epidemic and endemic diseases are ABO selective, and in many instances demonstrate ABO variation in morbidity, mortality, or microcharacteristics such as sub-strain preferentiality or level of inflammatory response. This includes cholera (O), smallpox (A), malaria (A) and influenza (variable subsets depending on strain). The influence of ABO polymorphism on infectious disease appears to stem from a multitude of unique factors. They are summarized on the following pages.

Strain	Susceptible phenotype	Comments
Amoebic dysentery	O, A	• Blood groups B and AB have a degree of resistance against developing severe or acute dysentery, especially the amoebic forms
Candida carriage	O, NS	• Candida carriage was associated with blood group O ($P < 0.001$) and, independently, with non-secretion of blood group antigens ($P < 0.01$) • Candida albicans extracellular polymeric material (EP) contains a mannoprotein adhesin with a lectin-like affinity for H (type 2) blood group antigen • There were a significantly higher number of non-secretors (48.9%) among 174 patients with either oral or vaginal candida infections compared with the proportion of non-secretors in the local population (26.6%). Non-secretor saliva actually seemed to enhance Candida attachment
Cholera	O, AB	• Blood group O individuals have a greater risk of infection with cholera and develop the most severe and life threatening forms of this illness. This has been documented in several studies • Type AB's on the other hand appear to have the highest degree of protection from cholera infections • Type O had more diarrhea-like stools per day than persons of other blood groups, were more likely to report vomiting and muscle cramps
Coccidioidomycosis	B	• Blood group B individuals are more prone to disseminated disease following exposure.
Dengue Fever	B	• According to researchers, blood group B was strongly associated with the severe form of dengue fever known as dengue hemorrhagic fever.
Dermatophytosis	A	• The fungus *Trichophyton rubrum*, isolated from 54.5% of the patients tested, was more frequent in individuals belonging to blood group A
E. Coli	Variable subsets	• It appears that many forms of E. coli capable of causing diarrhea are immunologically "B-like." This results in a substantially higher number of cases of diarrhea among individuals of blood group B and AB people • However, when it comes to the overall severity of infection with E. coli, type B and AB are not alone; type O's also are more likely to get a severe form of diarrhea

Figure 6.3.7 Influence on ABO polymorphism on susceptibility to various infectious agents. From: D'Adamo PJ, Kelly GS. Metabolic and immunologic consequences of ABH secretor and Lewis subtype status. Altern Med Rev 2001; 6(4):390-405

Strain	Susceptible phenotype	Comments
Giardia	A	• Blood group A is more susceptible to giardiasis especially the asymptomatic form, while blood group B was less susceptible to giardiasis.
Helicobacter pylori	O, non-secretor	• H. Pylori variants produce a variety of blood group antigens, including A, Lewis (a) and a variety of type 1 H like antigens (O) • Group O would be a moderate risk factor for infection by Helicobacter pylori, with more severe cases in men. • Group O has a more pronounced inflammatory reaction to H. pylori. Group O cells released significantly more IL-6 and TNF in response to H. pylori infection. • The Lewis (a + b-) non-secretor phenotype and blood group O are relevant genetic markers of peptic ulcer. • The Lewis (a+ b-) non secretor phenotype and blood group A were all positively associated with esophageal adenocarcinoma, with concurrent H. pylori infection
Hookworm	O	• A 1972 Egyptian study correlated type O with higher incidence of hookworm and strongyloidiasis.
Influenza	Variable subsets	• Blood group A: Generate a quick and substantial antibody response against influenza type A (H1N1) and especially A (H3N2). The antibody response against influenza B is not quite as dramatic. • Blood group AB: Relatively poor ability to generate high antibody levels against any of the influenza viruses. • Blood group B: Reasonable, but not great ability to generate an antibody response against influenza A (H1N1). Slowest (3-5 months) and weakest ability to generate antibodies against influenza A (H3N2 "Hong Kong") of any blood group. Against influenza B virus, blood group B has a significant advantage and responds differently from either blood group A or O. The blood group B immune response happens much earlier and persists longer. • Blood group O: Moderate ability to generate antibody response against influenza A (H1N1) and A (H3N2) viruses. Antibody response against influenza B is not as dramatic as blood group B.
Malaria	A, AB	• The evidence suggests that blood group A individuals might have a higher predisposition to infection with the Plasmodium vivax species while blood group B individuals tend toward higher infection rates with P. falciparum. • Malaria infected red blood cells sometimes bind to uninfected red blood cells to form clumps, called rosettes. The rosettes can obstruct flow in small blood vessels and lead to tissue damage and severe malaria disease. The tendency for malaria to be worse among A's and AB's is due primarily to a greater degree of rosette formation by RBC's with these antigens. • Von Willebrand Factor, always elevated type A, also enhances rosette formation

Figure 6.3.7 (continued) Influence on ABO polymorphism on susceptibility to various infectious agents. From: D'Adamo PJ, Kelly GS. Metabolic and immunologic consequences of ABH secretor and Lewis subtype status. Altern Med Rev 2001; 6(4):390-405

Strain	Susceptible phenotype	Comments
Neisseria gonorrhoeae	B	• The relation of infection with Neisseria gonorrhoeae to the blood groups A, B, AB, and O was examined in 584 women attending a prenatal clinic. The occurrence of gonorrhea was significantly higher in black patients with blood group B than in those with blood groups A, AB, or O. Depending on the ABO blood group, gonorrhea may affect the titers of isohemagglutinins compared with those of uninfected controls. • The isohemagglutinin titers in group O patients were significantly increased (P less than 0.001) against erythrocytes A, B, and AB. In group A patients, only the titer against AB erythrocytes was significantly increased. • In group B patients, the titer against AB erythrocytes was significantly lower (P less than 0.001) as compared with that in sera of healthy persons.
Norwalk Virus (Norovirus)	O	• It appears that group O RBC's are most easily bound by Noroviruses (NV) versus group B RBC's that are apparently little bound, if at all. Individuals with an O phenotype were more likely to be infected with NV. • The preferred binding sites are apparently the H type 2 antigen that functions as the viral receptor on human type O RBC's. • The Lewis B antigen (found in secretors) is also a binding site.
Schistosomiasis	A	• Group A tends to be more susceptible to infection, tends to get more intense symptoms following infection, and is much more likely to have damage to organs (like the liver) following infection.
Shigellosis	B, AB	• A strong association between blood group B (and AB to a slightly lesser degree) and shigellosis exists.
Smallpox	A	• Group A has higher mortality from smallpox infection • Group A individuals also have more reactions from smallpox vaccination. • The leukocytes of peripheral blood of group A individuals showed a poorer binding capacity with respect to the smallpox vaccine virus. • Blood group A also exhibited a high rate of chromosomal aberration after vaccination, resulting to some extent from increased proliferative ability of the cells.
Staphylococcus aureus	A	• Blood group A is much more likely to be a chronic carrier of Staphylococcus aureus. This is partly due to blood group A individuals having a decreased ability to mount an aggressive antibody (or immune) response against this organism.
Streptococcus (Group B)	B	• A blood group connection with neonatal group B streptococci infection exists for blood group B. Maternal blood group B associated with about a doubling of risk for infection among their infants.
Strongyloidiasis	O	• A 1972 Egyptian study correlated type O with higher incidence of hookworm and strongyloidiasis.
Tuberculosis	O	• Group O blood has a much higher rate of infection with tuberculosis (this is particularly true in individuals of European descent). • Tuberculosis runs a much more aggressive and detrimental course in blood group O, while type A are afforded the highest degree of protection. • Typically, during the first two years of infection with bacillary tuberculosis, there is a significant excess of infection among individuals with blood group O and AB.

Figure 6.3.7 (continued) Influence on ABO polymorphism on susceptibility to various infectious agents. From: D'Adamo PJ, Kelly GS. Metabolic and immunologic consequences of ABH secretor and Lewis subtype status. Altern Med Rev 2001; 6(4):390-405

Blood group	Uropathogenic Strains
A	• Staphylococcus saprophyticus
B	• Klebsiella pneumoniae • Proteus spp. • Pseudomonas sp.
AB	• Klebsiella pneumoniae • Proteus spp. • Pseudomonas spp. • Staphylococcus saprophyticus
Non-secretor	• Uropathogenic E. coli
As a rule, blood group B is most plagued by chronic or recurrent UTI's. Type AB is next on the susceptibility list, followed by type A Type O's are the most protected. Non-secretors are much more prone to repeated and severe UTI's.	

Figure 6.3.8 Influence on ABO polymorphism on susceptibility to various uropathic infectious agents. From: D'Adamo PJ, Kelly GS. Metabolic and immunologic consequences of ABH secretor and Lewis subtype status. Altern Med Rev 2001; 6(4):390-405

ABH secretor system

It was known early in the century that ABH substances occur in human tissues and secretions in two forms, water-soluble and alcohol-soluble, and that persons with these substances in saliva (*secretors*) have more water-soluble substances in their tissues than those lacking the substance in their saliva (*non-secretors*). One of the primary differences in physiology between secretors and non-secretors has to do with qualitative and quantitative differences in components of their saliva, mucus, and other body secretions. Two alleles, Se, and se control ABH secretion. *Se* is dominant and *se* is recessive (or amorphic). Approximately 80% of people are secretors (*SeSe* or *Sese*).

The term "ABH secretor," as used in blood banking, refers to secretion of ABO blood group antigens in fluids such as saliva, sweat, tears, semen, and serum. If people are ABH secretors, they will secrete antigens according to their blood groups. For example, group O people will secrete H antigen, group A people will secrete A and H antigens, etc. Soluble (secreted) antigens are called substances. To test for secretor status, an inhibition or neutralization test is done using saliva. The principle of the test is that if ABH antigens are present in a soluble form in a fluid (e.g., saliva) they will neutralize their corresponding antibodies and the antibodies will no longer be able to agglutinate red cells possessing the same antigens.

In the most rudimentary sense, the secretor gene (FUT2 at 19q13.3) codes for the activity of the glycosyltransferases needed to assemble aspects of both the ABO and Lewis blood groups. This it does in concert with the gene for group O, or H (FUT1). These enzymes are then active in places like goblet and mucous gland cells, resulting in the presence of the corresponding antigens in body fluids. (158)

ABH substances are secreted by mucous glands in many organs, including the upper respiratory tract, the gastrointestinal tract from the esophagus through the colon and the uterine cervix. (12) The secretor gene (*Se*) regulates synthesis of glycoprotein blood-group

substances in superficial glands of the gastric and small intestine mucosa. Large amounts of ABH material are found all secretors, (13-17) but abundant Le^b substance and no ABH substance is seen in non-secretors. Glands situated more deeply in the mucosa of the pylorus and small intestine (Brunner's glands) produce A and B substances without regard to secretor status. (14,16) The gastric parietal glands also produce A and B substances in both secretors and non-secretors. (17)

The prostate glands and the lactating mammary glands of secretors also produce ABH substances. (16) The pattern of secretion by the breast is unusual in that secretors of all ABH phenotypes produce abundant H substance, but much less A substance, and virtually no B substance, is detectable in the breast or in milk. (18)

Secretor status and auto-immune disorders

ABH non-secretors appear to have an increase in the prevalence of a variety of autoimmune diseases including ankylosing spondylitis, reactive arthritis, psoriatic arthropathy, Sjogren's syndrome, multiple sclerosis, and Grave's disease. This susceptibility towards autoimmune problems appears to be most pronounced among Lewis (a-b-) phenotypes. Among individuals with spondyloarthropathies, non-secretors are reported to make up 47% of the patient population. In the subgroup of these patients suffering from ankylosing spondylitis, ABH non-secretors account for 49% of patients. Since the control population had a prevalence of non-secretors of 27% (consistent with the expected percent in the general population), it appears that in spondyloarthropathies in general, and ankylosing spondylitis specifically, non-secretors are dramatically over represented. (159,160)

Among individuals with primary Sjogren's syndrome, Lewis blood group frequency differs from that of the general population, due mainly to an increased Lewis negative phenotype (Le (a-b-)) frequency. (161)

The inability to secrete the water-soluble glycoprotein form of the ABO blood group antigens into saliva is significantly more common in patients with Graves" disease than control subjects (40% versus 27%: p less than 0.025) but not among those with Hashimoto's thyroiditis or spontaneous primary atrophic hypothyroidism.

ABH non-secretors with Grave's disease were found to produce higher levels of anti-tubulin antibodies, while levels of other antibodies were similar to secretors. (162)

Secretor status and Crohn's disease

Fucosyltransferase 2 (FUT2) non-secretor status is associated with Crohn's disease. In an independent cohort of 1174 Crohn's disease cases and 357 controls between the four primary FUT2 SNPs and CD rs602662 and also association with *FUT2* W143X. These findings strongly implicate this locus in CD susceptibility and highlight the role of the mucus layer in the development of CD.

Secretor status and celiac disease

ABH non-secretors are at an increased risk for development of celiac disease. One study found 48 percent of patients with celiac disease were reported to be ABH non-secretors. (76) This appears to be especially true for the recessive Le (a-b-) phenotype. Evidence suggests an increased prevalence of complications and celiac-

associated abnormalities in the non-secreting and Lewis-negative celiac patients. (77)

Secretor status and Candida carriage

ABH non-secretors are much more likely to be carriers of Candida sp. and to have problems with persistent Candida infections. Blood group O non-secretors are the most affected of the non-secretor blood types. One of the innate defenses against superficial infections by Candida species appears to be the ability of an individual to secrete the water-soluble form of his ABO blood group antigens into body fluids. The protective effect afforded by the secretor gene might be due to the ability of glycocompounds in the body fluids of secretors to inhibit adhesins (attachment lectins) on the surface of the yeast. In attachment studies, preincubation of blastospores with boiled secretor saliva significantly reduced their ability to bind to epithelial cells. ABH non-secretor saliva did not reduce the binding and often enhanced the numbers of attached yeasts. (163,164) In one study, among individuals with Type II diabetes, 44% of ABH non-secretors were oral carriers of this yeast. (165)

Although non-secretors make up only about 26% of the population, they are significantly over represented among individuals with either oral or vaginal *Candida* infections, making up almost 50% of affected individuals. (168) The inability to secrete blood group antigens in saliva also appears to be a risk factor in the development or persistence of chronic hyperplastic candidosis. In one study, the proportion of non-secretors of blood group antigens among patients with chronic hyperplastic candidosis was 68%. (166)

Women with recurrent idiopathic vulvovaginal candidosis are much more likely to be ABH non-secretors. Combining both ABH non-secretor phenotype and absence of the Lewis gene, Lewis (a- b-), the relative risk of chronic recurring vulvovaginal candidosis is between 2.41-4.39, depending on the analysis technique and control group. (167)

Oral carriage of *Candida* is also significantly associated with blood group O ($p < 0.001$) and independently, with non-secretion of blood group antigens ($p < 0.001$), with the trend towards carriage being greatest in group O non-secretors. (168)

Secretor status and general immunity

Evidence suggests that ABH non-secretors have lower levels of IgG. In tests of 202 Caucasians, researchers found IgA concentrations to be significantly lower in non-secretors than in secretors.) This seems to imply that the ABH non-secretor state is associated with a "Defense in Depth" strategy (i.e. let the invader in and attempt to destroy it internally) versus the ABH secretor state, which implies a "Preclusive Strategy" (i.e. wall out the invader and don't allow entrance in the first place.) For example, the free ABH antigen on the mucosa barriers of ABH secretors acts as an effect anti-adhesive mechanism against ABH specific bacterial fimbriae lectins. (140,141)

On the other hand, the ability to secrete relatively different concentration of the components of the blood group substances as determined by secretors/non-secretor genetics seems to affect phagocytic activity of the leucocytes in a manner

that actually places non-secretors at somewhat of an advantage. In general, leukocytes of non-secretors have substantially greater ingestion power as compared to secretors. (142) Although this ability appears to be across the board for all non-secretors, blood group O and B non-secretors have the greatest advantage and highest phagocytic activity. Perhaps this is a compensatory mechanism for their more limited antigenic barrier in their body fluids and secretions.

Results suggest that the level of anti-I in the serum of normal individuals may be affected by the donor's ABO group, secretor status and sex. For individuals with blood group O, B and AB secretors have higher levels of an antibody presumed to be auto-anti-I (cold hemagglutinin). The level of this antibody is usually even higher among non-A female secretors than for males. (143)

Researchers have found that in individuals with insulin dependent diabetes mellitus, the mean level of C3c for non-secretors is significantly lower than that found for secretors. The level of C4 among ABH non-secretors was also significantly lower than that of ABH secretors. (144)

Secretor status influence on sialic acid recognition on erythrocytes

ABH antigens found on human erythrocytes modulate the specific interactions of sialic acid-recognizing proteins (such as human Siglec-2, 1918SC influenza hemagglutinin, and Sambucus nigra agglutinin) with sialylated glycans on the same cell surface. ABH antigens stabilize sialylated glycan clusters on erythrocyte membranes uniquely for each blood group, generating differential interactions of the three sialic acid-binding proteins with erythrocytes from each blood group. ABH antigens can also modulate sialic acid-mediated interaction of pathogens such as *Plasmodium falciparum* malarial parasite. Thus, ABH antigens can noncovalently alter the presentation of other cell surface glycans to cognate-binding proteins, without themselves being a direct ligand. (60)

Secretor status influence on breast milk composition

Human breast milk is the most heavily fucosylated of all the higher mammals. Specific human milk oligosaccharides, especially fucosylated neutral oligosaccharides, protect infants against specific microbial pathogens. The oligosaccharide composition of human milk varies considerably from person to person; however, and suggests the existence of many genotype subpopulations. This variation in individual oligosaccharide concentrations suggests that the protective activities of human milk could also vary among individuals and during lactation. (32) Fucosylation profiles of human milk oligosaccharides are known to vary by host ABO and Lewis blood groups and ABH secretor status via the *FUT2, FUT3,* and *FUT4* family of fucosyltransferases. (33,34)

During the first week of lactation, the ability to produce neuraminyloligosaccharides is linked to the ABH secretor groups. Moreover, the ability to produce oligosaccharides with Le (a) or Le (b) characteristics is linked to Lewis and Secretor systems. The consequences of this are that secretors will produce higher levels of N-acetylneuraminic acid and lower levels of galactose in their breast milk than non-secretors. In the ABH secretor groups, blood group A and O secretors also have higher N-acetylglucosamine contents than B and AB secretors (p less than 0.001), while the A and B secretors have higher galactose levels. A significantly higher level of fucose also distinguishes the Lewis secretor groups. The ABH (+), Le (a-b-) group had higher lactose contents than the other groups. (35)

Maternal ABO Blood Group, ABH secretor status and Lewis Phenotype	Composition of Breast Milk
A or O, Secretors, Lewis (a-b+)	• High amounts of n-acetylneuraminic acid and n-acetylglucosamine • Lower levels of galactose • Higher fucose • Lower lactose • Presence of Le(b) and either A or O substances
B or AB, Secretors, Lewis (a-b+)	• High amounts of n-acetylneuraminic acid • Lower n-acetylglucosamine • Moderate galactose • Higher fucose • Lower lactose • Presence of Le(b) and either B or AB substances
ABO non-secretors, Lewis (a+b-)	• Highest galactose • Lowest amounts of N-acetylneuraminic acid • Higher fucose • Lower lactose • Presence of Le(a) and absence of ABO substances
ABO, Lewis negative, Lewis (a-b-)	• No Lewis substances • Highest lactose • Quantities of ABO substances, galactose, n-acetylneuraminic acid and n-acetylglucosamine cannot be estimated

Figure 6.3.5 Differences in carbohydrate composition of human milk oligosaccharides by ABO and Lewis blood groups and ABH secretor status. (From D'Adamo PJ, Kelly GS. Metabolic and immunologic consequences of ABH secretor and Lewis subtype status. Altern Med Rev. Aug; 6(4):390-405; 2001)

The neuroplasticity that characterizes human adaptive learning may have a sweet tooth for fucose. Fucose is now garnering attention as an important component in learning: brain fucosylation skyrockets during periods of intense learning, which appear to correlate with increased ligand-like activity involving fucose at the neurosynaptic juncture. Inhibition of protein fucosylation using 2-deoxy-D-galactose causes amnesia in animals, presumably by blocking formation of fucose α(1-2) galactose linkages, whereas administration of free L-fucose in rats enhances memory retention and long-term potentiation (LTP), a widely accepted cellular model for memory. (36) It appears that what goes into holding the synapse together may be as important a factor in cognition and learning as what jumps across the synapse.

Subgroups of type A

Considerable numbers of variants of the A antigen are known, most of which are rare; the B antigen is less variable but several rare variants are known. The most important distinction is between A1, the commonest antigen, and A2, which has a frequency of several percent in most European, African, and West Asiatic populations. Though the distinction has been known for nearly 50 years, its basic nature is still not completely understood; but most of the facts are covered by the following conventional account. In 1930, it was observed that there were two varieties of the A antigen, A1 and A2, allowing the blood groups A and AB to be classified respectively as A1 and A2, and as A1B and A2B.

Both types of antigen react with the ordinary antibody anti-A, but only A1 reacts with anti-A1, while A2, fails to do so. Reaction of antigens is shown, as usual, by agglutination. (132)

Anti-A1 is present in the serum of most B persons together with ordinary anti-A. The latter antibody can be absorbed from a serum containing it, by means of A2 cells, leaving only anti-A1 behind, so that the serum becomes a specific anti-A1 reagent. An excellent anti-A1 reagent can also be prepared by extracting the lectin from *Dolichos biflorus* seeds with physiological saline.

The A1 and A2 antigens are produced by corresponding allelomorphic genes, so that what we have called the *A* gene is really of two possible kinds, *A1* and *A2*. In the genotype *A1A2,* the *A1* gene causes the production of A1 antigen, and thus the genotypes *A1A2*, *A1O,* and *A1A1* are indistinguishable by methods at present available, since all react both with anti-A and anti-A1.

As group A2 is intermediate in several of its properties between A1 and O, it would be of great interest to know whether this applies to its associations with disease; but unfortunately, very few investigators of associations have determined the subgroups of A in their patients.

RBC's of both react strongly with anti-A reagents in direct agglutination tests. The serologic distinction between A1 and A2 is based on results obtained in tests with reagent anti-A1, prepared from group B human serum or the lectin of *Dolichos biflorus* seeds.

Under prescribed testing conditions, anti-A1, reagents agglutinate A1, but not A2 RBC's. The RBC's of approximately 80% of group A or group AB persons are agglutinated by anti-A1, and, thus are classified as A1, or A1B. The remaining 20% RBC's are agglutinated by anti-A, but not by anti-A1, are called A2, or A2B.

Subgroups of A are phenotypes that differ from others of the same ABO group with respect to the amount of A antigen carried on red blood cells (RBC's), and, in secretors, present in the saliva.

Anti-A1 occurs in the serum of 1% to 8% of A2 persons and 22% to 35% of A2B persons. Anti-A1 can cause discrepancies in ABO testing and incompatibilities in crossmatches with A1 or A1B RBC's. It is considered clinically insignificant unless it reacts at 37°C. It is not necessary to test group A RBC's with anti-A1, to confirm their subgroup status except when working with samples from people whose sera contain anti-A1.

Subgroups weaker than A2 occur infrequently; and, in general, are characterized by decreasing numbers of A antigen sites on the red cells and a reciprocal increase in H antigen activity. The genes responsible constitute less than 1% of the total pool of A genes.

RBC's of the Ax Ael, Aint, or A3 subgroups are seen only infrequently in transfusion practice. Ax and Ael RBC's are readily recognized as subgroups of A by the discrepancies they produce between RBC and serum grouping tests. Ax RBC's are, in general, agglutinated by human anti-A,B but not by human anti-A. However, Ax RBC's react with some murine monoclonal anti-A reagents. Ael RBC's fail to react with anti-A or anti-A,B of any origin. Adsorption and elution studies are necessary to show that these RBC's carry the A antigen. RBC's of the Aint phenotype can be identified only if tests with anti-A1 are performed. Aint RBC's react more weakly than A1 RBC's with anti-A1, yet more strongly with anti-H than do A2 RBC's.

A3 RBC's produce a characteristic mixed-field pattern of small agglutinates among many free RBC's in tests with anti-A and anti-A,B. Weak subgroups of A such as Ax, Ael, and Aint,

cannot be identified on the basis of blood grouping tests alone. Saliva studies and adsorption/elution studies must be performed.

A 539G > C mutation represents a new molecular basis for the A2 blood group. The amino acid substitution from arginine to proline may have effect on the expression of A antigen. (136)

There is extensive sequence heterogeneity underlying the major ABO alleles that produce normal blood groups A, B, AB, and O when in correct combination with other alleles. Second, there is also extensive heterogeneity underlying the molecular basis of various alleles producing ABO subgroups such as A2, Ax, and B3. There are over 70 ABO alleles reported to date and these alleles highlight the extensive sequence variation in the coding region of the gene. (137)

The blood factor A is not exactly the same in different individual human bloods. For a number of years it has been known that there were two main varieties of A, which are designated as A1 and A2. (138) Of these, the latter gives weaker reactions with the average anti-A reagent, and the former gives stronger reactions. Since this is not a textbook on serology, we will not go further into the nature of the differences between the two subgroups of the gene A.

Although peoples of European stock seem to differ serologically from the populations of all or nearly all the rest of the world, except possibly the African, in that they have the subgroup A2 of the blood group A and have a considerable amount of rh' and rh genes; when and in what manner they acquired these differences is not easy to state. We do not know, at the present, of any human (or even anthropoid) group with much more A2, proportionally, than the average European. Perhaps A2 has some selective disadvantage, and under the conditions of stringent selection in Asia, it was eliminated before the ancestors of the American Indians left for this continent. In Paleolithic European man, the gene, although possibly inferior to A1, lingered on. Nevertheless, all this must be admitted to be in the highest degree conjectural. (139)

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Unique A2 distributions

A remarkable population isolate is that of the Flittas who live at Zemmora, southeast of Oran, and have a long history of fierce resistance to successive rulers of Algeria. They are probably of Berber origin, perhaps with some admixture at an early period, but they appear to have constituted a strict isolate for many hundreds of years. Their ABO frequencies are unique, with 18 percent of A2 genes, the highest frequency known except in the Lapps of northern Scandinavia. The total A gene frequency reaches the high level of 30 per cent, and M at 57 percent is well above the general North Africa level. The presence of 26 percent of cDe shows considerable Negro admixture, presumably long ago. The high A2 frequency remains a mystery—it is presumably the result of genetic drift or natural selection. But, why should only three known populations, the Lapps, the Flittas, and the Nagas of Burma (with 17 percent of A2), have evolved in this way, when the great majority

of isolates in Europe and the Mediterranean area, including the Arabs of Arabia and the Berbers of the Atlas Mountains, have minimal O and low total A frequencies? It would be of great interest to carry out A1/A2 subgrouping on the other high A peoples of North Africa. (139)

The Lapps are an ethnic group living in northern Norway, Sweden, Finland, and northwestern Russia. Some are reindeer herders and some are fisherfolk. They are mostly of Caucasoid appearance but with a very slight mongoloid tendency. They speak a Finno-Ugric language closely related to Finnish, but regarding this there are two points of view. One is that they formerly spoke a non-Finno-Ugric language now lost, and adopted Finnish that in the course of time became modified. The other is that their original language was a Finno-Ugric one but belonging to the Ugric sub-family. It became modified, however, by the very large-scale adoption of words from the language of their neighbors, the Finns. This distinction is an important one with regard to their possible relationship to the Samoyeds.

The Lapps have been very thoroughly studied from the point of view of blood groups. They are almost unique in their high frequency of A and totally so in having the highest A2 gene frequency known, reaching 42 per cent in one group tested. In this respect, they are super-Caucasoids, for the A2 gene is almost entirely confined to Caucasians. (139)

It is a striking fact that the subtype A2 does not seem to occur in the Australian aborigines, in China, Japan, in the Native Americans, or in the natives of the islands of the Pacific, in so far as can be determined from the somewhat sketchy studies made up to the present time. (139).

Lewis blood groups

Two broad categories of Lewis blood group exist. These are the "Lewis Positive" (either Le^{a+b-} or $Le^{a-b+)}$ and "Lewis Double Negative" (Le^{a-b-}) phenotypes. Depending on race, between 1% and 8% of the population is *Lewis Double Negative* (LDN)

In Lewis positive phenotypes, Lewis a is formed initially, and in the case of non-secretors (lacking the *Se* gene, *FUT2*), Lewis a substance is adsorbed onto the red cell, and they type as Lewis a. In the case of secretors, the *Se* gene activates the *H* gene, which causes an additional sugar to be added to Lewis a, converting it to Lewis b.

Among Lewis positive individuals, ABH secretors are always Le^{a-b+} since they convert all their Lewis a antigen into Lewis b. Conversely, among Lewis positives, ABH non-secretors are always Le^{a+b-} since they lack the *FUT2* dependent glycosyltransferase to accomplish this.

Thus it is often possible (and quite handy) to use the Lewis groups to infer ABH secretor status, since Lewis typing is quick to perform and easy to master when compared to the salivary inhibition test traditionally used. However, using Lewis typing to infer ABH secretor status only works in those individuals who are Lewis positive (about 9 out of 10 subjects). Lewis negative individuals can be either secretors or non-secretors. The Lewis negative patient, however, carries important metabolic consequences of their own, worthy of much attention.

Lewis Type	Category	ABH Secretor Status

Le (a+b-) Lewis a antigen but not Lewis b	Lewis Positive	ABH non-secretor
Le (a-b+) Lewis b antigen but not Lewis a	Lewis Positive	ABH secretor
Le (a-b-) Neither Lewis a nor Lewis b	Lewis Negative	Lewis outcome cannot determine ABH secretor status

Figure 6.3.3 Lewis blood groups and their relationship to ABH secretor/non-secretor status (10)

The ABH secretor system is a major determinant of the Lewis (Le) blood grouping system. This is due to the fact that, in addition to their function in creating the ABO glycoproteins, *FUT1* and *FUT2* also provides the glycans necessary for conversion of Lewis antigens as well.

Sialyl Lewis X (SLeX)

Sialylated forms of several Lewis variations (Sialyl Lewis A, Sialyl Lewis X) are oligosaccharide ligands now considered crucial to the initial adhesion of white blood cells to a site of injury mediated by E-selectins. Large quantities of SLeX have also been found on the surfaces of certain tumor and cancer cells and one of its variants (Sialyl 6-sulfo Lewis X) appears to be involved in routine homing processes involving a variety of chemokines. (55)

Selectins are cell-cell adhesion molecules that are involved in leukocyte-endothelial cell adhesive interaction, which is required for extravasation at target tissue sites. Three types of selectins have been discovered so far. L-selectins are generally expressed on almost all leukocytes. E-selectins are inducible on vascular endothelium upon stimulation with cytokines. P-selectins were originally found on activated platelets. Less known is the role of thrombomodulin as a c-type lectin with a domain that interferes with neutrophil adhesion to endothelial cells. Elevated levels of E-selectin ($P < 0.001$) and thrombomodulin ($P<0.001$) are linked with blood group A individuals. (19)

Eosinophil migration into and across the intestinal epithelium is dependent on adhesion molecules such as selectins. Human eosinophils express L-selectin and P-selectin counterligand P-selectin glycoprotein ligand-1 (PSGL-1). SLeX binds to all three selectins. (56)

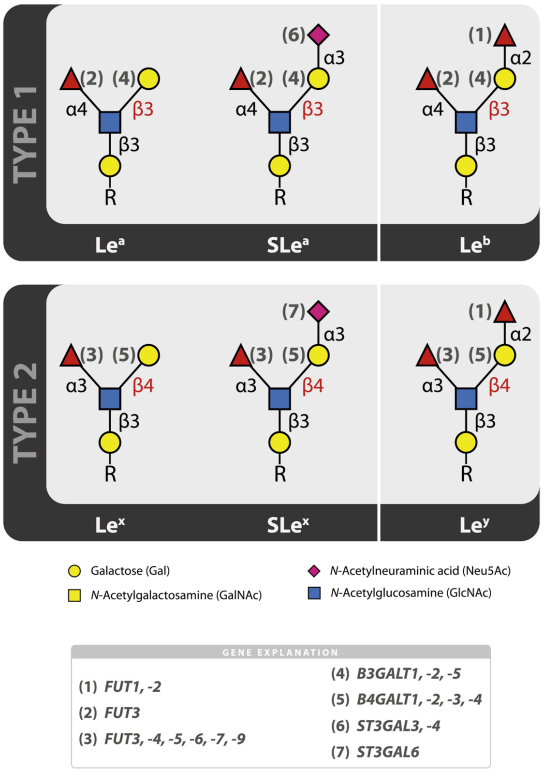


Figure 6.3.4 Lewis antigens. Lewis antigens are usually subdivided into two groups – type 1 and 2 – depending on whether the terminal galactose is bound to the preceding GlcNAc by a β3 or β4 bond (in red font). Epitopes in the latter category are considered as tumor-associated antigens. Both type 1 and 2 structures may appear on a variety of glycans (denoted as "R"). Therefore, they are important in interaction with other cells like endothelial cells. The main Lewis antigens are shown, alongside genes encoding key transferases in their synthesis. Modified from: Potapenko IO, Haakensen VD, Lüders T, Helland A, Bukholm I, Sørlie T, Kristensen VN, Lingjaerde OC, Børresen-Dale AL. Glycan gene expression signatures in normal and malignant breast tissue; possible role in diagnosis and progression. Mol Oncol. 2010 Apr; 4(2):98-118.

The presence of SLeX ligands on milk oligosaccharides together with their abundant distribution in human milk may suggest that they could be selectin ligands and they may modulate the inflammatory processes. (58) Excessive leukocyte infiltration causes severe tissue damage in a variety of inflammatory diseases. The initial step in leukocyte extravasation is mediated by selectins and oligosaccharides on their glycoconjugate ligands. Human milk is a rich source of lactose-derived oligosaccharides that are partly absorbed in the intestine and excreted with the urine. As these components contain binding determinants for the selectins specific oligosaccharides, including SLeX and other fucosylated glycans in human milk serve as anti-inflammatory components and might therefore contribute to the lower incidence of inflammatory diseases in human milk-fed infants. (57)

OTHER SEROLOGIC POLYMORPHISMS

MNS blood group

The MNS blood group system is second only to the Rh blood group system in its complexity. (64) It was the second blood group system to be discovered (1927). In a deliberate attempt to discover more blood group antigens, Landsteiner and Levine immunized rabbits with human red blood cells. The discovery and elucidation of inheritance was one of the most brilliant achievements in this field of biology; out of forty-one sera four were found to have a distinctive agglutinin that reacted independently of the then known ABO blood group types. By selective immunization and absorption, the serological specificities and inheritance of M and N were described. It was twenty years before the third antigen of the group, S, was identified, followed shortly by the discovery of the product of its antithetical allele, s. Because of this system's usefulness in testing inheritance within pedigrees, several newly discovered blood group antigens were found to be associated with this system; some being high incidence antigens (i.e., U, ENA) or, more frequently, low incidence antigens (i.e., Mg, He, Mta, etc.). To date, there have been over 43 antigens associated with this blood group system.

The MNS antigens are located on either glycophorin A, glycophorin B, hybrid or mutant structures of both of these sialoglycoproteins which are encoded by two highly homologous and closely linked genes on the long arm of chromosome 4. This system was the first non-water soluble blood group system to be biochemically investigated. Many of the low incidence antigens associated with hybrid structures could only have been assigned to this system through biochemical and DNA investigation.

The MNS antigens are found predominately on the red cells with some found on the renal endothelium and epithelium. Antibodies against M are fairly common, being the most frequently found antibody in non-transfused children, however antibodies against N are exceedingly rare (undoubtedly because N can be encoded by some forms of glycophorin A when the N gene is present as well as the most common form of glycophorin B). Even though anti–M antibodies are found in multiply transfused individuals and multiparous females, it rarely if ever is associated with hemolysis of red cells.

M phenotype appears correlated positively with an increased susceptibility to nonallergic asthma in all age groups, whereas N phenotype appears correlated positively with age at onset but in allergic asthma only. (61) M- twins have greater environmental variability in cholesterol levels than M+ pairs. (62) Males with the MN phenotype had significantly higher unadjusted systolic and diastolic blood pressures than those who were homozygous MM or NN. (63) Sharon. Weinberg and Husseini (1985) reported two groups of Arab patients with ankylosing spondylitis both of which had a very high incidence of homozygous MM (92% and 100%). (64) Interest has been revived in the human MN blood group system due to the discovery of the MN precursor substance (T-antigen) on certain malignant, but not benign or normal tissues. (65)

Rhesus blood group

Rh is the most complex of the blood groups systems, embracing over 45 distinct antigens, the absence or presence of which combine to exhibit an individual's Rh blood group type. The most clinical important antigen, D or Rho, was the first discovered in 1940, and it has been generally referred to as the Rh antigen, being present in over 85% of the random population. Those individuals that lack the D antigen are considered Rh negative.

The Rh antigens are encoded by two highly homologous and closely linked genes on the short arm of chromosome 1: the *RHD* gene producing the D antigen, or most of its components, and the *RHCE* gene producing the Cc and Ee antigens or their variants. The majority of the antigens within this system represent products of gene crossover, point mutations, or deletions within one or both genes.

The Rh antigens appear to be red cell specific, appearing early during development of red blood cells, and have not been found on other body tissues. Antibodies against the Rh antigens have caused severe and fatal transfusion reactions and hemolytic disease of the newborn. The importance of the Rh antigens in the erythroid membrane is exemplified by the fact that in many examples of autoimmune hemolytic anemia, auto-Rh antibodies are frequently found.

Moreover, in hematological testing the extremely rare (only 32 known throughout the world) individuals who have no detectable Rh antigens, *Rh null* individuals, a shortened red cell survival is quite common. *Rh null* cells exhibit stomatocytosis and spherocytosis, and have increased permeability to potassium suggesting that they lack a crucial membrane component. A current model suggests that Rh assembles in the membrane as a complex with CD47, LW, RhAG, and glycophorin B. Mutations of the RHAG gene accounts for most examples of *Rh null.*

It is truly ironic that this blood group system received this name because it was originally thought to be similar to an antibody produced in rabbits that had been immunized with rhesus monkey cells. By the time it was scientifically proven that they were two distinct antibody specificities there were too many publications referring to the Rh factor as the product of the D gene and the symbol Rh was well entrenched for this blood group system. Hence, the rhesus association to the system name had been made, but in fact, there is no association with rhesus monkeys what so ever. That antibody produced in rabbits to rhesus monkey cells and the similar human antibody specificities have been named after the two original investigators, Landsteiner and Wiener (refer to LW blood group system).

Expression of *RHCE*, *RHD,* and *RHAG* is confined to erythroid tissues; products of the non-erythroid homologues are expressed in kidney, liver, skin, testis, and brain.

Gene recombinations between the *RHD* and *RHCE* alleles, as well as other mutations at the *RHCED* locus are responsible for the origin of a large number of rare alleles whose expression is apparent from serological studies. Because of the opposite orientation of the two genes, it has been proposed that gene recombinations occur predominantly through gene conversion rather than unequal homologous recombination. In the *RHCED* locus, gene conversion is implicated in both large- and small-scale transfers of genetic material from donor to the recipient; they are defined as macroconversions or microconversion events, respectively. Unequal homologous recombination occurring within these “Rhesus Boxes” flanking the *RHD* gene may be responsible for its deletion (67). Other molecular

mechanisms include missense changes, nonsense mutations, and small in-frame and out-of-frame deletions.

The most common phenotypic alteration includes the absence of expression of the D antigen (RhD-negative, gene frequency in Caucasian population is 45%). This may be due to deletion of the entire gene, gene rearrangements, or mutations, as well as deletions or insertions resulting in frameshifts. In all these instances, the D epitope is absent or unavailable.

The incidence of variants of RHCE and RHD alleles in the population still lacks complete documentation. Whereas in some cases, less than 0.1% of the population tested show the variant phenotype, and the occurrence of some phenotypes has been documented in single families only; the incidence of other variants may be much higher. A recent report documents the prevalence of a RHD pseudogene in a large segment of RhD-negative African populations (68). In most cases, the sites of recombinations are known, but the breakpoints, which most often occur within introns, have not been defined or are ambiguous because of a high sequence identity.

Rh incompatible transfusion may result in death of patients. Rh incompatibility is still the leading cause of hemolytic disease of the newborn (HDN) and may involve some forms of graft-versus-host (GVH) disease in organ transplantation. Absence or severely reduced expression of all Rh30 polypeptides and/or their associated Rh antigens is referred to as Rh null or Rh mod (also known as Rh deficiency syndrome). This autosomal recessive disorder manifests a varying degree of compensated hemolytic anemia and spherostomatocytosis.

Rh incompatibility is the major cause of hemolytic disease of the newborn, however very few searches have been made for any other kinds of disease associations of the Rh groups. Rh proteins may influence glucose transport through red cell membrane and/or hemoglobin glycation. (69)

The Rh factor and Rh-associated glycoprotein (RhAG), with epithelial cousins RhBG and RhCG, form four subgroups conferring upon vertebrates a genealogical commonality. (70)

Although Rh+ and Rh- individuals are about equally likely to have colon cancer, Rh- individuals are more likely to have a localized disease, while Rh+ individuals are more likely to have metastatic disease. This suggests that Rh+ patients with colorectal cancer are less protected against tumor spread than Rh- patients are, especially with regard to regional lymph node metastases. (114) The absence of Rh factor (Rh-) was positively associated with the risk of breast cancer. (124)

Rh blood group might also influence NK cell activity. While some studies have not found an association, other researchers have observed a higher natural NK cytotoxicity against target cells in individuals with Rh- blood group. (125-127)

It has recently been demonstrated that two human Rh glycoproteins can correct ammonium transport deficiency in mutant yeast cells. Rh proteins are therefore ammonium transporters —a role that, in vertebrates, has remained previously uncharacterized. These data herald a new era in Rh protein research, beyond their role as blood group antigens, and into the characterization of ammonium transport mechanisms, notably in the kidney. (128)

Paleoserology of Rhesus blood groups

After the Second World War, William Boyd's baton as compiler of blood group data from around the world passed to the Englishman Arthur E. Mourant.

A native of Jersey in the Channel Islands, Mourant received a degree in geology, but as this was Depression-Era Britain, he was unable to find a job. His very strict Methodist upbringing had caused him considerable emotional unhappiness, which he hoped to resolve by becoming a psychoanalyst. To that end, he decided to begin by first studying medicine.

To avoid the German bombing raids on the capital, his medical school was moved from London to Cambridge, and it was here that he met Ronald Fisher, the most influential geneticist of his day. Fisher, a brilliant eccentric who we will meet again, had been working out the genetics of the new blood groups that were being discovered, and he had become fascinated by the particularly convoluted inheritance of one of them – the Rhesus blood group. Fisher found him a job at once, and the meticulous Mourant spent the rest of his working life compiling and interpreting the most detailed blood group frequency distribution maps ever produced. He never did become a psychoanalyst.

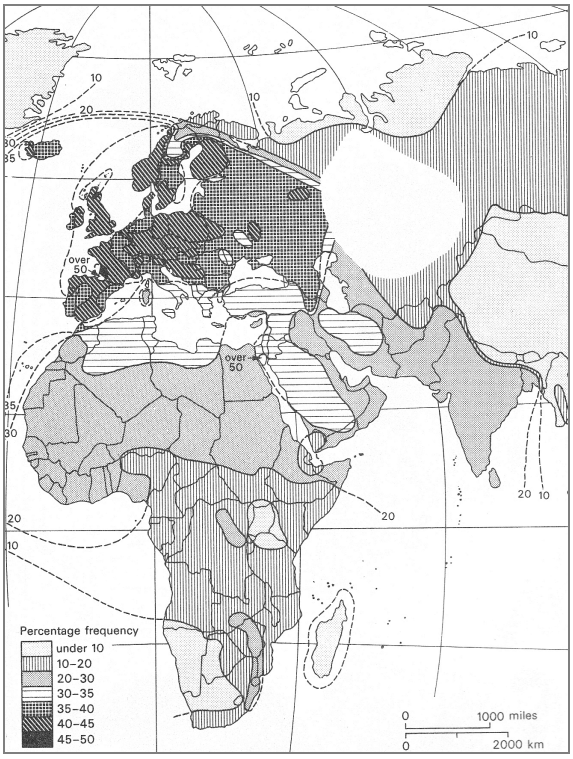


Figure 6.3.9 Rhesus blood group system. Distribution of the d gene in the indigenous populations of Europe, Western Asia, and Africa. (From Mourant, Blood Relations: Blood Groups and Anthropology, Oxford University Press 1985)

In the early 1600's Pierre de Lancre, a French witch hunter, speculated why the Basque area seemed to harbor so many witches. He thought the problem stemmed from their great numbers in the various Jesuit missionaries, with all their evangelizing, which had affected them with demons from far-off places that they had carried back to Spain. De Lancre also thought that their early adoption of tobacco use might also be working on their minds. He held Basque women in special contempt, saying that they produced only undersized and cursed children who died.

As Mark Kurlansky recounts in *The Basque History of the World*, this last accusation may have had a ring of truth to it, since Basques are renowned among anthropologists for their strikingly high percentage of individuals who have the Rhesus Negative (RH-) blood group genotype (dd): 60% compared to an average of 16% for the rest of Europe. When a mother is Rh- and she gives birth to Rh+ children, an immune reaction can occur which gives rise to a hemolytic ("blood destroying") anemia, and often would lead to the death of the child.

Mourant suggested that modern day Basques have other characteristics, which may mark them as descendants of the late Paleolithic population of Western Europe: They share a skeletal resemblance to Cro-Magnon man and they are the only Western European people who do not speak an Indo-European language. There is considerable evidence for this. The hemochromatosis gene (*HFE*) has a polymorphic variant (*C282Y*) that is found in very low incidence among the Basques, who instead have the H63 variant. The C282Y variant is sometimes called the Celtic polymorphism because it is found in a very large percentage of Celtic people. It has been speculated that the C282Y variant may have been an adaptation to decreased dietary iron in cereal grain-based Neolithic diets. Both homozygous and heterozygous carriers of the HFE C282Y mutation have increased iron stores and therefore possessed an adaptive advantage under Neolithic conditions. An allele age estimate places the origin of the C282Y mutation in the early Neolithic period in Northern Europe and is thus consistent with this hypothesis. C282Y seems to be the result of an effort to "accumulate" the non-heme iron found in plants as a response to the lowered intake of animal products occurring with the conversion of hunter-gathering to Neolithic agricultural practices.

The Basques are culturally and genographically unique, thought to be a Mesolithic remnant settling in the northern area of Spain before the LCM (Last Glacial Maximum). The high frequency of type O Rh- blood and the low incidence of the C282Y polymorphism may indicate that these three genes relate to preferences for heme (animal derived) iron

Duffy blood group

In 1950, the Duffy blood group was named for the multiply transfused hemophiliac whose serum contained the first example of anti-Fya. In 1951, the antibody to the antithetical antigen, Fyb, was discovered in the serum of a woman who had been pregnant three times. Using these antibodies three common phenotypes were defined: Fy(a+b+), Fy(a+b-), and Fy(a-b+). Differences in the racial distribution of the Duffy antigens were discovered four years later when it was reported that the majority of Blacks had the erythrocyte phenotype Fy(a-b-). This phenotype is exceedingly rare in Whites. The frequency of the Fy(a-b-) phenotype is 68 percent in American Blacks and 88-100 percent in African Blacks.

The Duffy system of blood groups is genetically simple, being controlled by four allelic genes at one locus, of which only three are sufficiently common to be of anthropological and medical significance. Because the letter D is used for the principal gene of the Rh system, the last two letters of the name Duffy are used in the gene symbols, Fya, Fyb, and Fy4. The Fy4 gene is very rare outside Africa and very common within that continent. Not

only is it therefore an important anthropological marker, but, it provides one of the few cases where we think we know, in terms of natural selection, why one population differs from another in their blood-group frequencies.

The Duffy genes, located on chromosome one at position 1922-23, have recently been cloned and sequenced. The difference between Fya and Fyb is a change in the amino acid at position 43 from aspartic acid (Fya) to glycine (Fyb). Studies have shown that blacks whose erythrocytes express Fyb antigen also have the antigen on the cells of their kidney, heart, muscle, brain, and placenta.

The Duffy genes code for proteins known as a *chemokine receptors*. Duffy has been found to act as a multispecific receptor for chemokines of both the C-C and C-X-C families, including: MGSA, regulated upon activation normal T expressed and secreted (RANTES; CCL5), monocyte chemotactic protein-1 (MCP-1; CCL2) and the angiogenic CXC chemokines interleukin-8 (Il-8, CXCL8), growth related gene alpha (GRO-α, CXCL1), neutrophil activating peptide-2 (NAP-2, CXCL7) and ENA-78 (CXCL5). Accordingly, the Fy protein is also known as DARC (Duffy Antigen Receptor for Chemokines). (129,130)

In DARC-transfected cells, DARC is internalized following ligand binding and this led to the hypothesis that expression of DARC on the surface of erythrocytes, endothelial, neuronal cells and epithelial cells may act as a sponge and provide a mechanism by which inflammatory chemokines may be removed from circulation as well as their concentration modified in the local environment. This hypothesis has also been questioned after knockout mice were created. These animals appeared healthy and had normal responses to infection. The Duffy antigen/receptor for chemokines (DARC) regulates prostate tumor growth. (131)

Sanger discovered that a high percentage of African blacks are of the phenotype Fy (a- b-), which is apparently a third gene termed Fyx that does not react with anti-Fya or anti-Fyb. In 1975, Miller was able to show that this type is probably specifically resistant to *Vivax malaria,* to which Africans have long been known to be resistant. (132)

The molecular basis for the Fy(a-b-) phenotype is the result of a point mutation in the erythroid specific promoter. On erythrocytes, the Duffy antigen acts as a receptor for invasion by the human malarial parasites *Plasmodium vivax* and *Plasmodium knowlesi*. Duffy negative individuals whose erythrocytes do not express the receptor are resistant to infection. This antigen may also play a role in erythrocyte invasion in the rodent malarial parasite *Plasmodium yoelii.* This racial variation in distribution of the Duffy system antigens provides one of the few known examples of selective advantage conferred by a blood group phenotype. The parasite-specific binding site, the binding site for chemokines and the major antigenic domains are located in overlapping regions at the exocellular N-terminal terminus.

Homozygous Duffy-deleted mice (Dfy -/-) are indistinguishable from their wild type in size, health, embryonic development and neurological behavior. The only difference noted is a diminution of neutrophil trafficking in the mutant mice. The human equivalents of the Dfy -/- mice are also healthy; they are individuals whose phenotype is Fy(a-b-) and who lack gp-Fy on erythrocytes; its level of expression on non-erythroid cells is not known.

The development of small molecule inhibitors of the Duffy antigen, the portal of infection of *Plasmodium vivax*, would be a novel and potentially effective approach for treating this form of malaria. (133) Studies of the interaction between Plasmodium vivax and the Duffy antigen provide the clearest example of the potential for basic research on blood groups and malaria to be translated into a vaccine that could have a major impact on global health. (134)

P blood group

P antigen system is a human blood group system based upon genes on chromosome 22. The P blood groups were discovered in 1927 during the course of the same investigations that defined the MN groups. It was at first thought that only one antigen, P, was involved, determined by a gene *P*, the allele *p* being an amorph, and the two genes having each a frequency of about 50 per cent in European populations. Further investigation has disclosed a system of considerable complexity, one feature of which will be described here. The antigen Tja was at first regarded as a product of a gene present in nearly all human beings, extremely rare allele being an amorph, with the homozygous usually having a strong anti-Tja antibody. Later work showed that Tja was part of the P system, with three alleles, *P1* (formerly *P*), *P2* (formerly *p*), and the new *p* (formally regarded as the amorph allele of Tja). These relationships are similar to those existing between *A1, A2,* and *O* of ABO system. P2 bloods sometimes show anti-P1, in the plasma usually with a very low titer, but the rare *p* bloods always have a high titer of anti-P+anti-P1. The P1 antigen is present in hydatid cyst fluid and in a considerable variety of worms, both parasitic and free living. Anti-P1 antibodies are not infrequently found in the plasma. P1 is a member of the neolacto-series glycosphingolipids with α-1,4-linked galactose at the non-reducing end. (135) The blood group P1 synthase gene is identical to the Gb3/CD77 synthase gene. In P2 individuals who produce anti-P1 antibodies, the response is typically due to worm infestation.

Women of genotype *pp*, who always have anti-P and anti-P1 in their plasma, are particularly subject to abortion, apparently resulting from the action of the antibody upon the almost invariably P-positive fetus.

Paroxysmal cold hemoglobinuria is due to the presence in the patient's plasma of a cold reacting autoantibody. It was shown that this usually has anti-P specificity. It is a receptor for the human pathogen Parvovirus B19. (178)

Kell blood group

The Kell blood group (also known as the Kell antigen system or Kell-Cellano system) is determined by a group of antigens on the human red blood cell surface. Kell antigens are targets for autoimmune or alloimmune diseases that destroy red blood cells. The Kell antigens are peptides found within the Kell protein, a 93-kilodalton transmembrane zinc-dependent endopeptidase that is responsible for cleaving endothelin-3. (150,151)

Several alleles exist of the gene that creates Kell protein. Two such alleles, K1 (Kell) and K2 (Cellano), are the most common. The Kell protein is tightly bound to a second protein, XK, by a disulfide bond. Absence of the XK protein (such as through gene deletion) leads to marked reduction of the Kell antigens on the red blood cell surface. Absence of the Kell protein (K0), however, does not affect the XK protein. (152)

Kell glycoprotein is a member of the Neprilysin (M13) sub-family of zinc endopeptidases whose principal function is the activation of bioactive peptides by specific proteolytic cleavage of inactive precursor polypeptides. It preferentially cleaves big endothelin-3, a 41 amino acid polypeptide, at Trp21-Ile22, creating bioactive endothelin-3. A crystal model of the Kell protein based on the crystal structure of the ectodomain of neutral endopeptidase indicates that Kell and NEP use the same homologous amino acids in coordination of zinc and in peptide hydrolysis, but different amino acids in substrate binding. The function of

XK is not yet known but its sequence analysis predicts a membrane transport protein. Its absence is associated with pathological conditions.

Kell antigens are important in transfusion medicine, autoimmune hemolytic anemia, and hemolytic disease of the newborn. Individuals who lack a specific Kell antigen may develop antibodies against Kell antigens when transfused with blood containing that antigen. Subsequent blood transfusions may be marked by destruction of the new cells by these antibodies, a process known as hemolysis. People without Kell antibodies (K0) must be transfused with blood from donors who are also K0 to prevent hemolysis.

Autoimmune hemolytic anemia (AIHA) occurs when the body produces an antibody against a blood group antigen on its own red blood cells. The antibodies lead to destruction of the red blood cells with resulting anemia. Similarly, a pregnant woman may develop antibodies against fetal red blood cells, resulting in destruction, anemia, and hydrops fetalis in a process known as hemolytic disease of the newborn (HDN). Both AIHA and HDN may be severe when caused by anti-Kell antibodies. (153)

1. Greenwell P. Blood group antigens: molecules seeking a function? Glycoconj J. 1997 Feb; 14(2):159-73.
2. Garratty G. Association of blood groups and disease: do blood group antigens and antibodies have a biological role? Hist Philos Life Sci. 1996; 18(3):321-44.
3. Landsteiner K. Zur Kenntnis der antifermentativen, lytischen und agglutinierenden Wirkungen des Blutserums und der Lymphe. Zentralbl Bakteriol. 1900; 27:357-362.
4. Sharon N. Lectins: past, present, and future. Biochem Soc Trans. 2008 Dec; 36(Pt 6):1457-60.
5. Freitas R. Nanomedicine, Volume I: Basic Capabilities, Landes Bioscience, Georgetown, TX, 1999.
6. Greenwalt TJ. A short history of transfusion medicine. Transfusion. 1997; 37(5):550-563.
7. http://www.bioc.aecom.yu.edu/bgmut/abo.htm
8. Sarafian V, Dimova P, Georgiev I, Taskov H. ABH blood group antigen significance as markers of endothelial differentiation of mesenchymal cells. Folia Med (Plovdiv). 1997; 39(2):5-9.
9. Chigira M. Origin of blood-group antigens: a self-declaration mechanism in somatic cell society. Med Hypotheses. 1996 Mar; 46(3):290-4.
10. D'Adamo PJ, Kelly GS. Metabolic and Immunologic Consequences of ABH Secretor and Lewis Subtype Status. Altern Med Rev 2001; 6(4):390-405.
11. Aird L, Bentall HH, Mehigan JA, Roberts JAF. Blood groups in relation to peptic ulceration and carcinoma of colon, rectum, breast, and bronchus. Brit M J. 1954; 2:315-321.
12. Szulman AE. Chemistry, distribution, and function of blood group substances. Ann Rev Med. 1966; 17:307-322.
13. Glynn LE, Holborow EJ, Johnson GD. Distribution of blood group substances in human gastric and duodenal mucosa. Lancet. 1957; 2:1083-1088.
14. Glynn LE, Holborow EJ. Distribution of blood-group substances in human tissue. Brit M Bull. 1959; 15:150-153.
15. Holborow EJ, et al. Distribution of blood group A antigen in human tissues. Brit J Exper Path. 1960; 41:430-437.
16. Szulman AE. Histological distribution of blood group substances A and B in man. J Exper Med. 1960; 111:785-800.
17. Idem E. Histological distribution of blood group substances in man as disclosed by immunofluorescence. 11. H antigen and its relation to A and B antigens. J Exper Med. 1962; 115:977-996.
18. Denborough MA, Downing IJ, McCrea MG. ABO system in milk and saliva. Australasian Ann Med. 1967; 16:320-325.
19. Blann AD, Daly RJ, Amiral J. The influence of age, gender and ABO blood group on soluble endothelial cell markers and adhesion molecules. Br J Haematol. 1996 Dec; 95(3):575-6.
20. Dintenfass L, et al. Blood rheology in patients with depressive and schizoid anxiety. Biorheology. 1976 Feb; 13(1):33-6.
21. Dintenfass L, et al. Dynamic blood coagulation and viscosity and degradation of artificial thrombi in patients with hypertension. Cardiovasc Res. 1970 Jan; 4(1):50-60.
22. Dintenfass L, et al. Effect of stress and anxiety on thrombus formation and blood viscosity factors. Bibl Haematol. 1975; (41):133-9.
23. Dintenfass L, et al. Genetic and ethnic influences on blood viscosity and capillaries in diabetes mellitus. Microvasc Res. 1977 Sep; 14(2):161-72.

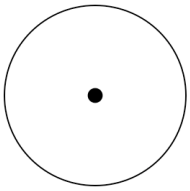
24. Dintenfass L, et al. Effect of fibrinogen on aggregation of red cells and on apparent viscosity of artificial thrombi in hemophilia, myocardial infarction, thyroid disease, cancer and control systems: effect of ABO blood groups. Microvasc Res. 1975 Jan; 9(1):107-18.
25. Dintenfass L, et al. Formation, consistency and degradation of artificial thrombi in severe renal failure. Effect of ABO blood groups. Thromb Diath Haemorrh. 1968 Nov 15; 20(1):267-84.
26. Dintenfass L. Some aspects of hemorrheology of metastasis in malignant melanoma. Haematologia (Budap). 1977; 11(3-4):301-7.
27. Blood groups and The Intestine. Editorial. *Lancet*. 1966 Dec 3; 2(7475):1232-3.
28. Arfors KE, Beckman L, Lundin LG. Genetic Studies of Blood Group-Associated Variations in a Human Serum Alkaline Phosphatase. Acta Genet Statist Med. 1965; 13, 89.
29. Mehta NJ, Rege DV, Kulkarni MB. Total serum alkaline phosphatase (SAP) and serum cholesterol in relation to secretor status and blood groups in myocardial infarction patients. Indian Heart J. 1989 Mar;41(2):82-85
30. Stepan J, Graubaum HJ, Meurer W, Wagenknecht C. Isoenzymes of alkaline phosphatase - reference values in young people and effects of protein diet. Experientia. 1976; 32(7):832-834.
31. Bayer PM, Hotschek H, Knoth E. Intestinal alkaline phosphatase and the ABO blood group system--a new aspect. Clin Chim Acta. 1980 Nov 20; 108(1):81-87.
32. Chaturvedi P, Warren CD, Altaye M, Morrow AL, Ruiz-Palacios G, Pickering LK, Newburg DS. Fucosylated human milk oligosaccharides vary between individuals and over the course of lactation. Glycobiology. 2001 May; 11(5):365-72.
33. Viverge D, Grimmonprez L, Cassanas G, Bardet L, Solere M. Discriminant carbohydrate components of human milk according to donor secretor types. J Pediatr Gastroenterol Nutr. 1990 Oct; 11(3):365-70.
34. Erney R, Hilty M, Pickering L, Ruiz-Palacios G, Prieto P. Human milk oligosaccharides: a novel method provides insight into human genetics. Adv Exp Med Biol. 2001; 501:285-97.
35. Viverge D, Grimmonprez L, Cassanas G, et al. Discriminant carbohydrate components of human milk according to donor secretor types. J Pediatr Gastroenterol Nutr. 1990 Oct; 11(3):365-70.
36. http://chemistry.caltech.edu/~fucose/Research%202.htm
37. D'Adamo, PJ, Zampieron, ER. AB0 bias may signal innate differences in "Natural" immunity. J Naturopath Med. 1991; 2(1): 11 – 16.
38. Sostaric V, Bozicevic D, Brinar V, Grbavac Z. Hereditary antigen characteristics of blood in ischemic cerebrovascular accident. Neurol Croat. 1991; 40(1):3-11.
39. Ionescu DA, Ghitescu M, Marcu I, Xenakis A. Erythrocyte rheology in acute cerebral thrombosis. Effects of ABO blood groups. Blut. 1979 Nov; 39(5):351-7.
40. Ionescu DA, Marcu I, Bicescu E. Cerebral thrombosis, cerebral hemorrhage, and ABO blood groups. Lancet. 1976 Feb 7; 1(7954):278-80.
41. Horby J, Gyrtrup HJ, Grande P, Vestergaard A. Relation of serum lipoproteins and lipids to the ABO blood groups in patients with intermittent claudication. J Cardiovasc Surg (Torino). 1989 Jul; 30(4):533-537.
42. Robinson WM, Roisenberg I. Venous thromboembolism and ABO blood groups in a Brazilian population. Hum Genet. 1980; 55(1):129-131.
43. Havlik RJ, et al. Blood groups and coronary heart disease. Lancet. 1969 Aug 2; 2(7614):269-70.
44. Slipko Z, Latuchowska B, Wojtkowska E. Body structure and ABO and Rh blood groups in patients with advanced coronary heart disease after aortocoronary bypass surgery. Pol Arch Med Wewn. 1994 Jan; 91(1):55-60.
45. Meshalkin EN, Okuneva GN, Vlasov IuA, Vel'tmander NN. ABO and Rh blood groups in cardiovascular pathology. Kardiologiia. 1981 Apr; 21(4):46-50.
46. Erikssen J, Thaulow E, Stormorken H, Brendemoen O, Hellem A. ABO blood groups and coronary heart disease (CHD). A study in subjects with severe and latent CHD. Thromb Haemost 1980 Jun 18; 43(2):137-140.
47. Allan TM. ABO blood groups, age, and work in ischemic heart disease. Atherosclerosis. 1975 May; 21(3):459-461.
48. Galeazzi L, Gualandri V. ABO blood-group phenotypes and pathogenesis of cardiovascular diseases. Congenital, rheumatic, and coronaric heart disease and arterial hypertension. Ital Cardiol. 1975; 5(5):744-751.
49. Rosenberg L, Miller DR, Kaufman DW, Helmrich SP, Van de Carr S, Stolley PD, Shapiro S. Myocardial infarction in women under 50 years of age. JAMA. 1983 Nov 25; 250(20):2801-2806.
50. Platt D, Muhlberg W, Kiehl L, Schmitt-Ruth R. ABO blood group system, age, sex, risk factors and cardiac infarction. Arch Gerontol Geriatr. 1985 Oct; 4(3):241-249.
51. Whincup PH, Cook DG, Phillips AN, Shaper AG. ABO blood group and ischemic heart disease in British men. BMJ. 1990 Jun 30; 300(6741):1679-1682.
52. Jorgensen G. ABO blood groups in physicians more than 75 years old. On the hypothesis concerning "little more fitness of blood group O" MMW Munch Med Wochenschr. 1974 Mar 29; 116(13):649-52.
53. Jorgensen G. Human genetics and infectious diseases. MMW Munch Med Wochenschr. 1981 Sep 25; 123(39):1447-52.
54. D'Adamo P. *The Complete Blood Type Encyclopedia*. GP Putnam and Sons, New York, NY. 2002.
55. Dohi T, Hashiguchi M. Fucosyltransferase producing sialyl Le(a) and sialyl Le(x) carbohydrate antigen in gastrointestinal cancer. Nippon Rinsho. 1995 Jul; 53(7):1781-5.
56. Michail S, Mezoff E, Abernathy F. Role of selectins in the intestinal epithelial migration of eosinophils. Pediatr Res. 2005 Oct; 58(4):644-7.
57. Bode L, Kunz C, Muhly-Reinholz M, Mayer K, Seeger W, Rudloff S. Inhibition of monocyte, lymphocyte, and neutrophil adhesion to endothelial cells by human milk oligosaccharides. Thromb Haemost. 2004 Dec; 92(6):1402-10.

58. Rudloff S, Stefan C, Pohlentz G, Kunz C. Detection of ligands for selectins in the oligosaccharide fraction of human milk. Eur J Nutr. 2002 Apr; 41(2):85-92.
59. D'Adamo PJ. Non-transfusion significance of ABO blood group. In: *Textbook of Natural Medicine*. Pizzorno J. and Murray M. Editors. Churchill Livingstone. 2006.
60. Cohen M, Hurtado-Ziola N, Varki A. ABO blood group glycans modulate sialic acid recognition on erythrocytes. Blood. 2009 Oct 22; 114(17):3668-76.
61. N Bottini, F Ronchetti, Fulvia Gloria-Bottini, L Stefanini, Egidio Bottini, N Lucarini. Atopic and nonatopic asthma in children. J Asthma. 2005 Feb; 42:25-8.
62. Martin NG, Rowell DM, Whitfield JB. MN and Jk systems influence environmental variability in serum lipid levels. Clin Genet. 1983 Jul; 24(1):1-14.
63. Gleiberman L, Gershowitz H, Harburg E, Schork MA. Blood pressure and blood group markers. Association with the MN locus. J Hypertens. 1984 Aug; 2(4):337-41.
64. Reid ME. MNS blood group system: a review. Immunohematology. 2009; 25(3):95-101.
65. Sharon R, Weinberg H, Husseini N. An unusually high incidence of homozygous MM in ankylosing spondylitis. J Bone Joint Surg Br. 1985 Jan; 67(1):122-3.
66. Howard DR. Expression of T-antigen on polyagglutinable erythrocytes and carcinoma cells: preparation of T-activated erythrocytes, anti-T lectin, anti-T absorbed human serum and purified anti-T antibody. Vox Sang. 1979; 37(2):107-10.
67. Wagner FF, Frohmajer A, Ladewig B, Eicher NI, Lonicer CB, Müller TH, Siegel MH, Flegel WA. Weak D alleles express distinct phenotypes. Blood. 2000 Apr 15; 95(8):2699-708.
68. Singleton BK, Green CA, Avent ND, Martin PG, Smart E, Daka A, Narter-Olaga EG, Hawthorne LM, Daniels G. The presence of an RHD pseudogene containing a 37 base pair duplication and a nonsense mutation in Africans with the Rh D-negative blood group phenotype. Blood. 2000 Jan 1; 95(1):12-8.
69. Gloria-Bottini F, Antonacci E, Bottini N, Ogana A, Borgiani P, De Santis G, Lucarini N. RH blood groups and diabetic disorders: is there an effect on glycosylated hemoglobin level? Hum Biol. 2000 Apr; 72(2):287-94.
70. Huang CH, Ye M. The Rh protein family: gene evolution, membrane biology, and disease association. Cell Mol Life Sci. 2010 Apr; 67(8):1203-18.
71. Matalliotakis I, Cakmak H, Goumenou A, Sifakis S, Ziogos E, Arici A. ABO and Rh blood groups distribution in patients with endometriosis. Arch Gynecol Obstet. 2009 Dec; 280(6):917-9.
72. Le Pendu J, Marionneau S, Cailleau-Thomas A, Rocher J, Le Moullac-Vaidye B, Clément M. ABH and Lewis histo-blood group antigens in cancer. APMIS. 2001 Jan; 109(1):9-31.
73. Ben Q, Wang K, Yuan Y, Li Z. Pancreatic Cancer Incidence and Outcome in Relation to ABO Blood Groups among Han Chinese Patients: A Case-control Study. Int J Cancer. 2010 Apr 27.
74. Risch HA, Yu H, Lu L, Kidd MS. ABO blood group, Helicobacter pylori seropositivity, and risk of pancreatic cancer: a case-control study. J Natl Cancer Inst. 2010 Apr 7; 102(7):502-5.
75. Amundadottir L, Kraft P, Stolzenberg-Solomon RZ, Fuchs CS, Petersen GM, Arslan AA, Bueno-de-Mesquita HB, Gross M, Helzlsouer K, Jacobs EJ, LaCroix A, Zheng W, Albanes D, Bamlet W, Berg CD, Berrino F, Bingham S, Buring JE, Bracci PM, Canzian F, Clavel-Chapelon F, Clipp S, Cotterchio M, de Andrade M, Duell EJ, Fox JW Jr, Gallinger S, Gaziano JM, Giovannucci EL, Goggins M, González CA, Hallmans G, Hankinson SE, Hassan M, Holly EA, Hunter DJ, Hutchinson A, Jackson R, Jacobs KB, Jenab M, Kaaks R, Klein AP, Kooperberg C, Kurtz RC, Li D, Lynch SM, Mandelson M, McWilliams RR, Mendelsohn JB, Michaud DS, Olson SH, Overvad K, Patel AV, Peeters PH, Rajkovic A, Riboli E, Risch HA, Shu XO, Thomas G, Tobias GS, Trichopoulos D, Van Den Eeden SK, Virtamo J, Wactawski-Wende J, Wolpin BM, Yu H, Yu K, Zeleniuch-Jacquotte A, Chanock SJ, Hartge P, Hoover RN. Genome-wide association study identifies variants in the ABO locus associated with susceptibility to pancreatic cancer. Nat Genet. 2009 Sep; 41(9):986-90.
76. Dickey W, Wylie JD, Collins JS, et al. Lewis phenotype, secretor status, and coeliac disease. Gut. 1994; 35:769-770.
77. Heneghan MA, Kearns M, Goulding J, et al. Secretor status and human leucocyte antigens in coeliac disease. Scand J Gastroenterol. 1996; 31:973-976.
78. Costantini M, Fassio T, Canobbio L, et al. Role of blood groups as prognostic factors in primary breast cancer. Oncology. 1990; 47(4):308-12.
79. Skolnick MH, Thompson EA, Bishop DT, Cannon LA. Possible linkage of a breast cancer-susceptibility locus to the ABO locus: sensitivity of LOD scores to a single new recombinant observation. Genet Epidemiol. 1984; 1(4):363-73.
80. Marinaccio M, Traversa A, Carioggia E, et al. Blood groups of the ABO system and survival rate in gynecologic tumors. Minerva Ginecol. 1995 Mar; 47(3):69-76.
81. Bjarnason O, Tulinius H. Tumors in Iceland. 9. Malignant tumors of the ovary. A histological classification, epidemiological considerations, and survival. Acta Pathol Microbiol Immunol Scand [A]. 1987 Jul; 95(4):185-192.
82. Nayak SK. ABO blood groups in different diseases. J Ind Med. 1971 Dec 16; 57(12):449-52.
83. Rybalka AN, Andreeva PV, Tikhonenko LF, Koval'chuk NA. ABO system blood groups and the rhesus factor in tumors and tumorlike processes of the ovaries. Vopr Onkol. 1979; 25(3):28-30. [Article in Russian]
84. Mori M, Kiyosawa H, Miyake H. Case-control study of ovarian cancer in Japan. Cancer. 1984 Jun 15; 53(12):2746-52.
85. Langer M, Gitsch E, Salzer H. Significance of ovulatory age in the pathogenesis of ovarian cancer. Geburtshilfe Frauenheilkd. 1984 Jun; 44(6):366-7.
86. Kaur I, Singh IP, Bhasin MK. Blood groups in relation to carcinoma of cervix uteri. Hum Hered. 1992; 42(5):324-6.
87. Llopis B, Ruiz JL, Server G, et al. ABO blood groups and bladder carcinoma. Eur Urol. 1990; 17(4):289-92.

88. Srinivas V, Khan SA, Hoisington S, Varma A, Gonder MJ. Relationship of blood groups and bladder cancer. J Urol. 1986 Jan; 135(1):50-2.
89. Orihuela E, Shahon RS. Influence of blood group type on the natural history of superficial bladder cancer. J Urol. 1987 Oct; 138(4):758-9.
90. Raitanen MP, Tammela TL. Relationship between blood groups and tumor grade, number, size, stage, recurrence and survival in patients with transitional cell carcinoma of the bladder. Scand J Urol Nephrol. 1993; 27(3):343-7.
91. Similar to most cancers, bladder cancer is characterized by a disappearance of normal ABO antigen expression and an appearance of specialized adherence molecules.
92. Orlow I, Lacombe L, Pellicer I, et al. Genotypic and phenotypic characterization of the histoblood group ABO(H) in primary bladder tumors. Int J Cancer. 1998 Mar 16; 75(6):819-24.
93. Orntoft TF, Wolf H. Molecular alterations in bladder cancer. Urol Res. 1998; 26(4):223-33.
94. Nayak SK. ABO blood groups in different diseases. J Ind Med. 19971; 87:449-52.
95. Klechova L, Gosheva-Antonova Ts. ABO and Rh blood group factors in thyroid gland diseases. Vutr Boles. 1980; 19(4):75-9. [Article in Bulgarian]
96. Csato E, Vass J. Frequency of non-secretor types among stomach cancer patients. Haematologia (Budap). 1986; 19(2):147-50.
97. Beckman L, Angqvist KA. On the mechanism behind the association between ABO blood groups and gastric carcinoma. Hum Hered. 1987; 37(3):140-3.
98. Kurtenkov O, Klaamas K, Miljukhina L. The lower level of natural anti-Thomsen-Friedenreich antigen (TFA) agglutinins in sera of patients with gastric cancer related to ABO(H) blood-group phenotype. Int J Cancer. 1995 Mar 16; 60(6):781-5.
99. Palli D, Caporaso NE, Shiao YH, et al. Diet, Helicobacter pylori, and p53 mutations in gastric cancer: a molecular epidemiology study in Italy. Cancer Epidemiol Biomarkers Prev. 1997 Dec; 6(12):1065-1069.
100. Falling into the category of the slightly mysterious, being a non-secretor is associated with a slight decrease in the prevalence of stomach cancer.
101. Csato E, Vass J. Frequency of non-secretor types among stomach cancer patients. Haematologia (Budap). 1986; 19(2):147-50.
102. Zampieri P, Meneghini F, Grigoletto F, et al. Risk factors for cerebral glioma in adults: a case-control study in an Italian population. J Neurooncol. 1994; 19(1):61-7.
103. Turowski K, Czochra M. ABO blood groups in glioblastoma multiforme. Neurol Neurochir Pol. 1979 Mar-Apr; 13(2):173-6. [Article in Polish]
104. Trouillas P, Menaud G, De The G, et al. Epidemiological study of primary tumors of the neuraxis in the Rhone-Alps region. Quantitative data on the etiology and geographical distribution of 1670 tumors. Rev Neurol (Paris). 1975 Oct; 131(10):691-708. [Article in French]
105. Romodanov SA, Gnedkova IA, Lisianyi NI, Glavatskii AIa. Efficacy of chemotherapy and immunochemotherapy in neuro-oncologic patients of various blood groups. Zh Vopr Neirokhir. 1989 Jan-Feb; (1):17-20. [Article in Russian]
106. Arseni C, Danaila L, Constantinescu AI. Considerations on the correlation between the blood groups and the incidence of cerebral tumors. Rev Roum Neurol. 1972; 9(3):135-8.
107. Arseni C, Danaila L, Constantinescu A. Correlations between the blood groups and the incidence of congenital neurosurgical diseases. Rev Roum Neurol. 1971; 8(5):335-8.
108. Pearce KM, Yates PO. Blood groups and brain tumors. J Neurol Sci 1965 Sep-Oct; 2(5):434-41.
109. Alcantara A, Kinzel W, Bube FW, Uhlenbruck G. Blood groups and brain tumors. Zentralbl Neurochir. 1965; 26(2):156-65. [Article in German]
110. Vidas I, Delajlija M, Temmer-Vuksan B, et al. Examining the secretor status in the saliva of patients with oral pre-cancerous lesions. J Oral Rehabil. 1999 Feb; 26(2):177-82.
111. Torrado J, Ruiz B, Garay J, et al. Blood-group phenotypes, sulfomucins, and Helicobacter pylori in Barrett's esophagus. Am J Surg Pathol. 1997 Sep; 21(9):1023-9.
112. Pyd M, Rzewnicki I, Suwayach U. ABO blood groups in patients with laryngeal and hypopharyngeal cancer. Otolaryngol Pol. 1995; 49 Suppl 20:396-8.
113. Yokota M, Ito N, Hatake K, et al. Aberrant glycosylation based on the neo-expression of poly-N-acetyllactosamine structures in squamous cell carcinomas of the head and neck. Histochem J. 1997 Jul; 29(7):555-62.
114. Xie X, Boysen M, Clausen OP, Bryne MA. Prognostic value of Le(y) and H antigens in oral tongue carcinomas. Laryngoscope. 1999 Sep; 109(9):1474-80.
115. Halvorsen TB. ABO blood groups, rhesus types, and colorectal adenocarcinoma. A retrospective study of 747 cases. Scand J Gastroenterol. 1986 Oct; 21(8):979-83.
116. Itzkowitz SH. Blood group-related carbohydrate antigen expression in malignant and premalignant colonic neoplasms. J Cell Biochem Suppl. 1992; 16G:97-101.
117. Xiros N, Northoff H, Anger B, Heit W, Heimpel H. Blood group change in a patient with blastic transformation of a myelodysplastic syndrome. Blut. 1987 May; 54(5):275-80.
118. Atkinson JB, Tanley PC, Wallas CH. Loss of blood group A in acute leukemia. Morphologic and biochemical studies of red cells. Transfusion. 1987 Jan-Feb; 27(1):45-8.
119. Uchikawa M. Alterations of ABH antigens in leukemic patients. Nippon Rinsho. 1997 Sep; 55(9):2369-73.
120. Hafner V, Coatmelec M, Niculescu R. Temporary changes and permanent changes in the erythrocyte blood-group antigens in malignant hemopathies. Rom J Intern Med. 1996 Jul-Dec; 34(3-4):183-8.
121. Karakousis CP, Evlogimenos E, Suh O. Blood groups and malignant melanoma. J Surg Oncol. 1986 Sep; 33(1):24-6.

122. Janardhana V, Propert DN, Green RE. ABO blood groups in hematologic malignancies. Cancer Genet Cytogenet. 1991 Jan; 51(1):113-20.
123. Nayak SK. ABO blood groups in different diseases. J Ind Med. 19971; 87:449-52.
124. Jackson N, Menon BS, Zarina W, Zawawi N, Naing NN. Why is acute leukemia more common in males? A possible sex-determined risk linked to the ABO blood group genes. Ann Hematol. 1999 May; 78(5):233-6.
125. Ronco AL, Stoll M, De Stéfani E, Maisonneuve JE, Mendoza BA, Deneo-Pellegrini H. Rh factor, family history, and risk of breast cancer: a case-control study in Uruguay. Cancer Detect Prev. 2009; 32(4):277-85.
126. Pross HF, Baines MG. Studies of human natural killer cells. I. In vivo parameters affecting normal cytotoxic function. Int J Cancer. 1982 Apr 15; 29(4):383-90.
127. Lasek W, Jakobisiak M, Plodziszewska M, Gorecki D. The influence of ABO blood groups, Rh antigens, and cigarette smoking on the level of NK activity in normal population. Arch Immunol Ther Exp (Warsz). 1989; 37(3-4):287-94.
128. Hersey P, Edwards A, Trilivas C, et al. Relationship of natural killer-cell activity to rhesus antigens in man. Br J Cancer. 1979 Mar; 39(3):234-40.
129. Marini AM, Matassi G, Raynal V, Andre B, Cartron JP, Cherif-Zahar B. The human Rhesus-associated RhAG protein and a kidney homologue promote ammonium transport in yeast. Nat Genet. 2000 Nov; 26(3):341-4.
130. Rot A. Contribution of Duffy antigen to chemokine function. Cytokine Growth Factor Rev. 2005 Dec; 16(6):687-94.
131. Gardner L, Patterson AM, Ashton BA, Stone MA, Middleton J. The human Duffy antigen binds selected inflammatory but not homeostatic chemokines. Biochem Biophys Res Commun. 2004 Aug 20; 321(2):306-12.
132. Shen H, Schuster R, Stringer KF, Waltz SE, Lentsch AB. The Duffy antigen/receptor for chemokines (DARC) regulates prostate tumor growth. FASEB J. 2006 Jan; 20(1):59-64.
133. Mourant, AE. *Blood Relations, Blood Groups, and Anthropology*. Oxford University Press, Oxford, UK 1983.
134. Handel TM, Horuk R. Duffy antigen inhibitors: useful therapeutics for malaria? Trends Parasitol. 2010 Apr 9.
135. Rowe JA, Opi DH, Williams TN. Blood groups and malaria: fresh insights into pathogenesis and identification of targets for intervention. Curr Opin Hematol. 2009 Nov; 16(6):480-7.
136. Iwamura K, Furukawa K, Uchikawa M, Sojka BN, Kojima Y, Wiels J, Shiku H, Urano T, Furukawa K. The blood group P1 synthase gene is identical to the Gb3/CD77 synthase gene. A clue to the solution of the P1/P2/p puzzle. J Biol Chem. 2003 Nov 7; 278(45):44429-38.
137. Chen DP, Tseng CP, Wang WT, Sun CF. Identification of a novel A2 allele derived from the A transferase gene through a nucleotide substitution G539C.Vox Sang. 2005 Apr; 88(3):196-9.
138. Yip SP. Sequence variation at the human ABO locus. Ann Hum Genet. 2002 Jan; 66(Pt 1):1-27.
139. Landsteiner K, Levine P. Proc Soc Exp Biol & Med. 1927; 24, 941-942.
140. Boyd WC. *Genetics and the Races of Man*, Little Brown and Company, Boston (1950).
141. Grundbacher FJ. Genetic aspects of selective immunoglobulin A deficiency. J Med Genet. 1972 Sep; 9(3):344-7.
142. Grundbacher FJ. Immunoglobulins, secretor status, and the incidence of rheumatic fever and rheumatic heart disease. Hum Hered. 1972; 22(4):399-404.
143. Tandon OP, Bhatia S, Tripathi RL, Sharma KN. Phagocytic response of leucocytes in secretors and non-secretors of ABH (O) blood group substances. Indian J Physiol Pharmacol. 1979 Oct-Dec; 23(4):321-4.
144. Dube VE, Tanaka M, Chmiel J, Anderson B. Effect of ABO group, secretor status, and sex on cold hemagglutinins in normal adults. Vox Sang 1984; 46(2):75-9.
145. Blackwell CC, Weir DM, Patrick AW, Collier A, Clarke BF. Secretor state and complement levels (C3 and C4) in insulin dependent diabetes mellitus. Diabetes Res 1988 Nov; 9(3):117-9.
146. Lauritsen JG, Grunnet N, Jensen OM. Materno-fetal ABO incompatibility as a cause of spontaneous abortion. Clin Genet 1975 Apr; 7(4):308-16.
147. Solish GI. Distribution of ABO isohemagglutinins among fertile and infertile women. J Reprod Fertil. 1969 Apr; 18(3):459-74.
148. Cantuaria AA. Blood group incompatibility and cervical hostility in relation to sterility. Obstet Gynecol 1978 Feb; 51(2):193-7.
149. Kulkarni AG, Ibazebe R, Fleming AF High frequency of anti-A and anti-B hemolysins in certain ethnic groups of Nigeria. Vox Sang 1985; 48(1):39-41.
150. D'Adamo PJ. ABO Bias in Natural Immunity. J. Naturopath. Med. 1991; 2:11-17.
151. Lee S, Wu X, Reid M, Zelinski T, Redman C. Molecular basis of the Kell (K1) phenotype. Blood. 1995 Feb 15; 85(4):912-6.
152. Lee S, Lin M, Mele A, Cao Y, Farmar J, Russo D, Redman C. Proteolytic processing of big endothelin-3 by the Kell blood group protein. Blood. 1999 Aug 15; 94(4):1440-50.
153. Yu LC, Twu YC, Chang CY, Lin M. Molecular basis of the Kell-null phenotype: a mutation at the splice site of human KEL gene abolishes the expression of Kell blood group antigens. J Biol Chem. 2001 Mar 30; 276(13):10247-52.
154. Wiener CP, Widness JA. Decreased fetal erythropoiesis and hemolysis in Kell hemolytic anemia. American Journal of Obstetrics and Gynecology. 1996; 174:547-55.
155. Osborne RH, Frances V, De George FV. The ABO Blood Groups in Neoplastic Disease of the Ovary. Am J Hum Genet. 1963 December; 15(4): 380–388.
156. Gates MA, Wolpin BM, Cramer DW, Hankinson SE, Tworoger SS. ABO blood group and incidence of epithelial ovarian cancer. Int J Cancer. 2010 Mar 22.
157. Marinaccio M, Traversa A, Carioggia E, Valentino L, Coviello M, Salamanna S, Dragone DC, Marinaccio L. Blood groups of the ABO system and survival rate in gynecologic tumors. Minerva Ginecol. 1995 Mar; 47(3):69-76.
158. Henderson J, Seagroatt V, Goldacre M. Ovarian cancer and ABO blood groups. J Epidemiol Community Health. 1993 Aug; 47(4):287-9.

159. Bals R, Woeckel W, Welsch U. Use of antibodies directed against blood group substances and lectins together with glycosidase digestion to study the composition and cellular distribution of glycoproteins in the large human airways. J Anat 1997 Jan; 190 (Pt 1):73-84.
160. Shinebaum R. ABO blood group and secretor status in the spondyloarthropathies. FEMS Microbiol Immunol. 1989 Jun; 1(6-7):389-95.
161. Shinebaum R, Blackwell CC, Forster PJ, et al. Non-secretion of ABO blood group antigens as a host susceptibility factor in the spondyloarthropathies. Br Med J (Clin Res Ed). 1987 Jan 24; 294(6566):208-10.
162. Manthorpe R, Staub Nielsen L, et al. Lewis blood type frequency in patients with primary Sjogren's syndrome. A prospective study including analyses for A1A2BO, Secretor, MNSs, P, Duffy, Kell, Lutheran, and rhesus blood groups. Scand J Rheumatol 1985; 14(2):159-62.
163. Toft AD, Blackwell CC, Saadi AT, et al. Secretor status and infection in patients with Graves" disease. Autoimmunity 1990; 7(4):279-89.
164. Blackwell CC, Aly FZ, James VS, et al. Blood group, secretor status and oral carriage of yeasts among patients with diabetes mellitus. Diabetes Res 1989 Nov; 12(3):101-4.
165. Thom SM, Blackwell CC, MacCallum CJ, et al. Non-secretion of blood group antigens and susceptibility to infection by Candida species. FEMS Microbiol Immunol 1989 Jun; 1(6-7):401-5.
166. Lamey PJ, Darwazeh AM, Muirhead J, et al. Chronic hyperplastic candidosis and secretor status. J Oral Pathol Med 1991 Feb; 20(2):64-7.
167. Chaim W, Foxman B, Sobel JD. Association of recurrent vaginal candidiasis and secretory ABO and Lewis phenotype. J Infect Dis 1997 Sep; 176(3):828-30.
168. Burford-Mason AP, Weber JC, Willoughby JM. Oral carriage of Candida albicans, ABO blood group and secretor status in healthy subjects. J Med Vet Mycol 1988 Feb; 26(1):49-56.
169. 168 Thom SM, Blackwell CC, MacCallum CJ, et al. Non-secretion of blood group antigens and susceptibility to infection by Candida species. FEMS Microbiol Immunol 1989 Jun; 1(6-7):401-5.
170. Al-Agidi SK, Shukri SM. Association between immunoglobulin levels and known genetic markers in an Iraqi population. Ann Hum Biol 1982 Nov-Dec; 9(6):565-9.
171. Shinebaum R. ABO blood group and secretor status in the spondyloarthropathies. FEMS Microbiol Immunol 1989 Jun; 1(6-7):389-95.
172. Grundbacher FJ. Immunoglobulins, secretor status, and the incidence of rheumatic fever and rheumatic heart disease. Hum Hered. 1972; 22(4):399-404.
173. Grundbacher FJ. Genetic aspects of selective immunoglobulin A deficiency. J Med Genet. 1972 Sep; 9(3):344-7.
174. Dube VE, Tanaka M, Chmiel J, Anderson B. Effect of ABO group, secretor status and sex on cold hemagglutinins in normal adults. Vox Sang 1984; 46(2):75-9.
175. Blackwell CC, Weir DM, Patrick AW, Collier A, Clarke BF. Secretor state and complement levels (C3 and C4) in insulin dependent diabetes mellitus. Diabetes Res 1988 Nov; 9(3):117-9.
176. Tandon OP, Bhatia S, Tripathi RL, Sharma KN. Phagocytic response of leucocytes in secretors and non-secretors of ABH (O) blood group substances. Indian J Physiol Pharmacol 1979 Oct-Dec; 23(4):321-4.
177. Potapenko IO, Haakensen VD, Lüders T, Helland A, Bukholm I, Sørlie T, Kristensen VN, Lingjaerde OC, Børresen-Dale AL. Glycan gene expression signatures in normal and malignant breast tissue; possible role in diagnosis and progression. Mol Oncol. 2010 Apr; 4(2):98-118.
178. McGovern DP, Jones MR, Taylor KD, Marciante K, Yan X, Dubinsky M, Ippoliti A, Vasiliauskas E, Berel D, Derkowski C, Dutridge D; International IBD Genetics Consortium, Fleshner P, Shih DQ, Melmed G, Mengesha E, King L, Pressman S, Haritunians T, Guo X, Targan SR, Rotter JI. Fucosyltransferase 2(FUT2) Non-Secretor Status is associated with Crohn's Disease. Hum Mol Genet. 2010 Jun 22.
179. Brown K, et al. Resistance to Parvovirus B19 Infection Due to Lack of Virus Receptor (Erythrocyte P Antigen) N Engl J Med. 1994 Apr 28; 330(17):1192-6.



Lectins

"Out of association grows adhesion, and out of adhesion amalgamation."

—Charlotte Bronte

Lectins, a class of sugar-binding and cell-agglutinating proteins, are ubiquitous in Nature, being found in all kinds of organisms, from viruses, to foodstuffs, to humans.

Lectins can have variety of biologic effects, including: induction of mitosis in lymphocytes, cellular agglutination via cross-linking of membrane sugars, preferential agglutination of malignant cells, precipitation of polysaccharides and glycoproteins and activation of complement. The binding of lectins to sugar is quite weak. It does not form a covalent bond, but is reversible, like enzyme-substrate or antigen-antibody reactions. When a lectin contains multiple binding sites, they can interconnect large numbers of cells, causing them to clump together or agglutinate. Each molecule of a lectin has two or more regions, perhaps clefts or grooves, each of which fits a complementary molecule of a sugar or several sugar units of an oligosaccharide. It is by means of these combining sites that the lectin attaches itself to the sugars on cell surfaces.

It is noteworthy that almost all saccharides recognized by lectins are typical constituents of animal cell surfaces. Broadly speaking, lectins can be classified into two classes: those that bind monosaccharides and oligosaccharides and those than only bind oligosaccharides. Because of their diversity, classifying lectins into families is a challenge. Lectin classification is still evolving, and general agreement has not been achieved yet.

Lectin-sugar reactions actually share many factors in common with antigen-antibody reactions, especially precipitation, which has prompted several investigators to suggest that lectins are "plant antibodies." They also may have a role as pattern-recognition receptors within the plant's innate immune system. Some of these lectins have been found to be toxic to insects, thus raising the possibility that they may serve as a deterrent to these pests and other pathogens. The fact that lectins are found in bacteria and plants as well as animals suggests that they appeared early in evolution.

Lectin property	Application
Specificity for human blood groups	Blood typing; structural studies of blood group substances; identification of new blood groups; diagnosis of secretors.
Toxicity in animals and humans	Studies of nutritional value of foodstuffs
Induction of mitosis in lymphocytes	Studies of chromosomal constitution of cells.
Agglutination of malignant cells	Investigation of architecture of cell surfaces.
Precipitation of polysaccharides and glycoproteins	Isolation, purification and structural studies of carbohydrate-containing polymers
Binding of sugars	Studies of specific combining sites on proteins

Figure 6.4.1 Properties and uses of lectins.

Lectin family	Typical saccharide ligands	Subcellular location
Calnexin	Glc_1Man_9	ER
M-type lectins	Man8	ER
L-type lectins	Various	ER, ERGIC, Golgi
P-type lectins	Man 6-phosphate, others	Secretory pathway
C-type lectins	Various	Cell membrane, extracellular
Galectins	β -Galactosides	Cytoplasm, extracellular
I-type lectins (Siglecs)	Sialic acid	Cell membrane
R-type lectins	Various	Golgi, Cell membrane
F-box lectins	$GlcNAc_2$	Cytoplasm
Ficolins	GlcNAc, GalNAc	Cell membrane, extracellular
Chitinase-like lectins	Chito-oligosaccharides	Extracellular
F-type lectins	Fuc-terminating oligosaccharides	Extracellular
Intelectins	Gal, galactofuranose, pentoses	Extracellular/cell membrane

Figure 6.4.2 Summary of lectin families

R-TYPE AND MANNOSE-SPECIFIC LECTINS

Peter Hermann Stillmark (1860–1923) at the University of Dorpat in Estonia first identified lectins in 1888. While investigating the toxic effects on blood of castor bean extract (*Ricinus communis*) he noticed that the red cells were being agglutinated. He isolated the material responsible for the agglutination and called it ricin. (1) Shortly, it was discovered that the toxic extract of the seed *Abrus precatorius* also caused cells to clump together. This new agglutinin was called abrin. This immediately caught the attention of the German bacteriologist Paul Ehrlich (1854-1915) who recognized that he could investigate certain immunologic problems with them rather than the then popular bacterial toxins. With these two agglutinins, some of the most basic principles of immunology were discovered, such as antibody specificity and species specificity.

The R-type CRD is the only sugar-binding protein module from animal lectins that is also present in prokaryotes. The R-type CRD is named after the castor bean protein ricin, which is a member of a group of toxic soluble plant lectins.

The R-type lectins are members of a superfamily of proteins, all of which contain a carbohydrate-recognition domain (CRD) that is structurally similar to the CRD in ricin. Ricin was the first lectin discovered and it is the prototypical lectin in this category. R-type lectins are present in plants, animals, and bacteria; and the plant lectins often contain a separate subunit that is a potent toxin.

Type-2 RIP lectins

These lectins also have R-type domains, belong to the RIP-II class, and kill cells in a manner similar to ricin

Ricin

Because of its incredible potency as a toxin and its ease of production, ricin has been in the news over the years as a possible lethal agent. The lethal dose (LD50) of ricin may be as low as 3–5 μg/kg per kilogram body weight, depending on the mode of exposure. Two different lectins have been purified from *R. communis* seeds, and in the original nomenclature, they were termed RCA-I and RCA-II. RCA-I is an agglutinin but a very weak toxin. RCA-II is commonly called ricin, and it is both an agglutinin and a very potent toxin. Ricin belongs to the group of type 2 ribosome-inactivating proteins (type 2 RIPs), which are distinguished from type 1 RIPs by the presence of the B chain.

Ricin consists of a Gal/GalNAc-binding subunit, B chain (32 kDa), and a toxic subunit, A chain (ca. 30 kDa), connected through a disulfide linkage. The A chain of ricin has RNA N-glycosidase activity to cleave a specific adenine base from ribosomal RNA, causing the inactivation of the ribosome and inhibition of protein synthesis. The lectin subunit, B chain, of ricin plays an important role of binding to the cell surface glycoconjugates of target cells and facilitates the internalization and translocation of the toxin to cytosol. Ricin binds to β-linked galactose and N-acetylgalactosamine, whereas RCA-I prefers β-linked galactose. The R-type domain is an ancient type of protein fold that is found in many glycosyltransferases as well as in bacterial and fungal hydrolases. (71,72)

Ricin has attracted considerable interest as an antitumor agent since the protein has been shown to be more toxic to transformed cells than to normal cells. (88) Ricin has been used for the construction of immunotoxins and its a chain conjugates have been used with some success in the treatment of a variety of T-cell leukemia and lymphomas. (89)

Abrin (Abrus precatorius, Abrus precatorius agglutinin, APA)

Seeds of *Abrus precatorius* contain two distinct but structurally related Type 2 RIP class lectins: abrin, an extremely potent toxin but a weak agglutinin, and *Abrus precatorius* Agglutinin (APA), a potent agglutinin with relatively low toxicity. APA strongly agglutinates human type A, B and O erythrocytes. (90) APA is a potent mitogen to thymus-derived lymphocytes. (91) Abrin has anticarcinogenic activity and since the toxicity of abrin is higher for certain tumor cells than for normal cells, abrin has been used to prepare immunotoxins. (92, 93)

There are other R-type plant lectins in the RIP-II class that are not toxic, and these include several lectins from several *Sambucus* species (but in particular *Sambucus nigra*, elderberry), such as nigrin-b, sieboldin-b, ebulin-f, and ebulin-r. SNA is unusual in that it is the only R-type lectins that bind well to α2–6-linked sialic acid–containing ligands and does not bind to α2–3-linked sialylated ligands.

Viscum album agglutinin (VAA)

Mistletoe is one of the few plants that contain lectins belonging to different lectin classes. As well as a complex mixture of Type 2 RIP lectins (ML-1, ML-2, ML-3) a small chitin binding lectin has also been identified (VisAlbCBA). Type 2 Viscum lectins kill cells in a manner similar to ricin. Mistletoe lectins are present in all mistletoe extracts in various concentrations.

With regard to antigenicity and chemical structure, there are three similar lectins in mistletoe plants. The most important and most often investigated lectin in mistletoe extracts is the galactoside-specific VAA-I. As shown in Fig. 1, it consists of a cytotoxic A-chain with a molecular weight of 29 kDa and a carbohydrate-binding B-chain of 34 kDa that is responsible for its immunomodulatory efficacy. (73)

The biological efficacy of mistletoe lectin can be regarded as directly cytostatic as well as having an immunomodulatory effect. In cultures of human peripheral mononuclear cells (PBMCs), VAA-I can stimulate cytokine production as well as apoptosis in approximately the same concentration as in vivo. (74) Recently, it was found that VAA-I is a potent inducer of human neutrophil apoptosis via caspase-3 activation. (75) VAA-I alters the mitochondrial transmembrane potential and increases intracellular levels of reactive oxygen species (ROS). Furthermore, the decrease of the expression of the antiapoptotic Mcl-1 and the degradation of cytoskeletal paxillin and vimentin proteins in VAA-I-induced neutrophil apoptosis have been described. (76)

In a 24-hour culture of peripheral blood mononuclear cells low and non-toxic lectin concentrations (with an optimum between 1 and 10 ng/ml) stimulate the release of pro-inflammatory cytokines such as interleukin (IL)-1, IL-6 and tumor necrosis factor (TNF)-alpha dose dependently. D-Galactose, a monosaccharide with the highest affinity for VAA-I, blocks TNF-α release competitively. In the case of cancer

patients, subcutaneous injections of mistletoe preparations with a lectin dose of 1 ng VAA-I/kg twice a week led to an elevation of cytotoxic activity and frequency of peripheral NK cells (CD3–/CD16+56+). (77)

Sambucus nigra agglutinins (SNA, elderberry)

Sambucus lectins belong to a distinct group of naturally occurring sialic-acid-binding lectins, including WGA, the selectins, most arthropod lectins, Cholera toxin (*Vibrio cholerae*), tetanus toxin (*Clostridium tetani*), botulinum toxin (*Clostridium botulinum*), pertussis toxin (*Bordetella pertussis*) Influenza A and B viruses hemagglutinins, polyomaviruses, rotaviruses and hemagglutinin neuraminidases and the Siglecs (sialic acid–binding, immunoglobulin-like lectins).

Elderberry contains lectins (SNA-I, SNA-II, SNA-III, SNA-L, SNA-V, SNA-I', SNA-IV, SNA-Vf, SNA-If) in almost all of its tissues. Bark, leaves, fruits and seeds express several lectins that are either Type 2 RIP lectins, lectins derived from Type 2 RIP precursors, or lectins encoded by truncated Type 2 RIP genes. Sambucus nigra lectins do not influence the frequency of apoptosis in the culture of human cells. (99) Embryonic thymic tissue experience changes in the glycosylation status of many cell surface molecules changes during the thymocyte maturation and selection processes. The immature cortical thymocytes were labeled by peanut agglutinin PNA, whereas medullary thymocytes were positive for SNA binding. (100) *Sambucus* lectin triggered basophils to release IL-4 at concentrations of up to 1ng/10(6) basophils. Lectins with high IL-4-inducing capacity also stimulated the release of IL-13 and histamine. (126)

SNA-I is found in the bark and has a slight preference for type A over type B and type O erythrocytes. SNA-I inhibits protein synthesis in a rabbit reticulocyte lysate and has RNA N-glycosidase activity. (94) SNA-I is slightly toxic for rats and inhibits the growth and development of some insects. (95) SNA-I is used for the isolation and fractionation of sialylated oligosaccharides and glycoconjugates. Lectin levels are much higher in the winter than in summer. (35)

SNA-I' is a variant of SNA-I, also found in the bark, lacking the extra cysteine on its B chain. It also agglutinates animal and human erythrocytes, inhibits protein synthesis and has RNA N-glycosidase activity. (97)

SNA-V is a non-toxic Type 2 RIP from elderberry bark. SNA-V agglutinates rabbit and human erythrocytes. The lectin has a slight preference for type B over type A and type O erythrocytes. SNA-V inhibits protein synthesis in animal cell-free systems and has RNA N-glycosidase activity. SNA-V is not toxic to mice. (97)

SNA-II agglutinates animal and human erythrocytes. The lectin has a slight preference for type B over type A and type O erythrocytes. (96) SNA-II does not inhibit cell-free protein synthesis and has no RNA N-glycosidase activity. Although in nutritional testing with rats the SNA-II preparation used had significant antinutrient effects, this lectin preparation is now known to have been contaminated with Type 2 RIP and therefore it is at present not clear which of the many *Sambucus* lectins were responsible for the nutritional toxicity. SNA-II is one of the most abundant bark proteins. SNA-II appears to recognize the Tn carcinoma antigen. (98)

SNA-III is a lectin isolated from the seed of *Sambucus nigra*. It is a Gal/GalNAc specific lectin. SNA-III agglutinates animal and human erythrocytes. The lectin has a slight preference for type A over type B and type O erythrocytes. (35)

Elderberry fruits contain at least three different lectins. Two of these are Type-2 RIP's that strongly resemble the bark lectins SNA-I and SNA-V. The third fruit lectin resembles the bark lectin SNA-II with regard to molecular structure and specificity, but it is encoded by a different gene. (35)

SNA-If agglutinates animal and human erythrocytes. Its agglutination properties are very similar to those of SNA-I. SNA-If inhibits protein synthesis in a rabbit reticulocyte lysate and has RNA N-glycosidase activity. SNA-If is the second-most abundant protein in elderberry fruits. (35)

SNA-Vf is a minor fruit protein that agglutinates human and rabbit erythrocytes. It inhibits protein synthesis in animal cell-free systems and has RNA N-glycosidase activity. (35)

SNA-IV is a minor fruit protein that agglutinates human and rabbit erythrocytes. It does not inhibit protein synthesis in animal cell-free systems and has no RNA N-glycosidase activity. SNA-IV is the predominant protein in the juice of elderberry fruits. (35)

Because cell surface sialylation is a common manifestation of the infective and malignant processes, SNA has enjoyed wide use as a "diagnostic probe" helping to elucidate the sialylation patterns of a variety of common and serious illnesses. These include the paradoxical nature of de-sialylation in thyroid cancer; (101) the distribution of sialylated receptors for H5NI Avian flu; (102) age-related changes in aortic valvular glycoproteins; (103) the sialylation patterns of the prostate specific antigen (PSA) and breast cancer. (105) Although the anti-viral effect of Sambucus is thought to result from the activities of the elderberry flavonoids, which compare favorably to the known anti-influenza activities of Oseltamivir/Tamiflu and Amantadine; (106) it is likely that *Sambucus* lectins also exert an anti-viral effect, either by competitively inhibiting viral attachment to sialic acid residues on host tissue, or by a mechanical corruption of these same receptors (such as is seen with receptor capping), thereby prohibiting the subsequent viral attachment.

MCL (Momordica charantia lectin, bitter pear melon)

Momordica charantia is a tropical and subtropical vine of the family *Cucurbitaceae,* widely grown for edible fruit, which is among the most bitter of all fruits. Two different lectins have been purified from the seeds of *Momordica charantia* by gel-filtration and ion-exchange chromatography. These two lectins appear to be composed of two subunits of 26,000 daltons. The lectin activity is maximal in the pH range 7.4-11.0, but decreases steeply below pH 7.0. The lectin activity is mostly unaffected in the temperature range 4-50 degrees C, but a sharp decrease is seen between 50 and 60 degrees C, which could be correlated to changes in the structure of the protein. (276)

Protein fraction I, but not II, showed agglutinating activity toward human type-O red blood cells. *Momordica* proteins have anti-HIV properties. (274) MCL is a T cell-independent B cell activator and a polyclonal Ig inducer. (275) MCL agglutinates group A erythrocytes at a titer of 1:256; group B erythrocytes at a titer of 1:512; group B erythrocytes at a titer of 1:512; by crude lectin preparation

Lectin isolated from the seeds of *Momordica charantia* (MCL) is a galactose-specific glycoprotein. MCL demonstrated antilipolytic and lipogenic activities in isolated rat adipocytes although it did not possess intrinsic lipolytic activity. The antilipolytic

activity was susceptible to destruction by heat, trypsin, chymotrypsin, glutathione and galactose, indicating that the integrity of the protein moiety, the disulfide linkages, and galactose, which is the sugar specifically bound by the lectin, all play an important role in interaction with the adipocyte leading to an expression of this insulin-like activity. (278)

Momordica lectins belong to the family of type-I ribosomal inactivating proteins (RIPs). Like every protein in the RIP family, they are N-glycosidases that depurinate the adenine base at position 2543 of 28S rat liver rRNA, thereby inhibiting ribosomal protein synthesis.

The reported anti-tumor and anti-HIV activities of MAP30 and other RIPs are believed to be distinct from their ribosome inactivating activity. MAP30 (*Momordic*a Anti-HIV Protein), alpha- and beta-momorcharins inhibit HIV replication in acutely and chronically infected cells and thus are considered potential therapeutic agent in HIV infection and AIDS. Further, MAP30 improved the efficacy of anti-HIV therapy when used in combination with other anti-viral drugs. MAP30 holds therapeutic promise over other RIPs because not only it is active against infection and replication of both HSV and HIV but is non-toxic to normal cells. Further, MAP30 improved the efficacy of anti-HIV therapy when used in combination with other anti-viral drugs. (277)

Cinnamomum camphora lectin

A Type 2 RIP has been isolated from the seeds of *Cinnamomum camphora*. *Cinnamomum* lectin shows RNA N-glycosidase activity and inhibits protein synthesis in the rabbit reticulocyte system. The seeds apparently also contain a Type 1 RIP as well. (279) The lectin shows remarkable inhibitory effects on the growth of cultured carcinoma cells. (35)

Animal R-type lectins

The R-type lectin domain is found in several animal lectins, including the mannose receptor (MR) family, discussed in detail below, and in some invertebrate lectins. EW29 is a galactose-binding lectin from the annelid (earthworm) *Lumbricus terrestris*. The R-type domain is also found in pierisin-1, which is a cytotoxic protein from the cabbage butterfly *Pieris rapae*. *Limulus* horseshoe crab coagulation factor G has a central R-type lectin domain.

Mannose receptor (MR) family of lectins

Proteins in the macrophage mannose receptor family also contain R-type CRD's, but have a very different architecture. There are four known members of the MR family in humans, all of which contain an R-type lectin domain, and based on a survey of the human genome, no other family members are predicted. The MR family includes the MR, the phospholipase A2 (PLA2) receptor, DEC-205/MR6-gp200, and Endo180/urokinase plasminogen activator receptor-associated protein. These receptors are unusual among animal lectins in that they can bind ligands in either a "cis" or "trans" fashion, which means they can bind to cell-surface glycoconjugates on the same cell or to those on other cells and to soluble ligands.

Mannose receptor (MR)

The MR (CD206) has important roles in the innate and adaptive immune systems. It is expressed at high levels on hepatic endothelial cells and Kupffer cells as well as on many other endothelial and epithelial cells, macrophages, and immature dendritic cells. The MR is part of the innate immune system and it facilitates the phagocytosis of mannose-rich pathogens. It also assists leukocytes in responding appropriately to antigens by promoting trafficking to the germinal center and is involved in antigen presentation. The MR can bind many different microorganisms, including *Candida albicans, Pneumocystis carinii, Leishmania donovani, Mycobacterium tuberculosis, and Klebsiella pneumoniae*. The MR also functions in adaptive immunity through its ability to deliver antigens to major histocompatibility (MHC) class II compartments and through its cleavage and release as a soluble protein into blood.

PLA2

The PLA2 receptor was discovered as a receptor for phospholipase A2 neurotoxins in snake venoms and was referred to as the M-type PLA2 receptor to distinguish it from the neuronal or N-type PLA2 receptor. The PLA2 receptor might be important in regulating production of pro-inflammatory cytokines by soluble phospholipase A2s. Thus, the PLA2 receptor might function in signal transduction mediated by phospholipase A2 binding.

DEC-205

DEC-205 is a 205-kD member of the MR family that is expressed by dermal dendritic cells and, at a lower level, by epidermal Langerhans cells. It is now designated as CD205. It is also expressed on some epithelial cells, on bone marrow stroma, and by endothelial cells.

Endo180

Endo180 is part of a trimolecular cell-surface complex with urokinase plasminogen activator (uPA) and its receptor (uPAR), and it originally was termed the UPAR-associated protein or UPARAP. It was also discovered as a novel antigen on macrophages and human fibroblasts. Like the MR, Endo180 is expressed on macrophages, but Endo180 is also expressed on fibroblasts and chondrocytes, some endothelial cells, and tissues undergoing ossification.

ppGalNAcT's

The UDP-GalNAc:polypeptide α-N-acetylgalactosaminyltransferases (ppGalNAcT's) are the only known glycosyltransferases that have a lectin and catalytic domain conjoined. Mucin-type O-glycans have the common core structure of GalNAcα1-Ser/Thr, which may be further modified by addition of galactose or N-acetylglucosamine residues.

L-TYPE LECTINS

The L-type lectins have a rich history that goes back to the end of the 19th century when it was found that extracts from the seeds of leguminous plants could agglutinate red blood cells. In 1908, Karl Landsteiner (1868-1943) reported that while small amounts of lentil *(lens culinaris)* agglutinin would clump rabbit erythrocytes, even high concentrations had no effect on pigeon red cells. Landsteiner had observed early on that these extracts did not always agglutinate the blood of different species equally and wrote, for publication in 1914, a paper entitled "Pflanzliche Hammagglutinine." This paper reached the stage of page proof; but owing to the war of 1914, it was never published. In the first edition of his book on the specificity of serological reactions (1933) "Die Spezifizitat der Scrologischen Reaktionen," Landsteiner summarized some of this paper's data. (2)

These agglutinins were found to be soluble proteins that are very abundant in the seeds of leguminous plants, and differences in hemagglutination specificity were found among agglutinins from different species of legumes. In 1945, William Boyd (1903-1983) of the Boston University School of Medicine discovered that the agglutinins could be blood group specific, being able to agglutinate the red cells of one type but not those of another. He discovered that lima bean (*Phaseolus lunatus)* agglutinin would agglutinate red cells of human blood group A but not those of O or B. (3) The word "lectin" was proposed by Boyd to describe a class of blood group specific agglutinins that had been found in certain plants. The word is allegorical to a degree, as it is of Latin derivation ("legere") meaning "to choose."

The seeds of *Lotus tetragonobolus* can agglutinate group O specifically, and *Bandeiraea simplicifolia* is specific to group B. The specificity of lectins is so sharply defined that they can differentiate among blood subgroups. *Dolichos biflorens* agglutinin reacts more vigorously with blood group Al than A2. Other blood groups can be distinguished by lectins, such as M and N types, and lectins can help distinguish and diagnosis *secretors:* individuals who secrete glycoproteins that have blood-group specificity into their urine, saliva and other body fluids

The L-type lectins are distinguished from other lectins primarily based on tertiary structure. In general, either the entire lectin monomer or the carbohydrate-recognition domains (CRD's) of the more complex lectins are composed of antiparallel β-sheets connected by short loops and β-bends, and they are usually devoid of any α-helical structure. These sheets form a dome-like structure related to the "jelly-roll fold," and it is often called a "lectin fold." The carbohydrate-binding site is generally localized toward the apex of this dome. All of these lectins require Ca++ and a transition metal ion (usually Mn++) for their carbohydrate-binding activity. L-type lectins are structurally diverse; examination of the amino acid sequence, molecular size and other molecular properties show that lectins have little in common other than they are all proteins. For example, soybean agglutinin is a glycoprotein with no disulfide bond; its molecular weight is 120,000. It consists of four subunits and has two binding sites. Wheat germ agglutinin is not a glycoprotein and is rich in disulfide bonds with a molecular weight of 36,000. It has two identical subunits and four binding sites for sugars.

The L-type lectins were first discovered in the seeds of leguminous plants, and they were found to have structural motifs that are now known to be present in a variety of glycan-binding proteins from other eukaryotic organisms. They are particularly abundant in legumes and cereals and they account for between 1.5 and 3 percent of the total protein content of soy and jack beans.

Plant-type L-lectins (phytohemagglutins)

Lectins are apparently most widely distributed in plants, where they were found in almost 1000 plants of some 3000 examined in recent years. (24) Of the plant lectins, the legume lectins as a group have been the most extensively studied. The first lectin to be purified was concanavalin-A (con-A) isolated from the jack bean. (1) In 1936, Sumner and Howell noted that the addition of Con-A to a solution of glycogen caused the sugar to precipitate, and that the agglutination of red cells by this lectin was inhibited by cane sugar. They suggested that the hemagglutination by Con-A might be the consequence of a reaction between the proteins and the carbohydrates on the surface of the red cells. In other words, lectins bind to sugars, and they agglutinate cells by means of this binding. For example, the agglutination of red cells by Con-A is specifically inhibited by the sugars mannose or glucose, indicating that Con-A binds mannose and glucose on the cell surface. It was soon discovered that lectins not only agglutinate red blood cells, but also other kinds of cells including lymphocytes, spermatozoa, bacteria, and fungi.

The plant lectins were previously classified into seven families based on the CRD's: amaranthins, Cucurbitaceae phloem lectins, lectins with hevein domains, jacalin-related lectins, legume lectins, mannose-binding lectins from monocots, and type-2 ribosome-inactivating proteins (30). For example, the legume (*Canavalia ensiformis*) lectin Concanavalin A (ConA) binds glucose/mannose residues; soybean (*Glycine max*) agglutinin (SBA), also known as soybean lectin (SBL), binds *N*-acetyl-d-galactosamine/galactose; gorse (*Ulex europaeus*) lectin (UEA1) binds l-fucose; and the hevein-domain cereal lectin wheat germ agglutinin (WGA) binds *N*-acetyl-d-glucosamine. (31)

Plant L-type lectins are primarily found in the seeds of leguminous plants where they constitute about 10% of the total soluble protein of the seed extracts. They are synthesized during seed development several weeks after flowering and transported to the vacuole where they become condensed into specialized vesicles called protein bodies. They are stable during desiccation of the seeds and can remain in that state indefinitely until the seeds germinate. In the seeds of legumes, most of the lectin is localized to the cotyledons in the protein bodies, subcellular organelles related to lysosomes. Besides seeds, lectins have been found in most types of vegetative tissue, where their levels are variable and exhibit seasonal changes. Levels in tissues other than seeds are typically lower, but can be as high as 30% in garlic bulbs or as low as 0.01% in leek leaves. (1)

Another common feature of the legume lectins is that they are secretory proteins and undergo cotranslational signal peptide removal, which accompanies their entry into the secretory system. All but the peanut agglutinin are N-glycosylated; the N-glycans undergo the normal posttranslational modifications that occur as they transit the Golgi apparatus. The lectins vary from one another as to whether the mature proteins contain oligomannose-type, complex-type, or a mixture of both types of N-glycans.

Plant lectins specific for monosaccharides are classified into five specificity groups according to the monosaccharides for which they show the highest affinity. The common monosaccharides and lectins associated with them are fucose (Fuc), galactose (Gal), N-acetyl glucosamine (GlcNAc), sialic acid (Neu), N-acetyl galactosamine (GalNAc), and mannose (Man). "Specificity groups" are distinguished by their preferential binding to more than one monosaccharide: the mannose/glucose binding lectins, such as Pea *(Pisum sativum)* lectin (PSL) and the galactose/N-acetylgalactosamine lectins (peanut agglutinin).

Overall, the legume lectins whose structures are known exhibit a similar protein-folding pattern, thus exhibiting three-dimensional structural conservation. In contrast to the generally conserved, legume lectin fold structures; the major cereal lectins, a less extensively characterized family of proteins than the legume lectins (32), are homodimers comprised of two subunits of ca. 18 kDa. In addition, the cereal lectins—WGA, rye, barley, and rice lectins—are highly homologous to one another. Rye and barley lectins are able to heterodimerize with WGA. (33)

Amaranthins

AHML, amaranthin

Amaranthus hypochondriacus lectin (AHML) is specific for N-acetyl-D-galactosamine as are the other Amaranthus lectins. AHML has no carbohydrate moiety and requires no metal ion for the hemagglutination activity. T-disaccharide and its alpha-linked glycosides (Gal beta 1,3GalNAc alpha-O-R, R = OH, methyl, (CH2)8-COOCH3, allyl, o-nitrophenyl, or benzyl) were the best inhibitors of AHML. In the human colon, binding of the lectin Amaranthus caudatus has been considered a marker of cellular proliferation and malignant progression. (82) Amaranthin recognizes T- (cryptic T) antigen. (81)

Cucurbitaceae phloem lectins

Cucurbita pepo lectins (pumpkin, summer squash, gourd, winter squash)

A lectin from the fruit of *Cucurbita pepo* (summer squash) is strongly inhibited by chitin but only weakly by NAcGlu. It has been postulated that the lectin may have anti-parasitic properties. This lectin has been shown to agglutinate rabbit erythrocytes. The lectin is not a glycoprotein, and it consists of a single polypeptide chain of a molecular weight of 20,000. It is a major protein (18% of the total) of the phloem exudate. (78,79) *Cucurbita maxima* (winter squash) lectin is specific for oligomers of NAcGlu and is not inhibited by any simple sugars. (80)

Lectins with hevein domains ("chitin-binding")

This group of lectins encompasses all chitin-binding lectins composed of one or more hevein domains. The name of their main domain is derived from hevein, a small chitin-binding protein from the latex of the rubber tree *Hevea brasiliensis.* Chitin-binding lectins with hevein domains have been isolated from several taxonomically unrelated plant families including the Gramineae, Urticaeae, Solanaceae, Papaveraceae, Euphorbiaceae, Phytolaccaceae, and Viscaceae. (35) Although usually referred to as chitin binding lectins, most of these lectins also react with GlcNAc, GlcNAc-oligomers, and N-acetyl-D-neuraminic acid.

Most people carry natural antibodies to WGA, although they do not interfere with its agglutinating properties. (36) Liposomal preparations of WGA have potential as oral vaccine carriers. WGA agglutinates group A erythrocytes at a titer of 1:16 and group B erythrocytes at a titer of 1:8 by crude lectin preparation (16mg/ml). (37) For additional characterization of tumor cell-specific "wheat germ" agglutinin, with special reference to its reaction with the blood group A substance, see Uhlenbruck, et al. (38)

Incorporation of WGA in the diet of rats reduced the digestibility and utilization of dietary proteins and the growth of rats. The lectin binds to the epithelial cells and is a growth factor for the intestine. WGA is also endocytosed by the small intestinal epithelial cells and transcytosed into the systemic circulation. (40) WGA is also used in other immunologic applications, such as the fractionalization of bone marrow cells, and separation of mouse spleen B- and T- cells by selective agglutination of the B-cells with WGA. (41)

WGA binds to extracellular network produced by cultured human fibroblasts. (42) WGA and *Ulex europaeus* I (UEA-1) stimulated the production of specific serum IgG and IgA antibody after three intramuscular or oral doses. (43)

WGA binding to pancreatic cancer cells in vitro results in toxicity. Lectin toxicity corresponded to membrane binding intensity, and was profound in the case of WGA. WGA exposure induced chromatin condensation, nuclear fragmentation and DNA release consistent with apoptosis. Important steps for WGA toxicity included binding to sialic acid on swainsonine-sensitive carbohydrate and lectin internalization. There was rapid cellular uptake and subsequent nuclear relocalization of WGA. (44) Parenterally administered WAG inhibits tumor growth and modulates the host immune response. The response of peripheral blood lymphocytes towards mitogenic stimulation was improved and enhancement of tumor cytolysis by peritoneal macrophages was noted following lectin treatment. (46) WGA also induces apoptosis of tumor cells. (47) WGA appears to inhibit *Herpes simplex* virus adsorption to susceptible cells. (45) (WGA) was found to stimulate DNA synthesis in human peripheral blood mononuclear cells at relatively low concentrations and to inhibit DNA synthesis at higher concentrations. (150)

WGA induces proliferation of T-cell colony forming units and growth factor production. Purified WGA was given to human volunteers and about 2% was recovered intact from the feces. It was speculated that the lectin escaped digestion by binding to the dietary fiber, and noted that a high fiber diet is also largely a high lectin diet. (16) Human thymocytes cultured for 5 days in interleukin 2 containing supernatants (IL 2 Sup) virtually become a population of mature T cells (T3+, HTA-) that acquire strong cytotoxic activity against NK-sensitive and NK-resistant target cells. The addition of WGA to the cultures strongly abrogated the expression of the cytotoxic activity and enhanced thymocyte proliferation. Lectin presence is not required at the onset of the culture but is required during the last 24 hours of the 5-day incubation period. Reversion of inhibition with full expression of cytotoxic activity can be obtained after removal of the lectin and subsequent culture in lectin-free conditions for at least an 18 to 24 hour period. (155) WGA inhibits proliferation of human peripheral blood mononuclear cells (PBMC) induced by mitogens and antigens. Although PBMC-proliferation was markedly suppressed by WGA, levels of IL 2 activity in WGA-inhibited cultures were not reduced, but instead were increased, suggesting failure to utilize IL 2. WGA markedly decreased the number of high-affinity IL 2 receptors per cell, suggesting that WGA inhibits lymphocyte

proliferation by binding to and decreasing the number of high-affinity IL 2 receptors displayed on T cells, without impairing IL 2 production. (156)

WGA has considerable insulin-like activity. (50) WGA produces several alterations in the ability of fat cells to bind and respond to insulin. Although WGA markedly stimulated glucose oxidation, it caused only a modest stimulation of glucose transport. Findings suggest that low WGA concentrations increase the affinity of the insulin receptor and the insulin sensitivity of the cells. At higher concentrations, the lectin appears to act at another site(s) to inhibit the activation of the transport system by insulin or other agents. (55) Within the hormone recognition area, peptide chains containing galactose, mannose and N-acetyl-glucosamine are strictly required for insulin-receptor interaction. WGA is effective in modifying the insulin with approximately 80% inhibition. (54)

The effect of insulin and WGA on [3H]glucosamine incorporation into pericellular glycosaminoglycans (GAG's) was investigated in two lines of cultured human dermal fibroblasts. WGA produced an insulin-like action stimulation of [3H]glucosamine incorporation into hyaluronic acid (HA) and heparan sulfate (HS) without any alteration of chondroitin sulfate (CS) and dermatan sulfate (DS) contents. (48) WGA stimulates insulin receptor beta-subunit autophosphorylation. (49) Selective transport of proteins is a major mechanism by which biochemical differences are maintained between the cytoplasm and nucleus; WGA completely inhibits the nuclear transport of fluorescently labeled nucleoplasmin. (51) Considerable number of patients with insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM) possess antibodies against WGA bound glycoproteins on the islet-cell membrane. The prevalence was highest for both IgG and IgM antibodies in IDDM patients within a year of the onset of disease. The prevalence of IgM antibodies was lower than that of IgG antibodies at all stages. In NIDDM patients, the prevalence of antibodies for both IgG and IgM was equal. (51) Like insulin, WGA has pleiotropic effects on sensitive cells, including the regulation of specific mRNA accumulation initiated by the binding of insulin to its plasma membrane receptor. (52) Brain insulin receptors react with WGA. (53) Transneuronal transport can be demonstrated for WGA, indicating that mechanisms exist whereby neurons exchange large molecules that could be involved in mediating trophic and other influences on target cells. (131) The binding of nerve growth factor (NGF) to specific cell surface receptors initiates a variety of effects that lead to the morphological and biochemical differentiation. WGA alters the characteristics of NGF-receptor interaction. WGA-induced changes in the NGF-receptor interaction reflect important alterations in the ability of the receptor to transmit biological signals, resulting in the abrogation of the biological effects of NGF on these cells. (151)

WGA considerably influenced the cell growth of all tested (MCF-7, T 47D, HBL 100, BT 20) human breast cancer cell lines. (142) All pancreatic cancer cell lines tested showed high WGA membrane binding, primarily to sialic acid residues. WGA exposure induced chromatin condensation, nuclear fragmentation, and DNA release consistent with apoptosis. (146)

Lectins that are especially rich in disulfide bonds such as WGA are very resistant to proteolytic enzymes, detergents, urea, alkalis, and acids. WGA is in fact one of the more heat sensitive lectins, being destroyed after 15 minutes at 75 degrees C, whereas other wheat lectins in gluten and gliadin resist autoclaving at 110 degrees C for 30 minutes. Researchers reported that the mucous membranes of celiac patients showed sugar residues that were capable of binding to the lectins in wheat germ, which resulted in a cytotoxic reaction. Rats treated with Con-A or WGA

developed a gut membrane that was paradoxically impermeable to small molecules, but very permeable to large, highly allergenic molecules, a situation that is mimicked in food allergies and celiac disease. WGA binds to microvilli in the intestinal crypts and to the goblet cells with an affinity that increases from the proximal to distal intestine.

Because it binds to specifically to sugars expressed also by gastrointestinal epithelial cells, WGA has been proposed as a carrier for oral drugs. (140) WGA enhances transport of some food ingredients (isoflavones, quercetin glycosides, carnosine) across Caco-2 cell monolayers. (141) Unlike most other lectins, which stimulate mast cells, WGA has inhibitory effect on mouse mast cell adhesion to fibronectin. (144)

A component of wheat, gliadin has been shown to bind preferentially to crypt epithelial cells of coeliac disease subjects but only rarely in health volunteers. This seems to result from an immaturity in the pattern of cell surface carbohydrates on the coeliac enterocytes, perhaps due to a genetically determined deficiency of a growth dependent enzyme, N-acetyl-glucoaminyltransferase, which renders celiac patients sensitive to the effects of the oligomannosyl-specific lectin gluten. The lectin properties of gluten are due to traces of WGA. (152)

Support for a role for WGA in rheumatoid disease has come from studies on the biochemistry of antibodies. The IgG from the sera of patients with chronic inflammatory diseases of autoimmune character or some chronic microbial infections is frequently deficient in galactose on N-linked glycans. Antibodies of the IgG class have carbohydrate side-chains that normally terminate in galactose. The IgG molecules in rheumatoid disease are defective; instead of terminating in galactose, they terminate with N-acetyl-glucosamine —the very sugar for which wheat lectin is specific. (56) This phenomenon, known as a "galactose deficient antibody" is a quite common concomitant with autoimmune disease.

At nanomolar concentrations, WGA stimulates the synthesis of pro-inflammatory cytokines and thus the biological activity of WGA should be reconsidered by taking into account the effects of WGA on the immune system at the gastrointestinal interface. These results shed new light onto the molecular mechanisms underlying the onset of gastrointestinal disorders observed in vivo upon dietary intake of wheat-based foods. (138) Incorporation of several lectins in the diet at the level of 7 g/kg reduced the apparent digestibility and utilization of dietary proteins and the growth of rats, with WGA being the most damaging. Because of their binding and endocytosis by the epithelial cells of the small intestine, all three lectins were growth factors for the gut and interfered with its metabolism and function to varying degrees. WGA was particularly effective; it induced extensive polyamine-dependent hyperplastic and hypertrophic growth of the small bowel by increasing its content of proteins, RNA and DNA. Furthermore, an appreciable portion of the endocytosed WGA was transported across the gut wall into the systemic circulation, where it was deposited in the walls of the blood and lymphatic vessels. WGA also induced the hypertrophic growth of the pancreas and caused thymus atrophy. (147)

WGA potentiates specific binding of platelet-activating factor to human platelet membranes, induces platelet-activating factor synthesis (148) and induces rapid protein-tyrosine phosphorylation in human platelets. (149) WGA induced serotonin release in human platelets without cell agglutination. (154)

Cholecystokinin (CCK) is a peptide hormone and plays a major role both in the regulation of pancreatic enzyme secretion and growth of the gastrointestinal tract.

The pancreatic CCK receptors are highly glycosylated membrane proteins that are able to bind plant lectins such as WGA, and which have been shown to inhibit their actions. (39) WGA binds to the glycosylated sites of these CCK receptors with the effect of blocking CCK binding and thus inhibiting the CCK-induced Ca2+ release and alpha-amylase secretion. Rat pancreatic acinar tumor cell line AR42J is a widely used model to study the secretion, proliferation and differentiation of cells under the influence of hormones. The so-called trophic hormones, CCK and gastrin stimulate the secretion and proliferation of AR42J cells within the autocrine loop via autostimulation of their CCK receptors. (139)

PHA and WGA decrease levels of heat shock stress proteins (HSP70, HSP72, and HSP90) in rat gut and enterocyte-like Caco-2 cells, leaving these cells less well protected against the potentially harmful content of the gut lumen. (145)

Urtica dioica agglutinin (UDA)

Stinging nettle root (*Urtica dioica* rhizome) contains a small-molecular-weight lectin Urtica dioica agglutinin (UDA) that can be purified from the root, and which is capable of modulating aspects of the immune system. The lectin in Urtica dioica might beneficially modulate an overactive immune system by reducing tumor necrosis factor-alpha and interleukin-1 beta. The lectin in *Urtica* root (UDA) is a T-cell mitogen, with the ability to discriminate a particular population of CD4+ and CD8+ T-cells that results in an increase in immune tolerance. Probably due to its ability to alter the production of anti-self antibodies, the lectin in *Urtica* root has been shown to protect the kidneys and cardiovascular system from the progression of experimentally induced autoimmune damage in mice. (63)

According to one source UDA is not blood group specific, but another claims that it agglutinates group B erythrocytes; its ability to agglutinate rabbit erythrocytes, however, is unquestioned. The lectin behaves as a superantigen for murine T cells, inducing the exclusive proliferation of Vbeta8.3(+) lymphocytes. (64) UDA is unique among known T cell superantigens because it can be presented by major histocompatibility complex (MHC) molecules of both classes I and II. UDA induces the proliferation of mouse T-lymphocytes. (65) It differs from the traditional mitogens, in that the UDA-induced activation of murine T-cells is characterized by late kinetics of proliferation, and only a limited portion of the CD4+ and CD8+ T-lymphocytes enter into the cell cycle. Therefore, UDA is regarded as a superantigen, although it does not possess the toxicity of the exogenous superantigens. UDA prevents the development of the systemic lupus erythematosus-like pathology of MRL lpr/lpr mice. (67) UDA and interleukin-2, the in vitro production of which is enhanced by this lectin, exhibited obvious preference for hyperplastic prostate cancer cells. (68)

Similar to the other superantigens, the clonal expression of T-cells is followed by the deletion of the majority of these lymphocytes by apoptosis whereas the remaining T-cells become anergic to UDA restimulation. In contrast to other superantigens, the clonal expansion of the T-cells does not occur in the thymus, but only in the spleen and lymph nodes. UDA induces the production of interferon-γ in fresh human lymphocytes. Incorporation of nettle lectin into the diet of rats reduces the apparent digestibility and utilization of dietary proteins and the growth of rats. UDA acts as a modest growth factor for the gut, although its effects are relatively modest.

Poke weed mitogen (PMW)

The lectin most studied in humans as regards to mitogenic effects is pokeweed mitogen (PWM), isolated from *Phytolacca americana*. Lymphocytes in mitosis are almost never found in peripheral blood, but they were observed in 1961 in the peripheral blood of a 3-year-old girl who had eaten a large number of pokeweed berries. PWM is one of the rare lectins that are mitogenic for both T- and B-lymphocytes. In vitro it triggers the production of IgE as well as other antibody isotypes. Recent studies on the plant show that salt water extracts of the plant yield six separate lectins designated Pa-l through Pa-5 and PLB. Pa-1 seems to be the only hemagglutinating lectin, and is powerfully mitogenic. Pa-2 and Pa-4 are the predominant mitogens in the roots. Pa-1 is mitogenic for both B and T cells, while the other five lectins are only mitogenic for T-cells. Interestingly, PWM blastogenesis is inhibited by other lectins such as WGA. *Benincasa cerifera*, used in Chinese medicine as an anti-inflammatory diuretic, was shown to contain a powerful anti-tumor mitogen termed "B. cerifera mitogen" (BCM). Salt-water extracts of the seed were shown to contain B cell mitogenic, adjuvant active and antitumor active substances. (69) Three mitogenic lectins, designated PL-A (Pa-2), PL-B and PL-C (Pa-4), were purified from the roots of pokeweed (Phytolacca americana). Although all of three lectins have mitogenic activities, PL-B is a mitogenic lectin with the most potent hemagglutinating and mitogenic activities, and PL-C has almost no hemagglutinating activity.

PWM suppresses erythroid burst activity, which could account for the anti-inflammatory activity traditionally ascribed to the plant. Human peripheral blood lymphocytes precultured with lipopolysaccharide from E. Coli (LPS) were shown to have a greatly enhanced blastogenic response when PMW was added to the suspension.

Pokeweed antiviral protein (PAP), a novel cap-binding protein, is a type I ribosome-inactivating protein (RIP-1) isolated from *Phytolacca americana*. Several cap-binding proteins mediate processes such as pre-mRNA splicing, translation initiation, and mRNA turnover. PAP also cleaves supercoiled DNA into relaxed and linear forms. B-cell precursor (BCP) leukemia represents one of the most radiation-resistant forms of human malignancy. PAP caused apoptosis of radiation-resistant primary BCP leukemia cells. (70)

LEA (tomato lectin, Lycopersicon esculentum)

LEA is a very stable glycoprotein containing about 50 percent arabinose and galactose. This lectin is composed of a single polypeptide of about 100,000 daltons that may form aggregates in solution. Like other lectins that bind N-acetylglucosamine oligomers, tomato lectin prefers trimers and tetramers of this sugar. LEA, although sharing some specificity with potato, *Datura* lectin and wheat germ agglutinin, has been reported to be dissimilar in many respects. LEA binds well to such glycoproteins as glycophorin and Tamm-Horsfall glycoproteins. LEA occurs in all tissues of the tomato plant but is located predominantly in the locular fluid of ripe plants. (108) Saline extracts of tomato seeds were able to agglutinate human types A, B, and O erythrocytes equally. (113)

LEA is an effective marker of blood vessels and microglial cells in rodents. LEA agglutinates human and animal erythrocytes. LEA is non-mitogenic for mouse and chicken lymphocytes but it dose-dependently inhibits the mitogenic effects of ConA, PHA and PWM in chickens. Tomato lectin resists digestion in the mammalian alimentary canal and binds to intestinal villi without deleterious effects. (112)

Tomato lectin has been used for the isolation of glycoproteins and the controlled oral delivery of drugs. The idea to use lectins for drug delivery came in 1988 from Woodley and Naisbett, who proposed the use of tomato lectin to target the luminal surface of the small intestine. (109, 110) The resistance of tomato lectin to denaturation by acid and by proteolytic enzymes was indicative of remarkable stability. Such characteristics may allow this lectin to survive intact in its passage through the gastrointestinal tract. The exposure of the U.S. population to a lectin from tomatoes cannot be dismissed lightly since the tomato is the primary vegetable source of vitamins and minerals in the U. S. diet. Total tomato consumption in the U. S. per person per year is about 74 pounds of which about 12.5 are eaten raw. The average person ingests at least 100 to 200 mg of the lectin a year. Some individual may be exposed to considerably higher quantities. (112)

Tomato lectin also recognizes a subclass of cells deep in the tectum, immediately adjacent to the ependymal cells lining the tectal ventricle, and disappears soon after migration along radial glia begins. This is where neural stem cells, cells that give rise to all the other neurons and glia of the nervous system, are known to be located. (107) LEA may have inhibitory effects, possibly through direct agglutination, on a variety of strains of Staphylococcus, including MERSA (methicillin-resistant Staphylococcus aureus). (111)

LEA has been shown to inhibit the transformation of peripheral lymphocytes challenged by recall antigens, and actually suppressed spontaneous DNA synthesis. Exogenously added Interleukin 1 and/or Interleukin 2 did not stop the inhibition of lymphocyte transformation, even at extremely high concentrations. This could be significant as the average American diet results in the ingestion of at least 200 mg. of tomato lectin annually, with vegetarians probably ingesting a far greater amount. (26)

STA (Solanum tuberosum lectin, potato)

STA exhibits specificity towards oligomers of GlcNAc, although GlcNAc itself does not inhibit it.

As expected from slow-growing tuber, potato lectin has significant antifungal properties, which were studied by following their effects against early developmental stages of *Fusarium oxysporum,* a fungal potato pathogen. The lectin content in the tubers varies considerable according to the variety. STA shares some sequence similarities with the extensin family and snake venom disintegrins (platelet aggregation inhibitors).

STA agglutinates group A erythrocytes at a titer of 1:64; group B erythrocytes at a titer of 1:128; group O erythrocytes at a titer of 1:256; by crude lectin preparation (114). STA agglutinates bovine, sheep, horse, pig, cat, guinea pig, rat, and mouse erythrocytes, as well as human. (115) STA agglutinates some strains of *Pseudomonas.* (116)

A major factor in non-allergic food hypersensitivity could be the interaction of dietary lectins with mast cells and basophils. Because immunoglobulin E (IgE) contains 10–12% carbohydrates, lectins can activate and degranulate these cells by cross-linking the glycans of cell-bound IgE. As potato lectin activates and degranulates both mast cells and basophils by interacting with the chitobiose core of IgE glycans, higher intake of potato may increase the clinical symptoms resulting from non-allergic food hypersensitivity in atopic subjects. (117) Diets containing

genetically modified (GM) potatoes expressing the lectin Galanthus nivalis agglutinin (GNA) had variable effects on different parts of the rat gastrointestinal tract. (118)

OSL (Oryza sativa lectin, rice bran)

This lectin is specific for GlcNAc and its oligomers. It agglutinates human and rabbit erythrocytes and is mitogenic to mouse splenic lymphocytes and human peripheral lymphocytes. It is anti-insecticidal. The lectin enhanced the rate of glucose oxidation and inhibited epinephrine-stimulated lipolysis in mouse adipocytes. Some characteristics of the lectin are compared with those of wheat germ agglutinin. (120) Transgenic rice engineered to include the mannose binding Allium sativum leaf agglutinin (ASAL) has been shown to be antifeedant and insecticidal against sap-sucking insects. (121) Effects of rice bran agglutinin (OSL) on human monoblastic leukemia U937 cells showed that that OSL induces chromatin condensation, externalization of membrane phosphatidylserine, and DNA ladder formation, features of apoptosis. A general inhibitor against caspases, which are known to play essential roles in apoptosis, inhibited DNA ladder formation. (143)

Hordeum vulgare lectin (barley) and Secale cereale lectin (rye)

Barley lectin has been isolated from seeds, roots, and leaves of the adult plant. It has many similar properties to WGA. (35) It agglutinates human and rabbit erythrocytes. (122) Like barley lectin, rye lectin is closely related to WGA and like WGA probably has a closer affinity for GlcNAc oligomers than for the monomer.

Jacalin-related lectins (JRLs)

JRLs exhibit high sequence conservation in their overall sequences and hence the same subunit structure, marked variations are observed in their abilities to recognize various carbohydrates. The data available so far have led to their broad classification into galactose-specific and mannose-specific classes. Some of them, however, are not exclusive to galactose or mannose, but their primary specificities are consistent with the classification. For example, jacalin, a galactose-specific lectin, has been shown to bind to mannose as well, albeit with a lower affinity. Until recently, it was believed that JRLs were found only in a few genera of the family Moraceae, to which the jackfruit plant (*A. integrifolia*) belongs. In the past few years, however, it has become evident that related lectins are found in many other families as well. (62)

Jacalin

The jackfruit (*Artocarpus integrifolia*) is a fast growing tree that can produce fruits within three years of planting. The fruits are borne on the main trunk and older branches. The jackfruit is the largest of all cultivated fruits. A well-ripe fruit emits a very pleasant smell, has a sweet taste and the flesh is waxy and golden-yellow in color. Juicy Fruit Gum was fashioned after the flavor of Jack Fruit. There are more than 100 seeds per fruit. Jackfruit is one of the most popular fruits in Kampuchea where they use green young Jackfruit to cook with curry and other soups. The seeds are as big as quail eggs, which are often boiled, fried, or roasted with salt.

The major lectin in the seeds and pulp is jacalin. Jacalin is a lectin composed of four subunits, two of approximately 10,000 daltons and two of 16,000 daltons each*).* Like peanut agglutinin (PNA), jacalin binds the Thomsen-Friedenreich antigen. However,

unlike PNA, jacalin will bind this structure even in a mono- or disialylated form. (60) This lectin has been used to purify human IgA, since no other human immunoglobulin class binds Jacalin. (57)

Increased cell surface expression of the Thomsen-Friedenreich antigen (TF antigen, Galbeta1-3GalNAcalpha-) is a common feature in malignant and pre-malignant epithelia. TF-binding lectins from peanut (*Arachis hypogea*) and edible mushroom (*Agaricus bisporus*) produce marked but different effects on human intestinal epithelial cell proliferation. Jacalin produced dose-dependent and non-cytotoxic inhibition of proliferation in HT29 human colon cancer cells with maximal effects of 46 +/- 4% at 20µg/ml. The lectin-mediated effects were inhibited by the presence of appropriate blocking sugars. These results provide further evidence that dietary TF-binding lectins can have marked effects on the proliferation of human malignant gastro-intestinal epithelial cells and hence may play a role in intestinal cancer development and treatment. (214)

Jacalin agglutinates all human red blood cells equally well. It also agglutinates erythrocytes from sheep, rabbit, mouse, buffalo, duck and pigeon as well as human and rat sperm. (58) Jacalin has potent mitogenic effects. It is a T-cell mitogen for human peripheral mononuclear cells and is specific for CD4+ lymphocytes. It also induces the production of gamma interferon and secretion of IL-2. (59)

Jacalin causes marked T-cell activation in response to T-cell receptor ligation and CD28 costimulation by binding to CD45. In contrast to Jacalin-stimulated T-cell activation, Jacalin induced human B-lymphocyte apoptosis, resulting in calcium mobilization and calpain activation, suggesting that the calcium-calpain pathway may mediate the Jacalin-induced apoptosis. (61)

Helianthus tuberosus lectin (Jerusalem artichoke)

This lectin is isolated from the dormant and non-dormant tubers of Jerusalem artichoke. The lectin is preferentially inhibited by N-acetyl galactosamine (GalNAc) and N-acetyl glucosamine (GlcNAc) and galactose. The lectin has the highest agglutination titer with human blood group A erythrocytes. (83)

BanLec (Musa paradisiac lectin, banana)

Banana lectin agglutinates rabbit red blood cells, and it has been shown to stimulate T-cell proliferation. (86) IgG4 antibodies are found to occur more frequently than expected. The most important antigen involved in this reaction is BanLec-I. (87) BanLec inhibits primary and laboratory-adapted HIV-1 isolates of different tropisms and subtypes. BanLec possesses potent anti-HIV activity, with IC_{50} values in the low nanomolar to picomolar range. BanLec inhibits HIV-1 infection by binding to the glycosylated viral envelope and blocking cellular entry. The relative anti-HIV activity of BanLec compared favorably to T-20 and maraviroc, two anti-HIV drugs currently in clinical use. (87)

Legume lectins

The L-type lectins were first discovered in the seeds of leguminous plants, and they were found to have structural motifs that are now known to be present in a variety of glycan-binding proteins from other eukaryotic organisms. The structures of many of these lectins

have been thoroughly characterized, and many L-type lectins are employed in a wide range of biomedical and analytical procedures.

PHA (Kidney bean lectin, phytohemagglutinin A, Phaseolus vulgaris)

In 1960, a researcher added PHA to a blood sample to agglutinate erythrocytes and thus encourage their removal and noticed to his annoyance that the lymphocytes had also been affected. He had discovered the mitogenic effect of PHA (and many other lectins) that was to be the key to the explosion of knowledge about lymphocyte physiology. (16) Lectins are probably the best biologic response modifiers (outside of monoclonal antibodies) found in nature.

PHA can induce the acquisition of T cell surface markers in peripheral blood in the absence of the normal maturation controls of the thymus. Con-A also acts as a mitogen producing a "mitogen induced erythroid burst promotion" due to monocyte blastogenesis. Interestingly PHA has been shown to suppress experimental autoimmune thyroiditis in mice for up to 7 weeks.

Lymphoid cells from patients with chronic lymphatic leukemia bind less PHA than do normal cells, and react poorly to the mitogenic activity of this and other lectins. PHA specifically stimulates many subpopulations of lymphocytes, separating mouse thymocyte populations into two groups: one that was agglutinated by peanut lectin and one that is not. The thymocyte population found not to be agglutinated by the lectin was found to resemble the adult circulating lymphocytes. Since blastogenesis can also occur in suppressor T-cell populations, it is quite feasible that significant suppression of graft versus host responses in tissue transplants can be accomplished by the use of lectins. Selection of hematopoietic stem cells can be used to prevent graft-versus-host disease (GVHD) after allograft transplantation.

PHA triggered basophils to release IL-4 at concentrations of up to $1ng/10^6$ basophils. Lectins with high IL-4-inducing capacity also stimulated the release of IL-13 and histamine. (126) PHA modulates the antibody response to type III pneumococcal polysaccharide, where a profound enhancement of IgG2a antibody response was noted after PHA was given. (132)

ConA (jack bean lectin, Canavalia ensiformis)

It has recently been shown that ConA causes a greatly enhanced secretion of mucous from the intestines of laboratory rats. It has been suggested that this "mucotractive" effect of lectins may have some usefulness in cystic fibrosis. One investigator ingested a 10mg dose of ConA in tap water. Later that day and on the next day he experienced moderate by quite intrusive bowel colic, with passage of foul smelling flatus of unfamiliar odor, and on day three passed a stool of normal size and texture, but thickly coated with mucous.

How mitogens work is still imperfectly understood. ConA has been shown to induce microtubule assembly in polymorphonuclear leukocytes. Lectins have been shown to cause early changes in cytoplasmic free Ca2+ and influence the lymphocyte membrane potential. Both Con-A and PHA were studied as to their effect on lymphocyte glycosyltransferase activity. The investigators found that this enzyme, associated with increased transport activity of sialic acids, galactose, and NAG was stimulated by ConA but not by PHA. Thus, the mitogenic effect of lectins on lymphocytes is not constant. PHA modulates the antibody response to type III pneumococcal polysaccharide, where a substantial increase in the magnitude of the IgG1 antibody response was noted after the administration of ConA. (132)

Transneuronal transport can be demonstrated for ConA, indicating that mechanisms exist whereby neurons exchange large molecules that could be involved in mediating trophic and other influences on target cells. (131)

ConA binds to viruses such as the Newcastle disease V4 strain. As bacterial binding and the attachment of parasites is, at least in part, through their lectin-adhesins, some of which are mannose-specific, infection and colonization of cells and tissues by these mannose-sensitive microbes can be effectively inhibited by the use of ConA. Recent examples include the inhibition of attachment of *Salmonella pullorum* to chicken gut and the blocking of attachment of *Giardia lamblia* trophozoites to Caco-2 cells. (238)

ConA activates basophil cells (237) and releases histamine from hamster mast cells, but can also inhibit exocytosis induced by other secretagogues. The lectin can bind both to IgE receptors of mast cells and to the carbohydrate side- chains of IgE. The morphological changes occurring after ConA binding to hamster mast cells and histamine release were recently established by electron microscopy. ConA reacts preferentially with IgA2, particularly in its polymeric form and with the secretory piece attached such as in sIgA in colostrum. ConA interacts with the insulin receptor of fat and liver cells, exhibits insulin-like activity, and stimulates the tyrosine kinase activity of the insulin receptor. (35)

There is an apparent correlation between the antinutritional effects and hemagglutination properties of ConA (239), and this association may partly be caused by its depressant effect on food intake. (240) ConA binds to brush-border membranes in vitro and in vivo, leading to increased shedding of brush-border membranes, accelerated cell loss and slight villus atrophy. The lectin is resistant to gut proteolysis, interferes with the reformation of brush-border membranes and causes some cytotoxic effects, particularly in young animals. However, all these effects can be reversed by the use of high enough concentrations of mannose. (35)

Griffonia simplicifolia lectins

GS-I is a mixture of five α-D-galactosyl binding lectins (A_4, A_3B, A_2B_2, AB_3 and B_4). (233,234) It is a reliable marker for human eosinophils. (232) The natural mixture of GS-I isolectins agglutinates type B and A erythrocytes, but not type O cells. GSI-B_4 is strictly blood group B specific, whereas GS-I-A_4 is specific for type A cells. Because of the strict blood group specificity of some of the *Griffonia* lectins, they are useful tools for blood typing. The lectin GS I-A_4 binds to terminal alpha-N-acetylgalactosaminyl (GalNAc) groups (which include the Tn antigen), but not to the closely related tumor-associated epitope, sialylated Tn antigen. Thus, GS-I-A_4 is cytotoxic to many colon cancer cell lines. (236) GS-I seeds contain a family of alpha-D-galactopyranosyl-binding isolectins that strongly agglutinate Ehrlich ascites tumor cells due to the presence of this determinant sugar on their cell surface glycoproteins. (235)

GS-II lectin does not agglutinate human ABO erythrocytes but reacts with acquired B, T-activated, and Tk polyagglutinable cells. GS-II shows a primary specificity for N-acetylgalactosamine.

The lectin GS-IV agglutinates type O Lewis b+ but not Lewis b- human erythrocytes. GS-IV is not mitogenic to mouse spleen cells or human peripheral lymphocytes. GS-IV is primarily directed against l-fucose.

SBA (soybean agglutinin, Glycine max)

Soy contains several hemagglutinating isolectins. The lectin, soybean agglutinin (SBA), exhibits the greatest affinity for N-acetylgalactosamine (GalNAc), its glycosides and oligosaccharides containing terminal GalNAc. The lectin is a mixture of tetrameric, glycosylated isolectins and is composed of two slightly different subunits. (243) ABA agglutinates red blood cells from human and several animal species. It also precipitates human A1 blood group substances but reacts poorly with A2 and B substances and not at all with H substance. High levels of soy agglutinin in the diet reduce the growth rate of young monogastric animals and induce dose and polyamine dependent and reversible hyperplastic growth of the small intestine and pancreatic hypertrophy. (241) The lectin stimulates pancreatic secretion via pancreatic cholecystokinin-A receptors and interferes with the absorption of iron. (244) Parenteral administration of SBA modulates the host's immune response and inhibits tumor growth. SBA is capable of differentiating between T-cells and stem cells in bone marrow. Natural spleen suppressor cells and cyclophosphamide-generated suppressor cells react preferentially with SBA and can therefore be isolated from the bone marrow by agglutination. (35)

Several investigators have noted a syndrome that is indistinguishable from celiac disease that is produced by soybeans. Investigators have also described a patient with soya intolerance whose severe diarrhea was ameliorated by ingesting sugar inhibitors of soybean agglutinin (SBA) such as galactose or lactose, whereas glucose or sucrose made it worse. Investigators noted violent reaction to soy protein formula in a 6-week-old infant. The infant developed (sequentially) fever, leukocytosis, cyanosis, vomiting, massive blood tinged mucosal diarrhea, dehydration and acidosis. All symptoms disappeared after discontinuing soymilk. The jejunal mucosa, previously normal, became inflamed and flat with the disappearance of the intestinal villi; however, it had regenerated by the fourth day after discontinuance. (16)

SBA is of vast importance to the field of clinical bone marrow transplantation. It binds bone marrow mononuclear cells, including mature myeloid, erythroid, and lymphoid cells, but has very low binding affinity and no toxic effect to the human hematopoietic cells. Peripheral blood stem cells (PBSC) are increasingly used for stem cell transplantation after high dose chemotherapy. CD34+ cell selection has also been done for use in autologous transplantation studies. Bone marrow may contain tumor cells at the time of harvesting, and on re-infusion, these cells could contribute to a subsequent relapse. Similarly, tumor cell contamination of PBSC collections has been found in a number of studies. Therefore, purging contaminating tumor cells may prevent cases of relapse. As most tumor cell types do not express CD34 antigen, one of the most widespread applications of CD34+ cell selection is likely to be in tumor cell purging. Bone-marrow-derived CD34+ hematopoietic progenitor cells can be enriched using SBA. (242) SBA bound to magnetic beads as a convenient tool for effective ex vivo purging of marrow aspirates contaminated with metastatic breast cancer cells in patients with advanced disease. (251) There is a correlation between SBA labeling, distribution, and the stage of lymphoid cell differentiation. (252)

SBA binds selectively to many blood cells by recognizing cell-surface sugars, which are dependent on the extent of cellular differentiation. SBA bound only the population of thymic lymphocytes that possessed a high content of sialic acid on its membrane, leading researchers to speculate that the attachment of sialic acid to the lymphocyte surface was a crucial maturation step. (27)

In both human adenocarcinomas and an experimental tumor system, most tumor cells that metastasize show preferential binding of PNA and SBA. (153)

Fibroadenomas are human benign breast tumors characterized by proliferation of epithelial and stroma cells of the terminal ductal unit. Expression of O-glycans seems to contribute to the proliferation and transformation events. Positive expression of CA15-3 and MUC1 is observed in fibroadenoma tissue, mainly in duct and stroma cells, whereas, in normal samples, staining was observed in duct cells. SBA recognized duct and stroma cells equally well, and it was the only lectin showing colocalization with anti-CA15-3 in healthy and tumor tissues. (231) Positive SBA staining was significantly correlated with tumor size, macroscopic tumor type, depth of invasion, lymph node metastasis, and venous invasion. (247) SBA may be used as differentiated markers in endometrial carcinoma and may be helpful in clinical diagnosis. (248) SBA is not useful in distinguishing prostatic hyperplasia from adenocarcinoma. (249) SBA binding displayed remarkable changes between the endometrial phases, SBA-positive glandular cells increasing in the secretory phase. (250)

SBA increased the transport of quercetin glycosides, although SBA did not change the transepithelial electrical resistance (TER) value of the Caco-2 cell monolayers. (241) SBA can bind to and be extensively endocytosed by intestinal epithelial cells, being nutritionally toxic for most animals. SBA is able to induce a local inflammatory reaction but has an anti-inflammatory effect when present in circulating blood. (245) Corneal endothelial cells bind SBA when undergoing repair, indicating that changes in the glycan structure (diminishment of GlcNAc and increased GalNAc) of the cornea are associated with corneal pathology. (246)

PNA (peanut agglutinin, Arachis hypogea)

Most people carry natural antibodies to PNA, although they do not interfere with its agglutinating properties. (36) PNA may be partially responsible for the atherogenicity of peanut oil. (172)

The lectin is widely used for the identification and separation of lymphocyte subpopulations in bone marrow transplantation and is a powerful tool in immunology. The loss of sialic acid from core 1 O-glycans on T-cell surface glycoproteins CD45, CD43, and CD8, a hallmark of immature thymocytes and activated peripheral T-cells is detected with PNA. (174)

The majority of human thymocytes (60-80%) bind PNA. The two thymocyte subpopulations (PNA+ and PNA-) were tested in the mixed lymphocyte reaction and with the phytohemagglutinin of Phaseolus vulgaris (PHA). The poor response of the PNA+ thymocytes to these stimuli indicates that these thymocytes are functionally immature. Examination of the peripheral blood lymphocytes of leukemia patients revealed that, in most acute leukemias, the PNA receptor is exposed on the blastic cells; whereas in most cases of chronic leukemia, the peripheral blood lymphocytes are PNA-negative. (185) PNA is a marker of early T cell subpopulations. Study of PNA binding during ontogenesis shows the occurrence of PNA-positive cells in the fetal liver before thymus constitution and in the very beginning of embryonic thymus and spleen development. (186) PNA binding is a useful marker for a subgroup of T-cell acute lymphoblastic leukemia with a better prognosis. (191)

Bone marrow and peripheral blood monocytes reacted weakly with PNA except in one case of acute monoblastic leukemia and two of chronic myelomonocytic

leukemia in which monocytes were strongly positive. Because of this, PNA may have potential as an agent to be used in myeloma for in vitro marrow purging prior to autologous transplantation in combination with high dose chemotherapy. (197)

The expression of two cell-surface PNA binding glycoproteins appeared to be related to the tumorigenic phenotype in human melanoma cell lines. (187) PNA receptors were seen in all well differentiated prostate carcinomas, 53% of microcarcinomas, and 50% of adenosis, while no such sites could be demonstrated in benign hyperplasia. This pattern of response also correlated with early changes in the expression of ABH isoantigens. (188)

PNA is frequently used for the identification of different cryptantigens. PNA has an extraordinary preference for gastrin-secreting cells as opposed to other stomach cells. A high positive rate was obtained in PNA binding to malignant cells particularly in cases of signet ring cell carcinoma and mucinous adenocarcinomas. (196) PNA showed weak binding to normal mucosal epithelial cells but showed strong binding to malignant cells in healthy subjects and patients with dysplastic and malignant lesions of the oral cavity. (201)

The brush border of pig small intestine is a local hotspot for beta-galactoside-recognizing lectins, as evidenced by its prominent labeling with fluorescent lectin PNA. (173) Lectins in the small intestine appear to encourage bacterial overgrowth. PNA has been used to isolate suppressor T-cells in vivo, these having been first induced by ConA. *Bacteroides vulgatus*, a predominant commensal bacterium in the gut, is thought to be responsible for the development of inflammatory bowel disease (IBD). Colonization of the mice by *B. vulgatus* increased the number of Peyer's patch (PP) cells bearing PNA (peanut agglutinin)+/anti-kappa+ phenotype, which represents plasma cell-like B cells. Treatment with *Bifidobacterium infantis* reversed this. (178) Gastric biopsies from patients with peptic ulcer disease classified into *Helicobacter pylori* positive and negative groups showed a statistically significant difference in peanut agglutinin (PNA) binding between the two groups attributable to exposure of sialic acid residues on gastric epithelium in the *H. pylori* positive group. (198)

Fetal and most colon cancer cells fail to produce mucin goblets and make incomplete glycoproteins. Most colonic adenomas consist of goblet cells, localize T antigen to the stalk, and probably make complete MN glycoprotein. However, in adenomas, nonmucinous columnar cells localize T antigen to the apical cytoplasm and/or glycocalyx region and represent incomplete blood group glycoprotein synthesis. The cytologic localization of T antigen by PNA binding corresponds to the cells' ability to produce mucin goblets. (191)

PNA is mitogenic for normal human colonic epithelium. Increased T antigen expression by premalignant epithelia allows stimulation of proliferation by dietary galactose N-acetylgalactosamine-binding lectins such as PNA, which could explain the increased colon cancer risk in ulcerative colitis. (199) This may also indicate a potential complementary role in colon cancer treatment, in that adding PNA to the diet (via peanut, peanut butter, etc.) during anti-metabolic treatment should catch significantly more neoplastic cells in their reproductive stages, rendering them more susceptible to chemotherapy drugs.

Unlike other lectins (LPA, Con A, RCA-II, SBA and BPA) PNA binding did not distinguish between “young” and “old” erythrocytes due to decreases in the number and distribution density of receptor sites on the cell surface were observed in aged erythrocytes. (192)

PNA binding can be demonstrated in specimens from fetal colons between the 4th and 5th week and the 5th and 6th lunar month. PNA-binding to fetal tissues is of interest since PNA binds to adult transformed colonic tissues, but not to normal ones. (182) PNA binding glycoantigen appeared in colon carcinoma and dysplastic lesion whereas only a small amount of both antigens was found in the non-dysplastic regenerating epithelium. Consequently, a very sharp contrast between the dysplastic lesion and non-dysplastic regenerating epithelium was demonstrated by the lectin staining. (189)

In both human adenocarcinomas and an experimental tumor system, most tumor cells that metastasize show preferential binding of PNA and SBA. (153) Enhanced PNA reactivity reflected mainly cytoarchitectural pattern of tumor growth, such as syncytial lobules, whorled formations, or trabecular arrangements of meningioma cells. (175) A loss of PNA-reactive oligosaccharides is closely associated with a poor prognosis in patients with Burkitt's lymphoma. (176) Progressive loss or alteration of T antigen expression as detected by PNA staining was correlated with unfavorable staging in bladder cancer. (184)

Expression of Tn antigen is closely related to the metastasis to regional lymph nodes and may reflect an important role of this carbohydrate in the process of metastasis of cervical cancer. The rate of Tn antigen expression as measured by PNA binding was significantly higher in the metastases than in the primary lesions. (200)

PNA binds the Thomsen-Friedenreich (TF) oncofetal carbohydrate antigen (galactose beta1-3N-acetylgalactosamine alpha) that shows increased expression in colon cancer, adenomas, and inflammatory bowel disease.) PNA is mitogenic, both in vitro and in vivo, for colon epithelial cells. In these cells, PNA binds predominantly to cell-surface TF antigen expressed by high molecular weight isoforms of the transmembrane glycoprotein CD44 that are generated in inflamed and neoplastic colonic epithelia by altered RNA splicing. (177) The expression of PNA binding carbohydrates correlated with a high lymph node metastatic rate in lung adenocarcinomas. (180)

PNA showed a binding pattern partly indicating disturbed secretory cell mechanisms. Further studies with a labeled antibody directed against a milk fat globule membrane glycoprotein, which was isolated by PNA affinity chromatography from human milk, confirmed the PNA receptor as a marker of a milk protein in breast carcinomas. Additionally, two other monoclonal antibodies that were raised against other components of the milk fat globule membrane were used for a more detailed characterization of the functional situation of mammary carcinomas. Tumors with a high secretory activity responded in about 80% to endocrine treatment, whereas breast carcinomas that exhibited no secretory function failed to respond to endocrine therapy. Thereby, the hormone dependence of tumor tissue showed an inverse correlation with the proliferative activity, as determined by the mitosis index. (181,183) Others have failed to reproduce this response, however. (194)

Cryptic receptor sites for peanut agglutinin (PNA) were stained in all cases of breast carcinomas, while free PNA sites stained only in a few cases of well-differentiated carcinomas. (193) Immortalization and transformation of normal human mammary epithelial cells transfected with the neomycin resistance gene (MCF-10Aneo) or with the c-Ha-ras activated oncogene (MCF-10AneoT) bound approximately ten times more PNA than did non-transformed cells. (195) Paget's disease showed cytoplasmic staining with PNA. (190)

PSL (Pisum sativa lectin, Pisum sativa agglutinin, PSA, pea)

Pea lectin is a mixture of isolectins that can be separated by ion exchange chromatography. Fucose residues were shown to be an important determinant in the tight binding of glycopeptides to pea lectin. The specificity of PSA is similar to *Lens culinaris* lectin and ConA.

Pea lectin is a mitogen. The lectin agglutinates human group A erythrocytes at a titer of 1:4; group B erythrocytes at a titer of 1:4; group B erythrocytes at a titer of 1:2; by crude lectin preparation. (114) Like lentil lectin, PSL agglutinates a wide variety of strains of *Candida albicans*. (124) Supernatants from mice spleen cells incubated in vitro for 24 hours with pea lectin induced the production of nitric oxide (NO) when added to macrophage cultures. NO release was blocked in the presence of IFN gamma antibodies and partially inhibited by TNF alpha antibodies. (125) Pisum sativum lectin triggered basophils to release IL-4 at concentrations of up to 1ng/106 basophils. Lectins with high IL-4-inducing capacity also stimulated the release of IL-13 and histamine. (126) PSL bound consistently to the luminal surface of all ducts and acinar cells of normal breast tissue and was reactive with all carcinoma but the staining profiles were similar regardless of the tumor differentiation. (127) Transneuronal transport can be demonstrated for PSL, indicating that mechanisms exist whereby neurons exchange large molecules that could be involved in mediating trophic and other influences on target cells. (131)

The reactivity of alpha-fetoprotein to PSL is considerably different in hepatocellular carcinoma when compared to benign liver disease. (128) Normal, dysplastic, and then malignant esophageal tissues react to PSL with increasing sensitivity. (129) Non-viable sperm are more sensitive to PSL than normal sperm. (130)

Similar to PSL, PAL is a glucose/mannose-specific lectin isolated from *Pisum arvense* seeds. PAL was observed to induce mononuclear cell migration in rats. (123)

LCL (Lens culinaris lectin, lentil)

LCL is a mixture of two isoforms designated as LCL-A and LCL-B. Both isoforms have identical molecular weights, agglutination properties and are serologically identical. Based on the inhibition of its agglutination activity, LCL belongs to the group of mannose/glucose-binding legume lectins. However, studies that are more detailed have demonstrated that the lectin binds more tightly to multiple sugar residues than a single α-mannose residue. Binding of glycopeptides to immobilized LCL revealed that the presence of a fucose residue attached to the asparagine-linked GlcNAc residue is essential for a high affinity binding. In addition to fucose, two α-mannose residues capable of interacting with the lectin must also be present. (281)

The percentage of fucosylated species of alpha-fetoprotein (AFP) in total AFP is a very useful diagnostic tool to distinguish AFP due to hepatocellular carcinoma from AFP due to non-neoplastic liver diseases. LCL is a useful tool for determining the degree of fucosylation of alpha-fetoprotein (AFP) (35) and might be useful as an adjunctive marker, in combination with AFP, to exclude the presence of hepatocellular carcinoma. (282) The detection of *Lens culinaris* agglutinin-reactive fraction of alpha-fetoprotein (AFP-L3) has a significant implication for the identification of benign or malignant liver disease and the early stage predictive diagnosis of hepatocellular carcinoma while AFP increases. (283,286) Spontaneous

regression of hepatocellular carcinoma is rare. There are few reports discussing spontaneous regression associated with elevations in serum LCL-reactive alpha-fetoprotein (AFP-L3). (285)

Measurement of *Lens culinaris* Agglutinin reactive thyroglobulin ratio in serum may be useful for distinguishing between thyroid carcinoma and benign thyroid tumor. (284)

LCL triggered basophils to release IL-4 at concentrations of up to 1 mg/10(6) basophils. Lectins with high IL-4-inducing capacity also stimulated the release of IL-13 and histamine. (126) Injections of lentil *(Lens culinaris)* lectin (LCL) into the knee joint cavity of non-sensitized rabbits resulted in the development of arthritis that was indistinguishable morphologically from rheumatoid. Studies show that lectins can be used exclusively to maintain transplants in animals for up to two years. Lentil lectin (LCL) induces striking transplant tolerance in both mice and humans. (24) In mice, LCL has been used to inhibit graft versus host reactions without suppressing hematopoietic activity. Repeated administration of the lectin decreased the number of lymphocytes and red blood cells and the amount of hemoglobin; other white blood cells increased in number. (280)

Transneuronal transport can be demonstrated for LCL, indicating that mechanisms exist whereby neurons exchange large molecules that could be involved in mediating trophic and other influences on target cells. (131) LCL can distinguish between alpha-fetoprotein produced as part of the hepatocellular carcinoma and benign forms. (135, 136,137)

The measurement of serum thyroglobulin (Tg) is widely used as a marker for recurrence of thyroid carcinoma following total thyroidectomy. However, this method cannot differentiate between benign and malignant disease. LCL binding patterns are able to distinguish between benign and malignant forms of the disease, binding being significantly lower in the thyroglobulin of benign tissue. (134)

Vicia faba agglutinin (VBA, broad bean lectin)

Broad bean lectin contains a mannose/glucose specific lectin for oligosaccharides containing these sugars in the alpha configuration. The lectin agglutinates human, mouse, rat, rabbit, and guinea pig erythrocytes, but not sheep erythrocytes. The lectin is mitogenic to lymphocytes. (210, 213)

The role of the epithelial cell adhesion molecule (EpCAM) was studied using a specific monoclonal antibody in blocking studies and Western blots. The cell line LS174T differentiated in the presence of broad bean lectin, *Vicia faba* agglutinin (VFA) into gland like structures. Expression of EpCAM itself was unaffected. VFA as well as wheat germ agglutinin (WGA) and the edible mushroom lectin (*A. bisporus lectin,* ABL) significantly aggregated LS174T cells but peanut agglutinin (PNA) and soybean agglutinin (SBA) did not. VFA stimulated an undifferentiated colon cancer cell line to differentiate into gland like structures. The adhesion molecule EpCAM is involved in this. Dietary or therapeutic VFA may slow progression of colon cancer. (209)

IL-8 production appeared to be specifically triggered upon stimulation with *Lens culinaris, Phaseolus vulgaris* and *Vicia faba* lectins. The IL-8 secreted may induce the extravasation of activated neutrophils and generate tissue damage. (211)

Like many lectins, the hemagglutinating activity of purified *Vicia faba* lectin was enhanced in the presence of gums; gum guar caused the highest enhancement. (212)

UEA (Ulex europaeus lectin, gorse, furze)

Seed extracts of *Ulex* contain two main lectins: the anti-H/O type UEA-I and the non-blood group specific UEA-II. Because of its blood group specificity UEA-I has been extensively used to study blood group active glycoconjugates as well as the isolation and characterization of other complex glycoconjugates. (35)

Purified UEA-I is glycosylated and shows high specificity for fucose or fucosylated structures. UEA-I preferentially binds to Fucα(1,2)Galβ(1,4)GlcNAc (H type-2 blood group antigen) with a cross reactivity to Fucα(1,2)Galβ(1,4)-[Fucα(1,3)]GlcNAcβ (Lewis Y-antigen), but not to internal αL-fucose units. UEA-I agglutinates blood group H(O) and has been shown to agglutinate human group A erythrocytes at a titer of 1:32 and group B erythrocytes at a titer of 1:16, by crude lectin preparation (16mg/ml). (37)

UEA-I binds to endothelial cells and has applications in vasoformative tumors such as angiosarcoma, or blood vessel invasion in follicular carcinoma of the thyroid, Tumor angiogenesis seems to be a useful prognostic indicator in many tumors as neovascularization is a hallmark of the more aggressive neoplasms. UEA-I is the preferred method used to outline blood vessels within tumors. (255)

UEA-II is not blood group specific, although it does precipitate blood groups A1, A2B, H, Lewis b, and Lewis a substances to varying degrees. (254)

UEA-I can be used as histochemical markers for normal and transformed parotid glands. Mucoepidermoid carcinoma stroma presented a direct relation between malignancy and staining intensity for UEA-I. (256)

Cervical mucins are glycosylated proteins that form a protective cervical mucus and carry multiple α(1,2)fucosylated glycans. UEA-I can distinguish mucins that partly protect from experimental vaginal candidiasis. (257)

Chronic gastritis and esophagitis are associated with changes in mucosal glycosylation patterns. Comparisons of lectin-staining scores between GERD and Barrett's esophagus revealed significant increases of UEA-I in both the stratum superficiale and stratum spinosum. Lectin UEA-I-binding proteins were specifically increased in the squamous epithelium of patients with Barrett's esophagus. (258) UEA I-binding patterns can provide further information in cases of oral squamous cell carcinoma regarding the potential for metastasis and tumor prognosis. (261)

UEA I-modified nanoparticles might serve as potential carriers for brain drug delivery, especially for mental therapeutics with multiple biological effects. Surface engineering of nanoparticles with lectins opened a novel pathway to improve the brain uptake of agents loaded by biodegradable PEG-PLA nanoparticles following intranasal administration. UEA I binds specifically to L-fucose, which is largely located in the olfactory epithelium. (259)

Mechanisms governing the normal resolution processes of inflammation are poorly understood. The removal of apoptotic cell material and their potentially toxic contents is a prerequisite of resolution. Engulfment by macrophages is an important disposal route. UEA I showed no cytotoxic activity and bound preferentially to dying

cells, indicating that l-fucose epitopes, in addition to N-acetylglucosamine and mannose epitopes are increasingly exposed on cells undergoing apoptosis. (260)

UEA-I can track the distribution of the H antigens of type 1, type 2, and type 3/4 chains of the ABO(H) histo-blood group system in human normal colon and in colon cancer. Mucosa of the normal colon from secretors, but not that from non-secretors, expressed only H type 1 and did not express H type 2 or H type 3/4. The expression of H type 1 in the normal colon and the aberrant expressions of H type 2 and H type 3/4 in colon cancer tissues were regulated by FUT2-encoded *Se* type α(1,2)fucosyltransferase. However, UEA-I-positive substance rather than H type 2 were uniquely expressed throughout the normal colon and in colon cancers from both secretors and non-secretors. (262) The frequency of high-grade dysplasia seen in the neoplastic cells of 52 colorectal adenomas was significantly greater in older patients and in samples with UEA-I positivity without neuraminidase pretreatment. UEA-I-reactive adenomas were generally characterized by high cell proliferation rates. A statistical model based on patient's age and UEA-I binding without neuraminidase treatment can generally predict grade of dysplasia in 83% of adenomas and particularly high-grade dysplasia in up to 93% of adenomas. Such a model may be potentially useful for the early detection of neoplasia, for instance in exfoliative cells from the large intestine. (263)

Total parenteral nutrition (TPN) for 2 weeks promoted intestinal atrophy and decreased absolute quantity of mucus gel. UEA-I staining correlates with changes in mucus gel. (264)

Glycosylation of IgG was suggested to be important in the etiology of rheumatoid diseases. Galactose and fucose are highly increased in the juvenile chronic arthritis. Fucosylation was analyzed using fucose-specific UEA I lectin. Fucose was found to be approximately 40% increased in RA patients with very high statistical significance ($p = 0.00095$). (265) Patients in whom juvenile chronic arthritis was currently active had significantly lower levels of galactose than those in remission, in whom galactose levels were comparable to the control group. Fucose levels in both groups of patients were significantly higher than in the control group. These results show that whereas degalactosylation is a good test to detect and measure the activity of juvenile chronic arthritis, increased fucosylation is a much more reliable measure for diagnosis of the disease itself. (267)

EA I binding to muscle fibers was observed in a small number of biopsies with inflammatory myopathy, but not in other diseases, including neurogenic muscular atrophies and muscular dystrophies. UEA I binding fibers were observed in 3 of 28 patients (11%) with other collagen diseases, 11 of 36 (31%) without these disorders, and 2 of 6 (33%) with inclusion body myositis. (266)

UEA-I binds to enteroendocrine cells in the ileum and caecum of humans, rabbits, rats, and mice. In all species investigated, numerous cells scattered in the crypt and villus epithelia intensely bound the UEA-I lectin. These cells proved to be argyrophilic, and they were identified as enterochromaffin cells and peptide tyrosine tyrosine (PYY) cells by immunohistochemistry. At the ultrastructural level, fucose binding sites were located in the matrix of the electron-dense secretory granules of these cells and in the glycocalyx covering their apical membrane. The presence of fucose residues in the apical membrane of enteroendocrine cells indicates that this membrane domain has a specialized composition of intramembranous glycoconjugates that could be involved in receptive and/or secretory functions. (268)

In breast cancer, UEA-I binding to the cancer cells can be a reliable indicator for axillary metastases, and the need for additional therapeutic interventions. Analysis of Formalin-fixed, paraffin-embedded tissues from 43 cases of breast carcinoma stained with UEA-I showed a significant relationship with blood vessel invasion ($P < 0.01$) and lymphatic vessel invasion ($P < 0.05$). (269)

Mannose-binding lectins from monocots

The monocot mannose-binding lectins are an extended superfamily of structurally and evolutionarily related proteins, which until now have been isolated from species of the Amaryllidaceae, Alliaceae, Araceae, Orchidaceae, and Liliaceae. The monocot mannose binding lectins have received much recent interest. The exclusive specificity of these lectins toward mannose has been exploited for the analysis and isolation of mannose-containing glycoconjugates. Other applications in biomedical research are based on the potent inhibitory effect of some monocot mannose-binding lectins on human and animal retroviruses (including HIV), and on their ability to block the adhesion receptors of mannose-fimbriated Escherichia coli in the small intestine of rats. Monocot mannose-binding lectins have also become an important tool in plant protection and plant biotechnology because their genes confer resistance against sucking insects and nematodes. (270) Characterization of the lectins from onion (*Allium cepa*), shallot (*A. ascalonicum*) and leek (*A. porrum*) has shown that these lectins differ from previously isolated Alliaceae lectins not only in their molecular structure but also in their ability to inhibit retrovirus infection of target cells. (271)

Allium cepa lectin (onion)

The lectin in onion represents a minor protein. Onion lectin readily agglutinates rabbit but not human erythrocytes. (35)

Allium porrum lectin (leek)

The lectin in leek represents a minor protein. Leek lectin readily agglutinates rabbit but not human erythrocytes. (35) Pure preparation of leek lectin strongly inhibits infection of MT-4 cells by HIV-1 and HIV-2 in vitro. Of all the Alliaceae lectins leek lectin proved to be the most effective inhibitor of HIV infection in vitro. (272)

ASA (Allium sativum lectin, garlic)

At present, four different lectins have been isolated from garlic. Two lectins (ASA-I and ASA-II) have been isolated from the bulb: one from the leaves (ASA-L) and one from the roots (ASA-RI.) Human antiserum contains natural antibodies to the mannose-specific lectins from garlic bulbs. (36) Bulb lectins appear to be storage proteins because the concentration is developmentally regulated and because of the abundance of the lectins in the bulb.

Mannose-specific agglutinins from garlic (*Allium sativum*) form part of a well-conserved super-family of bulb lectins. (273) Compared with other mannose-

binding lectins, ASAI and ASAIII bind to mannose very weakly. Methyl-α-d-mannopyranoside is six times better as an inhibitor than mannose. (274)

All garlic lectins agglutinate rabbit but not human erythrocytes. (35) They differ from each other in their specific agglutination activity. ASA-L is about 50 times more active than ASA-I, whereas ASA-II and ASA-RI are about 500 times less active than ASA-L.

Unclassifiable L-type plant Lectins

Zea mays lectin (corn)

The lectin in Zea mays found in prepared food (canned maize) was found to be autoclave resistant. The results of serological studies of Zea mays var. everta seed extracts with anti-B specificity showed that the lectin agglutinates A1B erythrocytes significantly more weakly than erythrocytes of B and A2B blood groups. (84) According to preferential monosaccharide specificity, salt-soluble lectins of corn seed comprise at least two distinct types: N-acetyl-d-galactosamine-interactive and mannose-interactive lectins. (85)

Psidium guajava lectin (guava)

Guava has a galactose-specific lectin that prevents adhesion of E. coli O157:H7 to red cells; this lectin is mediated by galactose. Prevention could also be due to its capacity of agglutinating E. coli by guava lectins. (119)

Bacterial lectins

Many bacterial species and genera express lectins, frequently more than one type and with distinct specificities. In Gram-negative bacteria, such as *E. coli, K. pneumoniae* and Salmonella species, the lectins are often in the form of sub-microscopic hair-like appendages, known as fimbriae or pili that protrude from the surface of the cell. Fimbrial surface lectins are also produced by Gram-positive bacteria among them are the oral *Actinomyces naeslundii* and *Actinomyces viscosus*. (2005) In rare cases, lectins are found in a predominantly intracellular distribution, as in the case of PA-IL and PA-IIL in *Pseudomonas aeruginosa.* (204*)*

Table 3.5 Bacterial surface lectins

Organism	*Carbohydrate specificity*	*Form*[a]
Actinomyces naeslundii	Galβ3GalNAc	GP
Campylobacter jejuni[b]	Fucα2Galβ4GlcNAc	GP
Escherichia coli Type 1	Manα3(Manα6)Man	GP
P	Galα4Gal	GSL
S	Neu5Acα2,3Galβ3GalNAc	GSL
CFA/1	Neu5Acα2,8-	GP
K1	GlcNAcβ4GlcNAc	GP
K99	Neu5Acα2,3Galβ4Glc	GSL
Haemophilus influenza	$(Neu5Ac\alpha2,3)_{0,1}$Galβ4GlcNAc-β3Galβ4GlcNAc	GSL
Helicobacter pylori	Neu5Acα2,3Galβ4Glc(NAc); Fucα2Galβ3(Fucα4)Gal	GP
Klebsiella pneumoniae	Man	GP
Mycoplasma pneumoniae	Neu5Acα2,3Galβ4Glc(NAc)	GP
Neisseria gonorrhoeae	Galβ4Glc(NAc)	GSL
Neisseria meningitidis	$(Neu5Ac\alpha2,3)_{0,1}$Galβ4GlcNAc – β3Galβ4GlcNAc	GSL
Salmonella typhimurium	Man	GP
Streptococcus pneumoniae	$(Neu5Ac\alpha2,3)_{0,1}$Galβ4 - GlcNAcβ3Galβ4GlcNAc	GSL
Streptococcus sanguis	Neu5Acα2,3Galβ3GalNAc	GP
Streptococcus suis	Galα4Galβ	GSL

Figure 6.4.3 Bacterial surface lectin specificities (from Sharon N and Lis H. *Lectins*. Second Edition. Kluwer Academic Publishers. 2003)

Viral lectins

Viruses contain sugar-specific surface proteins or glycoproteins that act as hemagglutinins, and they are therefore classified as lectins. Most of the information is available for the influenza and polyomaviruses, belonging to the orthomyxoviruses and papoviruses. Similar lectins, although less defined, are found in Newcastle disease, rotavirus, HIV and Herpes simplex.

Protozoan lectins

Two surface proteins of the pathogenic form of amoeba (*Entamoeba histolytica*) are specific for N-acetylglucosamine (GlcNAc) and for Gal/GalNAc, respectively. *Plasmodium falciparum* possesses two lectins: one specific for N-acetylneuraminic acid (NANA) and the other for GlcNAc. P. falciparum sporozoites possess a lectin specific for heparan sulfate. A variety of Trichomonas species have lectins specific for NANA.

Virus	*Specificity*
Corona viruses	
Bovine	$Neu5,9Ac_2$
Herpes viruses	
Herpes simplex	Heparan sulfate
Myxoviruses	
Orthomyxo	
Influenza A & B (human strains)	$Neu5Ac\alpha2,6Gal[\beta4Glc(NAc)]_{0,1}$
Influenza A & B (porcine strains)	$Neu5Ac\alpha2,3/6Gal[\beta4Glc(NAc)]_{0,1}$
Influenza C	$Neu5,9Ac_2$
Paramyxo	
Newcastle disease	$Neu5Ac\alpha2,3Gal[\beta4Glc(NAc)]_{0,1}$
Sendai	$Neu5Ac\alpha2,8Neu5Ac$
Rotavirus	$Neu5Ac^a$
Papoviruses	
Polyoma	$Neu5Ac\alpha2,3Gal[\beta4Glc(NAc)]_{0,1}$
	$Neu5Ac\alpha2,3Gal\alpha3(Neu5Ac\alpha2,6)$-GalNAc
Picornaviruses	
Foot-and-mouse disease	Heparan sulfate
Retroviruses	
HIV	Man-OS; heparin; dextran sulfate

Figure 6.4.4 Viral lectin specificities (from Sharon N and Lis H. *Lectins*. Second Edition. Kluwer Academic Publishers. 2003)

Fungal lectins

Fungal lectins are isolated from fruiting bodies and the mycelium of numerous species of macromycetes. The physiological role of fungal lectins covers, among other things, participation in the process of forming fruiting body primordia, the creation of mycelium structures easing the penetration of parasitic fungi into the host organism, as well as the identification of appropriate partners during the early stage of mycorrhization. Several fungal lectins, possessing antitumor, antiproliferative, and immunomodulatory activities are under clinical trial.

Agaricus bisporus lectin (ABAI-IV, common commercial mushroom)
A. campestris lectin (Meadow mushroom)

All ABA lectins were found to have quite similar carbohydrate-binding specificities. ABA lectin stimulates insulin release from pancreatic islets. Lectins from *Agaricus bisporus* and *Agaricus campestris* stimulate insulin and glucagon release from isolated rat islets in the presence of glucose. In the case of insulin release, maximal stimulation was observed at lectin concentrations above 58 mugs per milliliter (approximately 1 muM). *A. bisporus* PHA-B-stimulated insulin release was independent of a source of metabolic energy, but was abolished by deuterium oxide. (206)

It is proposed that the specific interaction between mushroom lectin and its receptors may lead to conformational changes in the structure of the membranes of the islet A2- and B-cells that facilitate exocytosis. Effects of both lectins are potentiated by amino acid alanine. Actions are "anti-hyperglycemic, insulin-releasing, and insulin-like." (207) Mushroom lectin also improved the hypoglycemic effect of exogenous insulin,

The lectin seems to have some reactivity towards human group A erythrocytes. (17)

The lectin from the common mushroom *Agaricus bisporus,* the most popular edible species in Western countries, has potent antiproliferative effects on human epithelial cancer cells, without any apparent cytotoxicity. *A. bisporus* lectin can alter the proliferation of colonic cells. This property confers to it an important therapeutic potential as an antineoplastic agent. A normal healthy cell is well differentiated, meaning that its characteristic features and structures are intact. Differentiation is regulated by glycosylated, adhesion molecules that are targets for lectin binding. Researchers from Hammersmith Hospital, London, assessed the effects of dietary lectins on the behavior of cancer cells in the colon. A. bisporus lectin is a reversible, noncytotoxic inhibitor of epithelial cell proliferation that deserves study as a potential agent for cancer therapy. This effect is thought to be a consequence of the selective blocking by ABL of nuclear localization sequence-dependent protein import, which is essential for cell functioning. The remarkable anti-neoplastic properties of ABL are due to the selectivity with which it binds the T-antigen disaccharide moiety, *i.e.* Galβ1–3GalNAc. (215)

As we've seen, Thomsen-Friedenreich antigen (Gal beta-1,3-GalNAc) the Class I core sequence in O-linked oligosaccharide chains, behaves as an oncofetal antigen showing increased expression in many epithelial malignancies. Previous work has shown that peanut agglutinin (PNA), a lectin that binds Gal beta-1,3-GalNAc, stimulates proliferation in HT-29 (human colon cancer) cells and normal human colonic epithelium and this implies that cell surface glycoproteins which express Gal beta-1,3-GalNAc may play an important role in the regulation of epithelial cell proliferation. *A. bisporus* lectin (ABL) another dietary Gal beta-1,3-GalNAc-binding lectin, differs from PNA in its ability to also bind to sialylated Gal beta-1,3-GalNAc. In contrast to PNA, ABL (25μg/ml) inhibited incorporation of [3H]-thymidine into DNA of HT29 colon cancer cells by 87% (95% confidence limit, 85-89%), Caco-2 colon cancer cells by 16% (95% confidence limit, 12-20%), MCF-7 breast cancer cells by 50% (95% confidence limit, 47-52%), and Rama-27 rat mammary fibroblasts by 55% (95% confidence limit, 51-60%) when these cells were grown for 24 hours in serum-free medium. (208)

The effect of the lectins that bind the T antigen on cell proliferation can be very pronounced and very different. For instance, the peanut agglutinin (*Arachis hypogaea* agglutinin) (PNA) stimulates the proliferation of human intestinal epithelial cells, whereas jacalin has the opposite effect, *i.e.* strong inhibition of cell growth (214) ABL has the remarkable property of reversibly inhibiting the proliferation of malignant epithelial cell lines without any apparent cytotoxicity for normal cells. This effect is thought to be a consequence of the selective blocking by ABL of nuclear localization sequence-dependent protein import, which is essential for cell functioning.

A second study, examining the effects of dietary lectins on differentiation, adhesion, and proliferation of colorectal cancer cells, assessed differentiation in three-dimensional gels, adhesion by aggregation assay, and proliferation by 3H thymidine

incorporation. The role of the epithelial cell adhesion molecule (EpCAM) was studied using a specific monoclonal antibody in blocking studies and Western blots. The cell line LS174T differentiated in the presence of broad bean lectin, *Vicia faba* agglutinin (VFA) into gland like structures. Expression of EpCAM itself was unaffected. VFA, as well as wheat germ agglutinin (WGA), and the edible mushroom lectin (A. bisporus lectin, ABL) significantly aggregated LS174T cells, but peanut agglutinin (PNA) and soybean agglutinin (SBA) did not. VFA stimulated an undifferentiated colon cancer cell line to differentiate into gland like structures. The adhesion molecule EpCAM is involved in this. Dietary or therapeutic VFA may slow progression of colon cancer. (209)

Candida albicans adhesins

The interaction of *Candida* species with their cognate host receptors is a key factor in the pathogenesis of different types of candidiasis. The recognition of different forms of *Candida albicans* by Toll-like receptors 2 and 4 on mononuclear leukocytes has recently been discovered to determine the function and activity of regulatory T-cells, determine the balance of Type 1 and Type 2 cytokines, and thereby influence the antifungal activity of both the innate and adaptive immune response. (220)

Different forms of *C. albicans* are also recognized by different lectins that are expressed on the surface macrophages. *C. albicans* and *Candida glabrata* express the ALS (agglutinin-like sequence) and EPA (epithelial adhesin) families of adhesins, respectively. The Candida adhesin, encoded by the EPA1 gene, is likely a glucan-cross-linked cell-wall protein and binds to host-cell carbohydrate, specifically recognizing asialo-lactosyl-containing carbohydrates (223) and fucose monosaccharides. (224) Protein adhesins of C. albicans are expressed preferably on the mycelial form.

Mannose-binding lectin (MBL), a component of the innate immune system, binds oligosaccharides on the surface of microorganisms to form complexes that activate the complement cascade and facilitate phagocytosis. Like macrophages, dendritic cells recognize Candida by the mannose-fucose receptor whereby the yeast is phagocytosed, killed, and processed for antigen presentation. (222) Problems with MBL play a role in recurrent vulvovaginal candidiasis (RVVC) and its production may correlate to vulvar/vaginal colonization by Candida, hormonal contraceptive use, and antifungal therapies. (216) RVVC due to deficient MBL production is more easily helped with antifungal medication than is RVVC due to some other mechanism.

The presence of RVVC can increase vaginal MBL level, which may be an immune response against *Candida albicans* infection; in women with RVVC, the low level of MBL in the vagina caused by mutation in the MBL gene may play a role in the recurrence of the infection. (221) The MBL2 codon 54 gene polymorphism is more frequent in Belgian women suffering from RVVC than in controls. The presence of the B allele is associated with a superior response to fluconazole maintenance therapy, as compared with RVVC patients without this polymorphism. (218)

Candida albicans binds l-fucose residues on cell surface glycoconjugates may represent recognition molecules for interactions between the yeast strain and blood group antigens. Glycans such as alpha-d-glucose/alpha-d-mannose, N-acetyl-d-glucosamine/N-acetylneuraminic acid and d-galactose/N-acetyl-d-galactosamine are components of fungal cell wall. (217)

The galectin family of lectins recognizes saccharide ligands on a variety of microbial pathogens, including viruses, bacteria, and parasites. Galectin-3, a galectin expressed by macrophages, dendritic cells, and epithelial cells, binds bacterial and parasitic pathogens. Galectin-3 can act as a pattern recognition receptor that recognizes a unique pathogen-specific oligosaccharide sequence. Galectin-3 bound only to *Candida albicans* species that bear beta-1,2-linked oligomannans on the cell surface. Surprisingly, binding directly induced death of *Candida* species containing specific beta-1,2-linked oligomannosides. (219)

The secretion of pro-inflammatory cytokines and expression of leukocyte adhesion molecules by endothelial cells, in response to *C. albicans* could enhance the host defense against this organism by contributing to the recruitment of activated leukocytes to sites of intravascular infection. *C. albicans* induced endothelial cells to express mRNA's encoding E-selectin, intercellular adhesion molecule 1, vascular cell adhesion molecule 1, interleukin 6, interleukin 8, monocyte chemoattractant protein 1, and inducible cyclooxygenase (cox2). (225)

VERTEBRATE AND INVERTEBRATE ANIMAL LECTINS

Invertebrate lectins

The biological roles of invertebrate agglutinins have been and remain subjects of unresolved controversy. Classical studies on invertebrate agglutinins have emphasized their hemagglutinating properties, an approach that has been criticized for its lack of biological relevance. Although erythrocyte agglutination has proven useful for determining various properties of invertebrate agglutinins, it does not address the question of their natural function. More recently, invertebrate agglutinins have been investigated for their ability to interact with pathogenic agents such as bacteria, yeast and protozoa. The lectin in edible snail has attracted considerable attention for its preferential binding proclivities for metastatically transformed cells.

HPA (Helix pomatia agglutinin, Roman snail, escargot)

HPA has a limited binding repertoire in that its combining site may be no larger than a single terminal non-reducing α-N-acetylgalactosamine residue (157), and it will thus recognize any glycoconjugate terminating in this structure. Preliminary analyses have indicated that HPA recognizes a heterogeneous range of glycoconjugates in breast cancer, including, amongst others, glycoproteins bearing the Tn epitope (N-acetylgalactosamine-O-Ser/Thr) and blood group A substance. (158) HPA-binding partners have been imprecisely characterized, and it remains unclear whether different tumor samples express the same, or different, profiles of HPA-binding molecules and whether any, or all, of these are expressed by “normal” cells. The innate specificity of the large HPA combining groove (aside from its avid reactivity with appropriately spaced GalNAc alpha-O-) remains obscure, despite careful investigation. This phenomenon was used successfully to accurately predict, in studies on 305 breast CA patients, early or late CA recurrence and patient survival time. (170)

The expression of the aberrant N-acetylgalactosamine (GalNAc) glycoconjugates, detected by binding of the lectin from HPA is reported to be associated with metastatic competence and poor prognosis in a range of human adenocarcinomas, but the functional significance of the glycoconjugates in metastatic mechanisms is unknown. HPA has emerged as a marker of altered glycosylation in cancer, where expression of glycoconjugates recognized by the lectin appears to be associated with poor patient prognosis. (159) All breast cancer cell lines exhibited, in addition to cell-surface HPA binding, a striking localization of the lectin to a discrete area in the perinuclear region of the cytoplasm, possibly corresponding to Golgi apparatus or endoplasmic reticulum. These observations may be consistent with increasing disruption in glycosylation pathways in the Golgi apparatus/endoplasmic reticulum, or in transport pathways, leading to a concentration of immature N-acetyl-galactosaminylated glycoforms in these cellular compartments. (167)

This association has been reported in breast cancer (160,161) and in other common adenocarcinomas including gastric cancer (162), carcinoma of the esophagus (163) colorectal carcinoma (164), carcinoma of the thyroid (165) and prostate cancer. (166) Around 80% of metastases arising from primary breast cancer are predictably HPA positive, but, intriguingly, around 20% do not express HPA binding glycoproteins reflecting the complexity of metastatic mechanisms and the further disruptions in cellular glycosylation that attend tumor progression. HPA binding is a

secondary prognostic factor, strongly associated with the presence of metastases in local lymph nodes. (171)

It appears plausible that the tumor oligosaccharides bound by HPA might be ligands for a putative lectin-like receptor on another cell type, and thus implicated in cell–cell adhesion. Alternatively, the alteration in cellular glycosylation represented by the expression of HPA-binding ligands may conversely result in a failure of recognition by putative oligosaccharide receptors resulting in loss of cell–cell adhesion, which could potentially be of significance in cancer cell migration at either the primary or the secondary site. Leathem and Brooks presumed that HPA recognizes a hitherto "undefined biological marker" that indicates a breast cancer's aggressiveness that they putatively termed "ligand like complex" (LLC). (170) The precise nature of the metastasis-associated HPA binding partner(s) is a question of some interest, but thus far remains unclear. HPA will recognize, for example, the Tn epitope and blood group A antigen, but its prognostic significance appears to be through recognition of a much broader and heterogeneous array of N-galactosaminylated glycoproteins. The results suggest that the prognostic significance of HPA binding in breast cancer is unlikely to be simply through recognition of blood group A antigen or Tn epitope on cancer cells, as first suggested by Springer. (1969) It has also been suggested (160) that exposure of terminal GalNAc, detectable by HPA, might result from a failure of sialylation mechanisms, sialic acid being a common terminal monosaccharide that could be masking sub-terminal GalNAc in HPA-negative tumors.

Aberrancies in IgA1 glycosylation have been linked to the pathogenesis of IgA nephropathy (IgAN), a kidney disease characterized by deposits of IgA1-containing immune complexes in the glomerular mesangium. HPA was shown to discriminate very effectively between the IgA1 secreted by cell lines derived from peripheral blood cells of patients with IgAN and of healthy controls. (168)

Electrophorus electricus lectin (electrolectin, electric eel)

Electrolectin, a beta-D-galactoside binding lectin, has been isolated from the electric organ of the electric eel Electrophorus electricus. Electrolectin is a dimer composed of two subunits. The molecular weight of the monomer is around 16,500 as determined by sodium dodecyl sulfate-gel electrophoresis and amino acid analysis. The molecular weight of the dimer determined by equilibrium sedimentation is 32,500 +/- 750. The electrolectin monomer is composed of 144 amino acids and 2.2 +/- 0.45 carbohydrates. It contains one tryptophan but cysteine and metals are absent. The exposure of electrolectin to O2 destroys its hemagglutination activity, abolishes its UV fluorescence, and shifts its UV absorption maximum from 287 nm to 250 nm. (202)

Antibodies to electrolectin, a lectin endogenous to embryonic skeletal muscle, have been used to study the distribution of electrolectin during myogenesis in L6 cells and rat primary muscle culture, indicating its role in myogenesis and synaptogenesis. (203)

Electrolectin was shown to prevent and effectively treat experimental autoimmune myasthenia gravis in rabbits, considered a good model for the human disease myasthenia gravis. Administration of electrolectin to the afflicted rabbits lead in all cases to complete recovery, presumably through modulation of the suppressor cell activity directed against acetylcholine receptor protein self antibodies. (24)

Electrolectin (β-galactoside-binding lectin, galaptin, L-14) depending on its source is now considered homologous to galectin-1.

Lumbricus terrestris lectin (earthworm)

Agglutinins in the coelomic fluid of Lumbricus terrestris are inhibited by a number of glycoproteins and polysaccharides. Naturally occurring agglutinins are inhibited most strongly by bovine submaxillary mucin and fetuin; and induced agglutinins are inhibited most strongly by thyroglobulin, bovine submaxillary mucin, hyaluronic acid, and fetuin.

Hemolytic activity in coelomic fluid of Eisenia fetida (ECF) is due to three proteins H1, H2, H3 with molecular weights of 46, 43, and 40kDa, respectively. H1 and H2 were shown to be stable in SDS and alpha-2-ME, whereas H3 splits into two fragments with molecular weights of 18 and 21kDa after SDS treatment. IEF indicates that each protein consists of different isoforms with protein isoelectric points (pIs) between 5.1 and 6.2. H3 was demonstrated to be a bifunctional protein that can lyse and agglutinate erythrocytes. (226)

Vertebrate lectins

Until the late 1980's, the major source of animal lectins was invertebrates. During the last decade, numerous lectins have been isolated from higher animals, and their number is fast growing. Unlike plant lectins, which can be grouped in families along taxonomic lines, animal lectins often exhibit structural similarities even when derived from diverse phyla. These lectins are therefore classified largely based on shared sequence characteristics of their carbohydrate recognition domains (CRD's). According to a recent count, at least 12 structural families of animal lectins are known to exist. The major ones are the C-type lectins (a superfamily), galectins, and Siglecs. However, not all animal lectins fall into any of the known families.

Galectins

The most widely occurring family of animal lectins is that of the galectins, (originally S-lectins), so-called because they bind specifically to beta-galactoside in a calcium-independent manner. Thirteen mammalian galectins have been described, as well as many additional ones from other species, including birds, lower vertebrates, worms and sponges. Galectins are probably unique among all types of animal lectins in that they can be found in the nucleus, cytoplasm, outer plasma membrane, and extracellular matrix. Galectins are probably the most ancient class of glycan-binding proteins, and they are found in all metazoans examined, from sponges and fungi to both invertebrates and vertebrates. Galectins can contribute to cell–cell and cell–matrix interactions, and galectin signaling at the cell surface can modulate diverse cellular functions.

Extracellularly, galectins are able to exhibit bivalent or multivalent interactions with cell-surface glycans on various immune cells and exert various effects. These include cytokine and mediator production, cell adhesion, apoptosis, and chemoattraction. In addition, they can form lattices with cell-surface glycoprotein receptors, resulting in modulation of receptor functions, including clustering and endocytosis. Intracellularly, galectins can participate in signaling pathways and modulate biologic responses. These include apoptosis, cell differentiation, and cell migration. Intracellular galectins may interact with

intracellular ligands to regulate cellular activities and may contribute to some fundamental processes such as pre-mRNA splicing.

A large body of literature indicates that galectins play important roles in the immune and inflammatory responses through regulating the homeostasis and functions of immune cells. Current research indicates that galectins play important roles in the development of acute inflammation as well as chronic inflammation associated with allergies, autoimmune diseases, atherosclerosis, infectious processes, and cancer. (228) Galectin-glycan lattices can determine receptor residency time by inhibiting endocytosis of glycoprotein receptors from the cell surface, thus modulating the magnitude or duration of signaling from the cell surface. (308)

The interactions of galectins with glycans are complex and several factors contribute to high-affinity binding, including the natural multivalency and oligomeric state of the galectins, the multivalency of their natural glycoconjugate ligands, and the mode of presentation of the glycans. Most members of the galectin family tested so far bind simple β-galactosides, such as disaccharides or trisaccharides, but the affinity is relatively weak. (230) Galectins are widely distributed in animals with a wide variety of functions, including inhibition of chronic inflammations, Graft versus Host, and allergic reactions.

Although the expression of galectins in animal tissues is tightly regulated, their expression can be induced and this may be especially important in innate immune responses. For example, both galectin-1 and galectin-3 are upregulated in gastric epithelial cells that are infected by *Helicobacter pylori*, and galectin-9 can be induced upon exposure of periodontal ligament cells to *Porphyromonas gingivalis* lipopolysaccharide.

Galectins have a developmental effect on phenotypic expression. Lack of galectin-3 in knockout mice is associated with several phenotypic changes, such as fatty liver disease, reduced mast cell function, reduced liver fibrosis upon induced liver damage, and age-dependent glomerular lesions. In contrast, lack of galectin-1 in mice is associated with a different set of interesting phenotypic changes, including decreased sensitivity to noxious thermal stimuli, altered primary afferent neural anatomy, aberrant topography of olfactory axons, and reduced muscle regeneration ability after injury.

Galectin-1 (Gal-1)

Gal-1 is differentially expressed by various normal and pathological tissues, and it appears to be functionally polyvalent, with a wide range of biological activity. The intracellular and extracellular activity of Gal-1 has been described. Evidence points to Gal-1 and its ligands as one of the master regulators of such immune responses as T-cell homeostasis and survival, T-cell immune disorders, inflammation and allergies as well as host–pathogen interactions. Gal-1 expression or overexpression in tumors and/or the tissue surrounding them must be considered as a sign of the malignant tumor progression that is often related to the long-range dissemination of tumoral cells (metastasis), to their dissemination into the surrounding normal tissue, and to tumor immune-escape. (227) Gal-1 can induce some anti-inflammatory cytokines, such as IL-5, IL-10, and transforming growth factor-β (TGF-β) in activated T cells, and it can inhibit production of pro-inflammatory cytokines, such as IL-2, tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ). (230)

Galectin-3 (Gal-3)

Gal-3 is associated with activation of T cells perhaps by interacting with the poly-N-acetyllactosamine-containing N-glycans on the T-cell receptor (TCR). Gal-3 can also

inhibit IL-5 production in several immune cells, including human eosinophils. On the other hand, Gal-3 can activate mast cells, neutrophils, and monocytes, in terms of mediator release and production of reactive oxygen species. Lack of galectin-3 in knockout mice is associated with reduced mast cell function, reduced accumulation of asthma-associated leukocytes in airway inflammation, and reduced peritoneal inflammatory responses. Gal-3 may be a culprit biomarker in heart failure. It was observed that Gal-3 is specifically upregulated in decompensated heart failure compared with compensated heart failure in animal models of heart failure. This has been associated with activation of fibroblasts and macrophages, which are a hallmark of cardiac remodeling. (229)

L-type lectins in vertebrates

L-type lectins in animal cells are involved in protein sorting in luminal compartments of animal cells. In humans and other mammals, there are four L-type lectins: ERGIC-53, ERGL, VIP36, and VIPL. ERGL is found only in mammals and VIP36 is restricted to vertebrates, but ERGIC-53 and VIPL are also found in invertebrates.

ERGIC-53

ERGIC-53 is a type I transmembrane protein with a single luminal L-type CRD and neck region containing a coiled-coil domain, named for its localization to the endoplasmic reticulum-Golgi intermediate compartment (ERGIC), a stepping-stone in the trafficking of proteins from the ER to the Golgi. ERGIC-53 binds in a calcium-dependent manner to the high-mannose glycans borne by folded glycoproteins in the ER lumen that are ready to exit the ER. It transports such proteins to the ERGIC, where the slightly lower pH triggers dissociation of receptor and cargo, allowing the receptor to recycle back to the ER. ERGIC-53 is only essential for the trafficking of a subset of glycoproteins. It is known to assist the ER exit of cathepsins C and Z and blood coagulation factors V and VIII. Mutations in the human ERGIC-53 gene cause a clotting deficiency because of the reduced secretion of these clotting factors into serum.

ERGL (ERGIC-53-like) is a mammalian protein related to ERGIC-53, which interestingly lacks a number of key residues identified in other animal L-type lectins as being critical for calcium- or sugar-binding activity. Expression of both human and rat ERGL (termed SLAMP) is restricted to a small number of specific tissues and cell types, where the protein is localized to the ERGIC, suggesting a specialized role in assisting the secretion of specific glycoproteins.

VIP36 (vesicular integral membrane protein of 36 kDa)

VIP36 is a type I transmembrane protein with a single luminal L-type CRD, which cycles between the ER and the Golgi. VIP36 exhibits shared sugar-binding specificity with ERGIC-53. It binds with highest affinity to high mannose N-linked glycans of the type found on glycoproteins that are correctly folded and have been processed by the glycosidases of the ER but not those of the Golgi. VIP36 shows a different pH-dependence of ligand binding to ERGIC-53, exhibiting optimum ligand binding at pH 6.5, at which pH level ERGIC-53 releases ligands, with reduced binding at more or less acidic conditions. VIP36 may function to traffic proteins from the ERGIC to the cis-Golgi, or may mediate retrograde transport of glycoproteins that have escaped correct glycan trimming and modification.

VIPL (VIP36-like) is very similar to VIP36 and the two proteins probably arose by gene duplication in an early vertebrate ancestor. Further gene duplication has resulted in fish possessing two VIPL proteins plus one VIP36. It is not clear whether VIPL cycles between the ER and the ERGIC/cis-Golgi, or whether it is confined to the ER. VIPL is reported to enhance secretion of a subset of glycoproteins and may function as a regulator of ERGIC-53

C-Type lectins in vertebrates

C-type lectin-like domains (CTLDs) of higher eukaryotes are protein modules originally identified as carbohydrate-recognition domains (CRD's) in a family of Ca2+-dependent animal lectins. Less closely related but still definitely homologous CTLDs have been identified in a variety of proteins that do not appear to have carbohydrate-binding activity. (253) They consist of three major classes; endocytic lectins, collectins, and selectins and one minor one, the lecticans; and are confined to specific species, organs, and tissues.

Collectins

Eight collectins have been identified including mannan-binding lectin (MBL), surfactant protein A (SP-A), surfactant protein D (SP-D), collectin liver 1 (CL-L1), collectin placenta 1 (CL-P1), conglutinin, collectin of 43 kDa (CL-43) and collectin of 46 kDa (CL-46). These molecules have been implicated as major modulators of the innate immune system where they have a key role in the first line of defense against invading microorganisms by binding to them so macrophages know to dispose of them.

Collectins cause the formation of aggregates of the microorganisms and opsonize the microorganisms to increase their phagocytosis and some upregulate of the activity of other pattern recognition receptors such as the mannose receptor. Collectins can also induce the production of pro-inflammatory molecules like cytokines, and reactive oxygen species in phagocytes by interacting with other cell surface receptors or by scavenging of bacterial molecules like lipopolysaccharide (LPS). Some collectins (e.g. MBL) activate of the lectin pathway of the complement system to increase membrane permeability of microorganisms and cause the destruction of that microbe.

Collectins and the lectin pathway of complement activation

The complement system can be activated via three distinct pathways: the classical, the lectin, and the alternative pathway, all of which converge to generate the same set of activation products. The lectin pathway is initiated when mannan-binding lectin (MBL), or ficolins, bind to carbohydrate moieties on bacterial surfaces. This binding promotes the activation of the MBL-associated serine proteases (MASPs) that lead to subsequent cleavage of the C4 and C4b-bound C2. Activation of the lectin pathway leads to the generation of the C3 converting enzyme complex C4b2a that, upon accumulation of the C3 cleavage product C3b, can develop C5 convertase activity. With the cleavage of C5, all enzymatically mediated activation steps are completed, while C5b, the major cleavage fragment of C5, initiates the assembly of the terminal activation steps of C6–C9, leading to the formation of the membrane attack complex (MAC) through a cascade of intermolecular rearrangements.

Ficolins

Ficolins are members of the collectin family of proteins that are able to recognize pathogen-associated molecular pattern (PAMP) on microbial surfaces. Upon binding to their specific PAMP, ficolins may trigger activation of the immune system by either binding to cellular receptors for collectins or by initiating activation of complement via the lectin pathway. For the latter, the human ficolins (i.e. L, H and M-ficolin) and murine ficolin-A were shown to associate with the lectin pathway-specific serine protease MBL-associated serine protease-2 (MASP-2) and catalyze its activation. This in turn activates C4 and C4b-bound C2 to generate the C3 convertase C4b2a. There is mounting evidence underlining the lectin nature of ficolins with a wide range of carbohydrate moieties recognized on microbial surfaces. (287)

Polymorphisms reported on human ficolins showed that, like MBL, single nucleotide polymorphisms (SNPs) in the promoter region of the L-ficolin gene lead to a significant variation in the plasma concentration of this lectin. On the other hand, two SNPs in the fibrinogen-like domain coding region (exon 8), which result in amino acid substitution, affect notably the binding capacity to GlcNAc In addition it was also reported that children with recurrent infections have low L-ficolin concentrations. The M-ficolin gene was also characterized and 12 SNPs were detected both in the promoter and in the structural regions albeit no amino acid exchanges were found. (288, 289)

Mannose binding lectin (MBL)

Mannose binding lectin (MBL), also named mannose or mannan-binding protein (MBP), is an important factor in innate immunity. Although MBL can form several oligomeric forms, there are indications that dimers and trimers are not biologically active and at least a tetramer form is needed for activation of complement. The most-recently discovered mannose-binding lectin pathway activates complement through the mannose-binding lectin protein. MBL binds to carbohydrates (specifically mannose and fucose residues) found on the surface of many pathogens.

MBL deficiency was first recognized in 1968, when a patient with a serum dependent defect in phagocytosis was described. A small girl suffered with severe dermatitis and persistent diarrhea. Almost no improvement was observed with antibiotic and steroid therapy. Isolated PMN cells from the patient revealed impaired ability to phagocytose heat killed yeast particles from *Saccharomyces cerevisiae,* rice starch and *Staphylococcus aureus* in autologous serum, while the same particles were ingested in heterologous serum suggesting a humoral serum dependent defect. Further studies have shown that the particular opsonic defect predisposes to respiratory infection, diarrhea, atopy and failure to thrive during infancy. (294)

The serum MBL level is variable in healthy population. The relative sufficiency of MBL function for any given individual is largely determined by polymorphisms within the *MBL2* gene, on chromosome 10. Three point mutations in exon 1 of the *MBL2* gene impair the expression of functional protein leading to a MBL deficient state, which leads to recurrent infections. It is known now that the mean serum MBL level in an individual is genetically determined by three single nucleotide substitutions in exon-1 of the human MBL gene located at codons 52, 54 and 57 also referred as D. B, and C mutations whereas the wild type allele is referred as A. These mutations result in amino acid substitution in the collagen-like domain, which

results in dominant decreases of functional serum MBL level. In addition, three pairs of allelic dimorphisms can occur in the downstream promoter and the 5'-untranslated region of the mbl-2 gene at positions -550, 221, and +4. The -550 and -221 promoter region polymorphisms form the haplotypes H Y. HX, LY, and LX, when inherited in cis with a normal coding region (A). The HYA, LYA, LXA haplotypes are associated with high, intermediate, and low serum MBL levels. (291-293)

The balance of evidence suggests that MBL deficiency is most relevant when immunity is already compromised because of immunological immaturity, for example, in young children (295) or is impaired by co-morbidity or medical therapy, such as in cystic fibrosis (296) after chemotherapy (297,298) or following transplantation. (299-301) In the pediatric population, MBL exerts greatest influence during an immunological "window of vulnerability", between the decline in maternal passive immunity but before the development of a fully mature adaptive immune system. There is a strong association between MBL deficiency and childhood infection, which has been found for both milder respiratory tract infections managed within the community, as well as more severe infections requiring hospitalization. In cystic fibrosis (CF), innate immunity is compromised in part by impaired mucociliary clearance and bronchiectasis. In one series of CF patients, those with mutant *MBL2* alleles had worse pulmonary function and shorter survival to end-stage CF. (296) The same investigators reported successful MBL replacement in the management of one patient with rapidly progressive CF. Several studies have shown an association between MBL deficiency and risk or severity of infection following chemotherapy. (297,298)

A number of autoimmune disorders are associated with MBL. This may in part relate to the role of MBL in removing pathogens and apoptotic bodies, thus minimizing the emergence of cross-reactivity or auto-immunogenic epitopes. (302) Inherited deficiencies within the classical complement pathway predispose to systemic lupus erythematosus (SLE), thus it was logical to evaluate the role of MBL in this condition. A recent meta-analysis concluded that deficient MBL2 genotypes increase the risk of developing SLE. Other studies have shown that MBL deficiency increases the risk of SLE-related complications, such as arterial thrombosis. The effect of variant MBL and risk of vascular complications extend beyond patients with SLE. (303,304)

Inflammatory bowel disease (IBD) is a pathological spectrum encompassing ulcerative colitis (UC), Crohn's disease (CD), and indeterminate colitis. The resultant IBD phenotype is the consequence of multiple interactions between environmental factors, particularly enteric flora, and the host response to this environment, determined by immunogenetic, epithelial, and other non-immune genetic factors. MBL, as an important component of innate immunity, has engendered considerable research interest. In an early study of 340 unrelated patients with IBD genotyped for MBL2 exon 1 coding mutations, the frequency of deficient alleles was significantly lower in patients with UC than either the control group ($P = 0.02$), or those with CD ($P = 0.01$). This suggests that MBL deficiency could be protective against UC. Alternatively, it could be interpreted that MBL deficiency, in individuals otherwise predisposed to IBD, may skew the phenotype away from the UC spectrum of disease towards CD. (305)

In one study, 117 patients with histologically and serologically confirmed coeliac disease were genotyped for MBL2 exon 1 mutations, and compared to a healthy blood donor population. There was a significant difference in the frequency of the O/O genotype between those with celiac disease (13%) and the control group (5%). The association between MBL and celiac disease —and indeed other autoimmune

conditions— could relate to impaired apoptosis, whereby MBL deficiency impairs the normal removal and clearance of apoptotic cells, which may subsequently reveal previously hidden self-antigen, causing loss of self-tolerance and spreading of autoimmunity. (309) The association between variant MBL2 alleles and coeliac disease has also been confirmed within the Finnish population. (306,307)

Selectins

The selectins (E, L and P) are membrane lectins found on vascular endothelium, platelets and on leukocytes.

L-Selectin

L-Selectin, also known as CD62L, is a cell adhesion molecule found on leukocytes. It is cleaved by ADAM metallopeptidase domain 17 (ADAM17). L-Selectin acts as a "homing receptor" for leukocytes to enter secondary lymphoid tissues via high endothelial venules. Ligands present on endothelial cells will bind to leukocyte expressing L-Selectin, slowing leukocyte trafficking through the blood, and facilitating entry into a secondary lymphoid organ at that point. L-Selectin is present on essentially all blood monocytes and neutrophils, on the majority of blood borne T and B cells and on a subset of natural Killer (NK) cells. However, its expression is variable and depends on different factors, including the developmental stage of the cell. On B-cells L-Selectin occurs relatively late in development, well after immunoglobulin gene rearrangement and just before the mature, virgin, immunocompetent B cells migrate out of the bone marrow.

Naive T-lymphocytes, which have not yet encountered their specific antigen, need to enter secondary lymph nodes to encounter their antigen. Central memory T-lymphocytes that have encountered antigen express L-Selectin to localize in secondary lymphoid organs. Here they reside, ready to proliferate upon re-encountering antigen. Effector/memory T-lymphocytes do not express L-Selectin, as they circulate in the periphery and have immediate effector functions upon encountering antigen.

E-selectin

Endothelial (E)-selectin (CD62E), formerly known as ELAM-1, is synthesized de novo by endothelial cells in response to IL-1, lipopolysaccharide, TNF-α, or G-CSF and is, therefore, detectable either after or concurrently with P-selectin to augment leukocyte recruitment. In humans, E-selectin is encoded by the *SELE* gene. E-selectin recognizes and binds to sialylated carbohydrates present on the surface proteins of certain leukocytes. These carbohydrates include members of the Lewis X and Lewis A families found on monocytes, granulocytes, and T-lymphocytes.

E-selectin is a heavily glycosylated transmembrane protein. E-selectin, recognizes several diverse and structurally distinct glycoconjugates on various hematopoietic and carcinomatous cells in affinity or binding assays. These ligands may include cutaneous lymphocyte-associated antigen (CLA) a distinct glycoform of P-selectin glycoprotein ligand-1 (PSGL-1), L-selectin, E-selectin ligand-1, CD43, hematopoietic cell E- and L-selectin ligand (a specialized glycoform of CD44), β2 integrins, and

glycolipids. (309) Recently, death receptor-3 (DR3) expressed on colon carcinoma cells has been identified as a new E-selectin ligand. (310)

During inflammation, E-selectin plays an important part in recruiting leukocytes to the site of injury. The local release of cytokines IL-1 and TNF by damaged cells induce the over-expression of E-selectin on endothelial cells of nearby blood vessels. Leukocytes in the blood, expressing the correct ligand, will bind with low affinity to E-selectin, causing the leukocytes to "roll" along the internal surface of the blood vessel as temporary interactions are made and broken. As the inflammatory response progresses, chemokines released by injured tissue enter the blood vessels and activate the rolling leukocytes, which are now able to tightly bind to the endothelial surface and begin making their way into the tissue. E-selectin binds sialyl Lewis X (SLeX).

ABO is a major locus for serum soluble E-selectin levels. E-selectin is higher in O/O than O/A heterozygotes, which likewise have higher levels than A/A genotypes. Analysis of subgroups of A alleles reveals heterogeneity in the association, and even after this was accounted for, an intron 1 SNP remained significantly associated. Additional findings indicate that the genetic variants at ABO locus affect plasma soluble E-selectin levels and diabetes risk. (312,313)

P-selectin

P-selectin is the largest of the known selectins at 140kDa. P-selectin is expressed in a-granules of activated platelets and granules of endothelial cells. Within minutes of stimulation of the endothelial cells by inflammatory mediators, such as histamine, thrombin, or phorbol esters, P-selectin is surface-expressed. The expression is short-lived, reaching its peak after only ten minutes. Additional synthesis of P-selectin is brought about within two hours by cytokines such as interleukin-1 (IL-1) or tumor necrosis factor a (TNF-a). The primary ligand for P-selectin is PSGL-1 (P-selectin glycoprotein ligand-1) which is constitutively found on all leukocytes. Other ligands for P-selectin include CD24 and uncharacterized ligands. As with all selectins, the transient interactions between P-selectin and PSGL-1 allow leukocytes to roll along the venular endothelium. Accordingly, P-selectin is largely responsible for the rolling phase of the leukocyte adhesion cascade. P-selectin can also mediate and capture when L-selectin is not present.

P-selectin and E-selectin tend to have overlapping functions. In mice that are deficient for P-selectin, it is necessary to block E-selectin function to reduce rolling significantly; and in E-selectin knockouts, an antibody against P-selectin must be introduced to reduce rolling. Correspondingly, no leukocyte rolling is observed in E-selectin/P-selectin double deficient mice treated with TNF-a. Although P- and E-selectin seem to have redundant functions, observations of rolling flux fraction and rolling velocity indicate that P-selectin is responsible for early rolling while E-selectin allows slow rolling and more adhesion.

P-selectin levels are heavily influenced by ABO blood group, being highest in group A1. (311)

Lecticans

The proteoglycan group of CTLD-containing extracellular matrix proteins has four members in both human and mouse, which are also known as *lecticans* or *hyalectans*. Neurocan and brevican are expressed in the central nervous system, aggrecan is found principally in cartilage, and versican has a wide tissue distribution. The N-terminal region of a lectican polypeptide consists of an immunoglobulin domain and multiple link modules. The central region, which is divergent and varies significantly in length between different lecticans, serves as an attachment region for glycosaminoglycan chains, primarily chondroitin sulphate. The C-terminal region consists of a CTLD sandwiched between one or two epidermal growth factor (EGF)-like domains and a complement control domain. Brevican is also produced in a truncated, glycosylphosphatidyl inositol-anchored form.

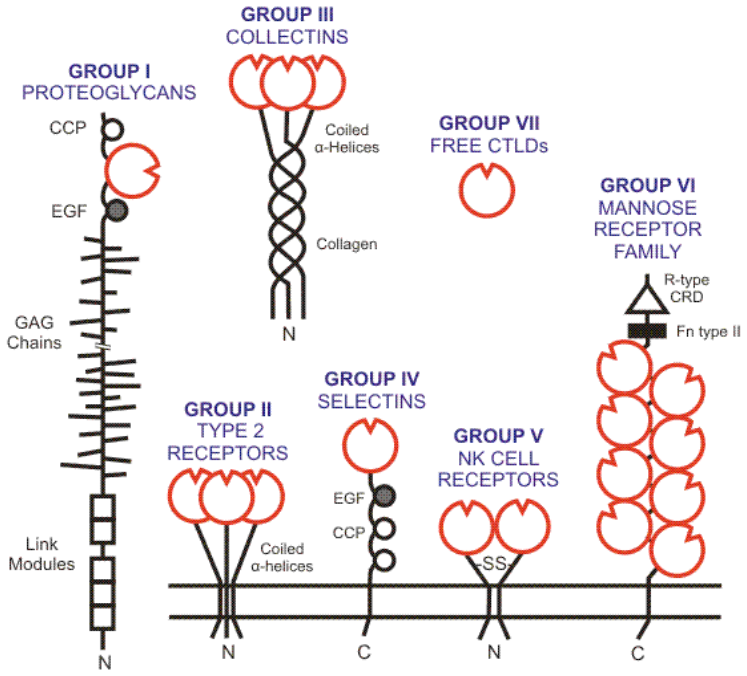


Figure 6.4.5 Domain organization of major mammalian proteins containing CTLDs

Chaperone and quality control L-type lectins

Calnexin (CNX) and calreticulin (CRT) are homologous protein chaperones that mediate quality control of proteins in the endoplasmic reticulum (ER). CNX is membrane-bound and is perhaps closely associated with the protein-translocating channel that is involved in importing nascent proteins into the ER. CRT is a soluble ER luminal component.

The ER chaperones can be categorized into three groups: (a) chaperones of the heat-shock protein family and the co-chaperones, (b) chaperone lectins like CNX and CRT, and (c) substrate-specific chaperones such as HSP47. Calreticulin protein is also highly expressed in the developing heart, but it is only a minor component of the mature heart. Traditional

knockout approach, creating homozygous deletion of ER chaperones such as CRT, results in embryonic lethality. (20) Interestingly, CNX deficient mice are viable, but 50% of the CNX knockout mice died within 2 days of birth and the surviving mice were smaller than their littermates and exhibit obvious motor disorders. (21)

These two proteins bind to monoglucosylated, high-mannose-type glycans and prevent their exit from the ER until they are properly folded and assembled into correct quaternary structures. During the binding and dissociation from CRT or CNX, if the glycoprotein folds correctly, then glucose removal by glucosidase-II allows its passage out of the ER. In the event that a glycoprotein misfolds or aggregates, it is reglucosylated by UDP-Glc:glycoprotein glucosyltransferase (UGGT); this enzyme only recognizes misfolded or aggregated glycoproteins. Following reglucosylation, the monoglucosylated protein binds again to CRT or CNX. Thus, there is a cycle of glucose removal and addition by the alternating actions of glucosidase-II and UGGT and interactions with CNX/CRT. (23)

CNX and CRT are found in all multicellular eukaryotic organisms, as well as some types of yeast. The role of these proteins in glycoprotein quality control seems to have evolved about the same time the N-linked glycosylation pathway took shape. The assembly of major histocompatibility complex (MHC) class I molecules with peptides is orchestrated by several assembly factors including CNX and CRT. (22)

Pentraxins

The pentraxins are a family of proteins characterized by calcium dependent ligand binding and a distinctive flattened β-jellyroll structure similar to that of the legume lectins. The name *pentraxin* is derived from the Greek word for five (*penta*) and berries (*ragos*) relating to the radial symmetry of five monomers forming a ring approximately 95Å across and 35Å deep. The "short" pentraxins include Serum Amyloid P component (SAP) and C reactive protein (CRP). The "long" pentraxins include PTX3 (a cytokine modulated molecule) and several neuronal pentraxins.

C-reactive protein (CRP)

C-reactive protein (CRP) is a protein found in the blood, the levels of which rise in response to inflammation (an acute-phase protein). Its physiological role is to bind to phosphocholine expressed on the surface of dead or dying cells (and some types of bacteria) in order to activate the complement system. CRP is a member of the class of acute-phase reactants, as its levels rise dramatically during inflammatory processes occurring in the body. This increment is due to a rise in the plasma concentration of IL-6, which is produced predominantly by macrophages as well as adipocytes. Recent research suggests that patients with elevated basal levels of CRP are at an increased risk of diabetes, hypertension, and cardiovascular disease.

Amyloid P component, serum (SAP)

Amyloid P component, serum (SAP) is a 25kDa pentameric protein first identified as the pentagonal constituent of in vivo pathological deposits called "amyloid." In amyloidosis, AP makes up 14% of the dry mass of amyloid deposits, and it is thought to be an important contributor to the pathogenesis of a related group of diseases called the amyloidoses. These conditions are characterized by the ordered aggregation of normal globular proteins and peptides into insoluble fibers that disrupt tissue architecture and are associated with cell death. AP is thought to

decorate and stabilize aggregates by preventing proteolytic cleavage and hence inhibiting fibril removal via the normal protein scavenging mechanisms.

EFFECTS OF DIETARY LECTINS

The surface epithelium of the gut is extensively glycosylated (9) mainly because most membrane proteins, including hormone and growth factor receptors, transport proteins and brush-border enzymes, are glycosylated before being embedded in the brush-border membrane. Additionally, membrane lipids and gangliosides are also glycosylated, and all secreted mucins are carbohydrate-rich glycoproteins. Thus, the scope of potential lectin-carbohydrate interactions is quite wide. However, not all lectins react with the epithelium and even those which react vary in their ability to recognize and bind to specific types of carbohydrate receptors. Since lectin reactions are quite specific, it is imperative that the correct carbohydrate structures be present on the surface of the gut mucosa.

Although many lectins are destroyed by normal cooking --which is why grains and beans are edible, many are not, and in fact a few, such as the lectin in bananas, have their activity enhanced by heating and was inhibitable by n-acetyl glucosamine (NAG) and GalNAc (blood group A antigen) glycoproteins. In over 100 food plants found to contain active lectins, seven were autoclave resistant (apple, carrot, wheat bran, canned corn, pumpkin seeds, banana, and wheat flour). Nachbar and Oppenheim (17) also noted high levels of lectin activity in dry roasted peanuts, Corn Flakes, Rice Krispies, and Kellogg's Special K. Phytohemagglutinins from kidney beans can resist mild cooking and retain lectin activity even at 90 degrees C for 3 hours. Pre-soaking the beans however resulted in complete loss of lectin activity. Several investigators noted year-to-year and batch-to-batch variations in the lectin content of foods, so the occasional lectin is likely to occur even with foods normally considered safe.

Eighty-eight common food items were examined for lectin-erythrocyte agglutination activity, which was observed in thirty-eight. Many foods showed agglutinating activity so substantial that the extracts could be diluted several fold. Crude extracts of various foods, such as tomato, lettuce, cucumber, wheat bran and whole wheat, sesame and sunflower seeds, vanilla yogurt, coconut, banana and baby food banana, carrot, onion, apple, alfalfa and soya protein have also been found to bind, and in some instances precipitate the components of human saliva, including cellular debris and bacteria. This may have some significance in the development of caries. Interestingly, avocado lectin inhibited the sucrose dependent adherence of Streptococcus mutans to plaque pellicle. Approximately 1 to 5% of the ingested dietary lectins are absorbed into the blood stream. Here they can clump and bind to red and white blood cells, destroying them. It has been proposed that many of the low-grade anemias seen in the Third World may be resulting from destruction of red blood cells by lectin-rich grain and bean diets.

Foodstuffs are naturally rich in fiber and can be an important cause of allergies. Dietary lectins stimulate mast cells, which can degranulate and release stored histamine, leading several researchers to ascribe a role for dietary lectins in the genesis of food allergy. It is not generally known, however, why some individuals become sensitized to food in their diets. In an attempt to clarify this, celiac disease has been extensively studied, since patients with this disease usually normalize when placed on a gluten free diet.

Lectin damaged areas of the jejunum have been observed to be characteristically heavily infected with coliform bacteria. It is worth noting that most human microbial pathogens and parasites are able to overcome normal gut motility by lectin-like attachments. It has been hypothesized that these ingested lectins that are cytotoxic (via alternative complement activation) and are likely to first damage the lymphocytes of the mesenteric nodes, thus making bacterial overgrowth and eventual food allergy more likely.

Factors influencing glycosylation in the intestines, hence activity of dietary lectins (10)

- Animal species
- Blood group specificity
- Age
- Particular area of the small intestine
- Position along the crypt/villi axis
- State of cell maturation
- Diet
- Bacterial status
- Sickness or pathology

Most lectins in our diet are resistant to breakdown during gut passage and are bound and endocytosed by epithelial cells. These lectins are powerful exogenous growth factors for the small intestine, can induce dramatic shifts in its bacterial flora, and interfere with its hormone secretion. In addition, lectins that are transported across the gut wall into the systemic circulation can modulate the body's hormone balance, metabolism, and health. In contrast to dietary proteins, lectins resist degradation in the small intestine and are resistant to breakdown by most gut bacteria. Thus, most lectins survive at least in part the passage through the digestive tract in an immunologically and functionally intact form. (14)

Although lectin binding is most frequently studied in the small intestine, similar binding can occur throughout the entire digestive tract, from the stomach to the distal colon. However, as surface glycosylation varies in the different functional parts of the gut, lectin binding is not uniform in the digestive tract. Binding of lectins and their endocytosis by enterocytes occurs throughout the gut, although endocytosis in the small intestine becomes appreciable only in the presence of large numbers of commensal bacteria. As expected, endocytosis of lectins is more extensive in the colon where bacterial counts are high.

Dietary lectins block ion transport in the intestinal tract by blocking phospholipase C (PLC) and protein kinase C (PKC) resulting in inhibition of the epithelial Na(+) channel (ENaC). This may help explain why some lectins have been shown to induce diarrhea and hypersecretion in human airways. This effect may be therapeutically useful in patients suffering from cystic fibrosis. (28)

Eighty-five samples from fifteen different legume seed lines generally available in the UK were examined by measurements of their net protein utilization by rats and by hemagglutination tests with erythrocytes from a number of different animal species. From these results, the seeds were classified into four broad groups. Group A seeds, from most varieties of kidney beans, showed high reactivity with all cell types and were highly toxic. Group B, which contained seeds from lima or butter beans and winged bean, agglutinated

only human and pronase-treated rat erythrocytes. These seeds did not support proper growth of the rats although the animals survived the 10-day experimental period. Group C consisted of seeds from lentils, peas, chickpeas, black-eyed peas, pigeon peas, mung beans, field or broad beans, and azuki beans. These generally had low reactivity with all cells and were non-toxic. Group D, represented by soya and pinto beans, generally had low reactivity with all cells but caused growth depression at certain dietary concentrations. This growth depression was probably mainly due to antinutritional factors other than lectins. (133)

Lectin content is a prime area of manipulation for the production of transgenic foods (18) so it would appear that they would continue to occupy to attention of nutritionally oriented physicians well into the future.

The variety of reported effects resulting from the ingestion of dietary lectins is summarized in the following table.

Action	Description
Induction of interleukins	• Dietary lectins are known to induce interleukins IL-4 and IL-13. Since lectins can enter the circulation after oral uptake, they might play a role in inducing the so-called early IL-4 required to switch the immune response towards a Th2 response and type I allergy. (6,8)
Induction of autoimmunity	• The interaction of dietary lectins with enterocytes and lymphocytes may facilitate the translocation of both dietary and gut-derived pathogenic antigens to peripheral tissues, which in turn causes persistent peripheral antigenic stimulation. In genetically susceptible individuals, this antigenic stimulation may ultimately result in the expression of autoimmune disease. (9)
Interference with protein digestion	• Aminopeptidase activity (the enzyme that breaks down polypeptides into amino acids) is inhibited by several dietary lectins. (10)
Interaction with the brush border membrane	• Lectins, which bind avidly to the brush border membrane, are potent hyperplastic growth factors for the gut. (11) • Lectin-binding to the epithelium is obligatory for growth stimulation, and their growth factor activity is determined mainly by the strength and intensity of their binding (12).
Anti-nutrient effects	• Rats fed a diet comprised principally of raw navy bean flour were smaller and had 50% less ability to absorb glucose and utilize dietary protein than a control group who were fed navy beans in which the lectin had been inactivated. (11)
Enhancement of gut permeability	• In one study, rats fed on diets containing kidney beans showed increased intestinal permeability to serum proteins that had been injected into their blood stream. After challenge with kidney bean proteins, it was found that the protein injected into their blood stream was detected in both the lumen (open space) and the walls of the small intestine. It was suggested that dietary lectins may be responsible, at least in part, for loss of serum proteins and may contribute to other food intolerance secondary to the loss of gut integrity. (12)
Activation of gut hormones	• Cholecystokinin is induced by several dietary lectins. (13)
Hormone and growth factor mimicry	• As surface membrane receptors of cells are glycosylated, lectins are good mimics of the effects of endogenous growth factors, hormones, and cytokines in all types of cells (14). • Lectins can mimic the effect of the natural ligands and induce similar physiological reactions. In addition, bound lectin may induce conformational changes in the receptor and/or physically block the active site of the receptor, thereby attenuating or completely abolishing the physiological effect of the natural ligand.
Mucotractive effects	• Many lectins stimulate the production of mucus. This is possibly a protective function or an allergic response, and was for a time thought to be an action of lectins that promised some therapeutic benefit that could be applied to patients with the disease cystic fibrosis. (16)
Mitogenic effects	• Several foodstuffs and herbal medicines are known to contain lectins which are capable of inducing T or B cell blastogenesis

Figure 6.4.6 Reported effects of dietary lectins.

The studies on molecular evolution of leptin indicate that adaptation of rodent leptin and insufficient adaptation of human leptin to a diet including large amounts of seeds from grass. This adaptation and lack thereof could also involve the leptin receptor, since leptin and leptin receptor coevolved due to interdependency for signaling. An adaptation of the leptin gene could thus be to avoid disturbed function of either leptin or the leptin receptor. It would be interesting to see results from studies on molecular evolution of the leptin receptor, but such studies are unfortunately lacking.

However, when considering direct lectin interaction with leptin or the leptin receptor, this interaction could be with either or with both. Lectins binding to sugar structures of a membrane receptor can mimic or block the effect of the physiological ligand. Leptin is not glycosylated, but the leptin receptor is highly glycosylated; and lectins binding to different leptin receptor glycosylations might explain different leptin binding affinity. Thus, dietary lectins could possibly bind to the leptin receptor and affect its function, which could translate into diseases of affluence as indicated by studies on effects of single nucleotide polymorphisms on the function of leptin and the leptin receptor. (288)

1. Stillmark H. Ueber Rizin, ein giffiges Ferment aus dem Samen von Ricinlis commurris L. und einigen anderen Euphorbiaceen. Inaug Diss Dorpat. (1888)
2. Landsteiner, K. *The Specificity of Serological Reactions*. Second edition. Harvard Univ Press. Cambridge, Mass. (1945)
3. Boyd WC. *Fundamentals of Immunology*. 2nd. edit. Interscience Publishers. NY (1947)
4. Pusztai A, Ewen SW, Grant G, Peumans WJ, Van Damme EJ, Coates ME, Bardocz S. Lectins and also bacteria modify the glycosylation of gut surface receptors in the rat. Glycoconj J. 1995 Feb; 12(1):22-35.
5. Pusztai A, Bardocz, S. Biological Effects of Plant Lectins on the Gastrointestinal Tract: Metabolic Consequences and Applications. Trends Glycosci Glycotechnol. 8:149-165.
6. Haas H, Falcone FH, Schramm G, Haisch K, Gibbs BF, Klaucke J, Poppelmann M, Becker WM, Gabius HJ, Schlaak M. Dietary lectins can induce in vitro release of IL-4 and IL-13 from human basophils. Eur J Immunol. 1999 Mar; 29(3):918-27.
7. Cordain L, Toohey L, Smith MJ, Hickey MS. Modulation of immune function by dietary lectins in rheumatoid arthritis.Br J Nutr. 2000 Mar; 83(3):207-17. Review.
8. Erickson RH, Kim J, Sleisenger MH, Kim YS Effect of lectins on the activity of brush border membrane-bound enzymes of rat small intestine. J Pediatr Gastroenterol Nutr 1985 Dec;4(6):984-91
9. Pusztai, A. (1991) in Plant Lectins, Cambridge, Cambridge University Press
10. Pusztai, A. Ewen, S.W.B., Grant, G., Peumans, W.J., Van Damme, E.J.M., Rubio, L., and Bardocz, S. (1990) Digestion 46(suppl. 2), 308-316
11. Weinman MD, Allan CH, Trier JS, Hagen SJ Repair of microvilli in the rat small intestine after damage with lectins contained in the red kidney bean. Gastroenterology 1989 Nov;97(5):1193-204
12. Greer F, Pusztai A Toxicity of kidney bean (Phaseolus vulgaris) in rats: changes in intestinal permeability. Digestion 1985; 32(1):42-6
13. Jordinson M, Playford RJ, Calam J. Effects of a panel of dietary lectins on cholecystokinin release in rats. Am J Physiol. 1997 Oct; 273(4 Pt 1):G946-50.
14. Pusztai A. Dietary lectins are metabolic signals for the gut and modulate immune and hormone functions. Eur J Clin Nutr. 1993 Oct; 47(10):691-9.
15. Bardocz S, Grant G, Ewen SW, Duguid TJ, Brown DS, Englyst K, Pusztai A. Reversible effect of phytohemagglutinin on the growth and metabolism of rat gastrointestinal tract. Gut. 1995 Sep; 37(3):353-60.
16. Freed DJ "Dietary Lectins" in *Food Allergy and Intolerance.* Brostoff and Callacombe, Editors. Bailliere Tindall Publishers, London
17. Nachbar MS, Oppenheim JD. Lectins in the United States diet: a survey of lectins in commonly consumed foods and a review of the literature. (SV)Am J Clin Nutr 1980 Nov;33(11):2338-45 Prog Food Nutr Sci 1989;13(3-4):317-48
18. Ewen SW, Pusztai A. Health risks of genetically modified foods. Lancet. 1999 Aug 21; 354(9179):684.
19. Etzler ME and Cummings RD. L-type Lectins. In: Varki A, Cummings R, Esko J, Freeze H, Stanley P, Bertozzi C, Hart G, and Etzler M. *Essentials of Glycobiology*, 2nd edition, Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2009

20. Mesaeli N, Nakamura K, Zvaritch E, Dickie P, Dziak E, Krause KH, Opas M, MacLennan DH, Michalak M. Calreticulin is essential for cardiac development. J Cell Biol. 1999 Mar 8; 144(5):857-68.
21. Denzel A, Molinari M, Trigueros C, Martin JE, Velmurgan S, Brown S, Stamp G, Owen MJ. Early postnatal death and motor disorders in mice congenitally deficient in calnexin expression. Mol Cell Biol. 2002 Nov; 22(21):7398-404.
22. Raghavan M, Del Cid N, Rizvi SM, Peters LR. MHC class I assembly: out and about. Trends Immunol. 2008 Sep; 29(9):436-43.
23. Caramelo JJ, Parodi AJ. Getting in and out from calnexin/calreticulin cycles. J Biol Chem. 2008 Apr 18; 283(16):10221-5.
24. D'Adamo PJ. Lectins and Mitogens. In: Gut Ecosystems. Townsend Letter for Doctors. Port Townsend WA (August 1990)
25. D'Adamo PJ. Non-transfusion significance of ABO blood group. In: *Textbook of Natural Medicine*. Pizzorno J, Murray M. Editors. Churchill Livingstone. 2006
26. Nachbar MS, Oppenheim JD. Tomato (Lycopersicon esculentum) lectin. Methods Enzymol. 1982;83:363-8
27. Yura H, Kanatani Y, Ishihara M, Takase B, Nambu M, Kishimoto S, Kitagawa M, Tatsuzawa O, Hoshi Y, Suzuki S, Kawakami M, Matsui T. Selection of hematopoietic stem cells with a combination of galactose-bound vinyl polymer and soybean agglutinin, a galactose-specific lectin. Transfusion. 2008 Mar; 48(3):561-6.
28. Kunzelmann K, Sun J, Schreiber R, König J. Effects of dietary lectins on ion transport in epithelia. Br J Pharmacol. 2004 Aug; 142(8):1219-26.
29. http://www.imperial.ac.uk/research/animallectins/ctld/lectins.html#Summary
30. Van Damme EJ, Barre A, Rougé P, Peumans WJ. Cytoplasmic/nuclear plant lectins: a new story. Trends Plant Sci. 2004 Oct; 9(10):484-9.
31. De Hoff PL, Brill LM, Hirsch AM. Plant lectins: the ties that bind in root symbiosis and plant defense. Mol Genet Genomics. 2009 Jul; 282(1):1-15.
32. Sharon N. Lectin-carbohydrate complexes of plants and animals: an atomic view. Trends Biochem Sci. 1993 Jun; 18(6):221-6.
33. Etzler ME. Plant lectins: molecular and biological aspects. Annu Rev Plant Physiol 36:209–234 (1995)
34. Sharon N. Lectins: past, present and future. Biochem Soc Trans. 2008 Dec;36(Pt 6):1457-60
35. Van Damme EJM, Peumans WJ, Pusztai A, Bardocz S. *Handbook of Plant Lectins.* Wiley and Sons. 1998.
36. Tchernychev B, Wilchek M. Natural human antibodies to dietary lectins. FEBS Lett. 1996 Nov 18; 397(2-3):139-42.
37. Tomita M, Osawa T, Sakurai Y, Ukita T. On the surface structure of murine ascites tumors. I. Interactions with various phytoagglutinins. Int J Cancer. 1970 Sep 15; 6(2):283-9.
38. Uhlenbruck G, Otten H, Reifenberg U. Additional characterization of tumor cell specific "wheat germ" agglutinins, with special reference to its reaction with the blood group A substance. Z Krebsforsch. 1968; 70(4):350-3.
39. Damm I, Mikkat U, Kirchhoff F, Böckmann S, Jonas L. Inhibitory effect of the lectin wheat germ agglutinin on the binding of 125I-CCK-8s to the CCK-A and -B receptors of AR42J cells. Pancreas. 2004 Jan;28(1):31-7
40. Pusztai A, Ewen SW, Grant G, Brown DS, Stewart JC, Peumans WJ, Van Damme EJ, Bardocz S. Antinutritive effects of wheat-germ agglutinin and other N-acetylglucosamine-specific lectins. Br J Nutr. 1993 Jul; 70(1):313-21.
41. Sharon N. Lectin receptors as lymphocyte surface markers. Adv Immunol. 1983; 34:213-98.
42. Nizheradze KA. Binding of wheat germ agglutinin to extracellular network produced by cultured human fibroblasts. Folia Histochem Cytobiol. 2000; 38(4):167-73.
43. Lavelle EC, Grant G, Pusztai A, Pfüller U, O'Hagan DT. Mucosal immunogenicity of plant lectins in mice. Immunology. 2000 Jan; 99(1):30-7.
44. Schwarz RE, Wojciechowicz DC, Picon AI, Schwarz MA, Paty PB. Wheat germ agglutinin-mediated toxicity in pancreatic cancer cells. Br J Cancer. 1999 Aug; 80(11):1754-62.
45. Marchetti M, Mastromarino P, Rieti S, Seganti L, Orsi N. Inhibition of herpes simplex, rabies and rubella viruses by lectins with different specificities. Res Virol. 1995 May-Jun; 146(3):211-5.
46. Ganguly C, Das S. Plant lectins as inhibitors of tumor growth and modulators of host immune response. Chemotherapy. 1994 Jul-Aug; 40(4):272-8.
47. Kim M, Rao MV, Tweardy DJ, Prakash M, Galili U, Gorelik E. Lectin-induced apoptosis of tumor cells. Glycobiology. 1993 Oct; 3(5):447-53.
48. Yevdokimova NY, Yefimov AS. Effects of wheat germ agglutinin and concanavalin A on the accumulation of glycosaminoglycans in pericellular matrix of human dermal fibroblasts. A comparison with insulin. Acta Biochim Pol. 2001; 48(2):563-72.
49. Wilden PA, Morrison BD, Pessin JE. Wheat germ agglutinin stimulation of alpha beta heterodimeric insulin receptor beta-subunit autophosphorylation by noncovalent association into an alpha 2 beta 2 heterotetrameric state. Endocrinology. 1989 Feb; 124(2):971-9.
50. Finlay DR, Newmeyer DD, Price TM, Forbes DJ. Inhibition of in vitro nuclear transport by a lectin that binds to nuclear pores. J Cell Biol. 1987 Feb; 104(2):189-200.
51. Kitano N, Taminato T, Ida T, Seno M, Seino Y, Matsukura S, Kuno S, Imura H. Detection of antibodies against wheat germ agglutinin bound glycoproteins on the islet-cell membrane. Diabet Med. 1988 Mar; 5(2):139-44.

52. Messina JL, Hamlin J, Larner J. Insulin-mimetic actions of wheat germ agglutinin and concanavalin A on specific mRNA levels. Arch Biochem Biophys. 1987 Apr; 254(1):110-5.
53. Shemer J, LeRoith D. The interaction of brain insulin receptors with wheat germ agglutinin. Neuropeptides. 1987 Jan; 9(1):1-8.
54. Caron M, Lascols O, Picard J, Cherqui G, Capeau J. Further evidence for a role of carbohydrates in insulin binding: studies in lectin-purified receptors. J Recept Res. 1983;3(3):423-37
55. Livingston JN, Purvis BJ. Effects of wheat germ agglutinin on insulin binding and insulin sensitivity of fat cells. Am J Physiol. 1980 Mar; 238(3):E267-75.
56. http://www.drdavidfreed.co.uk/sc-rheumaticpatches.html
57. http://www.dadamo.com/lecster2/Lecster-read.pl?show=70
58. Kabir S, Daar AS. The composition and properties of jacalin, a lectin of diverse applications obtained from the jackfruit (Artocarpus heterophyllus) seeds. Immunol Invest. 1994 Apr; 23(3):167-88.
59. Roque-Barreira MC, Campos-Neto A. Jacalin: an IgA-binding lectin. J Immunol. 1985 Mar; 134(3):1740-3.
60. Sastry MV, Banarjee P, Patanjali SR, Swamy MJ, Swarnalatha GV, Surolia A. Analysis of saccharide binding to Artocarpus integrifolia lectin reveals specific recognition of T-antigen (beta-D-Gal(1----3)D-GalNAc).J Biol Chem. 1986 Sep 5;261(25):11726-33.
61. Ma BY, Yoshida K, Baba M, Nonaka M, Matsumoto S, Kawasaki N, Asano S, Kawasaki T. The lectin Jacalin induces human B-lymphocyte apoptosis through glycosylation-dependent interaction with CD45. Immunology. 2009 Aug; 127(4):477-88.
62. Raval S, Gowda SB, Singh DD, Chandra NR. A database analysis of jacalin-like lectins: sequence-structure-function relationships. Glycobiology. 2004 Dec; 14(12):1247-63.
63. Galelli A, Truffa-Bachi P. Urtica dioica agglutinin. A superantigenic lectin from stinging nettle rhizome. J Immunol 1993; 151:1821-1831.
64. Galelli A, Delcourt M, Wagner MC, Peumans W, Truffa-Bachi P. Selective expansion followed by profound deletion of mature V beta 8.3+ T cells in vivo after exposure to the superantigenic lectin Urtica dioica agglutinin. J Immunol. 1995 Mar 15; 154(6):2600-11.
65. Le Moal MA, Colle JH, Galelli A, Truffa-Bachi P. Mouse T-lymphocyte activation by Urtica dioica agglutinin. II.--Original pattern of cell activation and cytokine production induced by UDA. Res Immunol. 1992 Sep; 143(7):701-9.
66. http://www.dadamo.com/lecster2/Lecster-read.pl?show=637
67. Musette P, Galelli A, Chabre H, Callard P, Peumans W, Truffa-Bachi P, Kourilsky P, Gachelin G. Urtica dioica agglutinin, a V beta 8.3-specific superantigen, prevents the development of the systemic lupus erythematosus-like pathology of MRL lpr/lpr mice. Eur J Immunol. 1996 Aug; 26(8):1707-11.
68. Kayser K, Bubenzer J, Kayser G, Eichhorn S, Zemlyanukhina TV, Bovin NV, Andre S, Koopmann J, Gabius HJ. Expression of lectin, interleukin-2 and histopathologic blood group binding sites in prostate cancer and its correlation with integrated optical density and syntactic structure analysis. Anal Quant Cytol Histol. 1995 Apr; 17(2):135-42.
69. Waxdal MJ. Isolation, characterization, and biological activities of five mitogens from pokeweed. Biochemistry. 1974 Aug 27; 13(18):3671-7.
70. Waddick KG, Myers DE, Gunther R, Chelstrom LM, Chandan-Langlie M, Irvin JD, Tumer N, Uckun FM. In vitro and in vivo antileukemic activity of B43-pokeweed antiviral protein against radiation-resistant human B-cell precursor leukemia cells. Blood. 1995 Dec 1; 86(11):4228-33.
71. Lord, J M et al: Ricin: structure, mode of action, and some current applications. FASEB J. 8 , 201-208, 1994
72. Hartley, M R et al: The structure and function of ribosome-inactivating proteins. Trends Plant Sci, 1, 254-259, 1996
73. Hajtó T, Hostanska K, Berki T, Pálinkás L, Boldizsár F, Németh P. Oncopharmacological Perspectives of a Plant Lectin (Viscum album Agglutinin-I): Overview of Recent Results from In vitro Experiments and In vivo Animal Models, and Their Possible Relevance for Clinical Applications. Evid Based Complement Alternat Med. 2005 Mar; 2(1):59-67.
74. Janssen O, Scheffler A, Kabelitz D. In vitro effects of mistletoe extracts and mistletoe lectins. Cytotoxicity towards tumor cells due to the induction of programmed cell death (apoptosis) Drug Res 1993; 43: 1221–7
75. Savoie A, Lavastre V, Pelletier M, Hajto T, Hostanska K, Girard D. Activation of human neutrophils by the plant lectin Viscum album agglutinin-I: modulation of de novo protein synthesis and evidence that caspases are involved in induction of apoptosis. J Leukoc Biol. 2000; 68: 845–53.
76. Lavastre V, Pelletier M, Saller R, Hostanska K, Girard D. Mechanisms involved in spontaneous and Viscum album agglutinin-I-induced human neutrophil apoptosis: Viscum album agglutinin-I accelerates the loss of antiapoptotic Mcl-1 expression and the degradation of cytoskeletal paxillin and vimentin proteins via caspases J Immunol 2002; 168: 1419–27
77. Hajto T, Hostanska K, Herrmann R. Immunomodulatory potency of mistletoe lectins in cancer patients. Results of a dose finding study Allergy (Suppl) 1993; 48: 548
78. Allen AK. A lectin from the exudate of the fruit of the vegetable marrow (Cucurbita pepo) that has a specificity for beta-1,4-linked N-acetylglucosamine oligosaccharides. Biochem J. 1979 Oct 1; 183(1):133-7.
79. Lorenc-Kubis I, Klimczak K, Morawiecka B. Lectins from squash (Cucurbita ficifolia) seedlings. Acta Biochim Pol. 1993; 40(1):103-5.

80. Read SM, Northcote DH. Eur J Biochem. 1983 Aug 15; 134(3):561-9.Subunit structure and interactions of the phloem proteins of Cucurbita maxima (pumpkin).
81. Rinderle SJ, Goldstein IJ, Matta KL, Ratcliffe RM. Isolation and characterization of amaranthin, a lectin present in the seeds of Amaranthus caudatus, that recognizes the T- (or cryptic T)-antigen. J Biol Chem. 1989 Sep 25; 264(27):16123-31.
82. Boland CR, Chen YF, Rinderle SJ, Resau JH, Luk GD, Lynch HT, Goldstein IJ. Use of the lectin from Amaranthus caudatus as a histochemical probe of proliferating colonic epithelial cells. Cancer Res. 1991 Jan 15; 51(2):657-65.
83. Nakagawa R, Yasokawa D, Ikeda T, Nagashima K. Purification and characterization of two lectins from callus of Helianthus tuberosus. Biosci Biotechnol Biochem. 1996 Feb; 60(2):259-62.
84. Prodanov P, Atanasova N. An anti-B lectin from Zea mays everta. Folia Haematol Int Mag Klin Morphol Blutforsch. 1984; 111(1):84-5.
85. Janković M, Cuperlović M, Hajduković L. Salt-Soluble Lectins of Corn Grain. Plant Physiol. 1990 Aug; 93(4):1659-1662.
86. Koshte VL, van Dijk W, van der Stelt ME, Aalberse RC. Isolation and characterization of BanLec-I, a mannoside-binding lectin from Musa paradisiac (banana). Biochem J. 1990 Dec 15; 272(3):721-6.
87. Koshte VL, Aalbers M, Calkhoven PG, Aalberse RC. The potent IgG4-inducing antigen in banana is a mannose-binding lectin, BanLec-I. Int Arch Allergy Immunol. 1992; 97(1):17-24.
88. Swanson MD, Winter HC, Goldstein IJ, Markovitz DM.A lectin isolated from bananas is a potent inhibitor of HIV replication. J Biol Chem. 2010
89. Lord JM, Roberts LM, Robertus JD. Ricin: structure, mode of action, and some current applications. FASEB J. 1994 Feb;8(2):201-8
90. Olsnes S, Pihl A. Isolation and properties of abrin: a toxic protein inhibiting protein synthesis. Evidence for different biological functions of its two constituent-peptide chains. Eur J Biochem. 1973 May; 35(1):179-85.
91. Olsnes S. Toxic and nontoxic lectins from Abrus precatorius. Methods Enzymol. 1978; 50:323-30.
92. Lin JY, Lee TC, Tung TC. Inhibitory effects of four isoabrins on the growth of sarcoma 180 cells. Cancer Res. 1982 Jan; 42(1):276-9.
93. Thorpe PE, Blakey DC, Brown AN, Knowles PP, Knyba RE, Wallace PM, Watson GJ, Wawrzynczak EJ. Comparison of two anti-Thy 1.1-abrin A-chain immunotoxins prepared with different cross-linking agents: antitumor effects, in vivo fate, and tumor cell mutants. J Natl Cancer Inst. 1987 Nov; 79(5):1101-12.
94. Biochem J. 1984 Jul 1; 221(1):163-9. A lectin from elder (Sambucus nigra L.) bark. Broekaert WF, Nsimba-Lubaki M, Peeters B, Peumans WJ.
95. Pusztai A, Ewen SW, Grant G, Peumans WJ, van Damme EJ, Rubio L, Bardocz S. Relationship between survival and binding of plant lectins during small intestinal passage and their effectiveness as growth factors. Digestion. 1990; 46 Suppl 2:308-16.
96. Kaku H, Peumans WJ, Goldstein IJ. Isolation and characterization of a second lectin (SNA-II) present in elderberry (Sambucus nigra L.) bark. Arch Biochem Biophys. 1990 Mar; 277(2):255-62.
97. Van Damme EJ, Barre A, Rougé P, Van Leuven F, Peumans WJ. Characterization and molecular cloning of Sambucus nigra agglutinin V (nigrin b), a GalNAc-specific type-2 ribosome-inactivating protein from the bark of elderberry (Sambucus nigra). Eur J Biochem. 1996 Apr 15; 237(2):505-13.
98. Maveyraud L, Niwa H, Guillet V, Svergun DI, Konarev PV, Palmer RA, Peumans WJ, Rougé P, Van Damme EJ, Reynolds CD, Mourey L. Structural basis for sugar recognition, including the Tn carcinoma antigen, by the lectin SNA-II from Sambucus nigra. Proteins. 2009 Apr; 75(1):89-103.
99. Kovalenko OO, Lukash LL. Effect of lectins on induction of apoptosis in the populations of mammalian cells in vitro. Tsitol Genet. 2007 Sep-Oct; 41(5):48-53.
100. Balcan E, Tuğlu I, Sahin M, Toparlak P. Cell surface glycosylation diversity of embryonic thymic tissues. Acta Histochem. 2008; 110(1):14-25.
101. Krzeslak A, Gaj Z, Pomorski L, Lipinska A. Sialylation of intracellular proteins of thyroid lesions. Oncol Rep. 2007 May; 17(5):1237-42.
102. Yao L, Korteweg C, Hsueh W, Gu J. Avian influenza receptor expression in H5N1-infected and noninfected human tissues. FASEB J. 2008 Mar; 22(3):733-40.
103. Przybyło M, Stepień E, Pfitzner R, Lityńska A, Sadowski J. Age effect on human aortic valvular glycoproteins. Arch Med Res. 2007 Jul; 38(5):495-502.
104. Kosanović MM, Janković MM. Sialylation and fucosylation of cancer-associated prostate specific antigen. J BUON. 2005 Apr-Jun; 10(2):247-50.
105. Wang XY, Lin SQ, Li JW, Kemmner W, Ding YQ. Effect of cell surface sialic acid and their linkages on adhesion of mammary carcinoma cells. Nan Fang Yi Ke Da Xue Xue Bao. 2006 Jun;26(6):74
106. Roschek B Jr, Fink RC, McMichael MD, Li D, Alberte RS. Elderberry flavonoids bind to and prevent H1N1 infection in vitro. Phytochemistry. 2009 Jul;70(10):1255-61
107. http://faculty.tamu-commerce.edu/fmiskevich/Research/tomato_lectin_research.html
108. Oguri S, Amano K, Nakashita H, Nagata Y, Momonoki YS. Molecular structure and properties of lectin from tomato fruit. Biosci Biotechnol Biochem. 2008 Oct; 72(10):2640-50.
109. Bies C, Lehr CM, Woodley JF. Lectin-mediated drug targeting: history and applications. Adv Drug Deliv Rev. 2004 Mar 3; 56(4):425-35.
110. Woodley JF. Lectins for gastrointestinal targeting—15 years on. J Drug Target. 2000; 7(5):325-33.
111. Jarløv JO, Rosdahl VT, Yeo M, Marples RR. Lectin typing of methicillin-resistant Staphylococcus aureus from Singapore, England and Wales, and Denmark. J Med Microbiol. 1993 Oct; 39(4):305-9.

112. Kilpatrick DC, Pusztai A, Grant G, Graham C, Ewen SW. Tomato lectin resists digestion in the mammalian alimentary canal and binds to intestinal villi without deleterious effects. FEBS Lett. 1985 Jun 17; 185(2):299-305.
113. Nachbar MS, Oppenheim JD, Thomas JO. Lectins in the U.S. Diet. Isolation and characterization of a lectin from the tomato (Lycopersicon esculentum). J Biol Chem. 1980 Mar 10; 255(5):2056-61.
114. Tomita M, Osawa T, Sakurai Y, Ukita T. On the surface structure of murine ascites tumors. I. Interactions with various phytoagglutinins. Int J Cancer. 1970 Sep 15; 6(2):283-9.
115. Allen AK, Neuberger A. Potato lectin. Methods Enzymol. 1978;50:340-5
116. Sequeira L and Graham TL. Agglutination of avirulent strains of Pseudomonas solanacearum by potato lectin. Physio Plant Pathol 11-43-54 (1977)
117. Pramod SN, Venkatesh YP, Mahesh PA. Potato lectin activates basophils and mast cells of atopic subjects by its interaction with core chitobiose of cell-bound non-specific immunoglobulin E. Clin Exp Immunol. 2007 Jun; 148(3):391-401.
118. Ewen SW, Pusztai A. Effect of diets containing genetically modified potatoes expressing Galanthus nivalis lectin on rat small intestine. Lancet. 1999 Oct 16; 354(9187):1353-4.
119. Coutiño-Rodríguez R, Hernández-Cruz P, Giles-Ríos H. Lectins in fruits having gastrointestinal activity: their participation in the hemagglutinating property of Escherichia coli O157:H7. Arch Med Res. 2001 Jul-Aug; 32(4):251-7.
120. Tsuda M. Purification and characterization of a lectin from rice bran. J Biochem. 1979 Nov; 86(5):1451-61.
121. Saha P, Majumder P, Dutta I, Ray T, Roy SC, Das S. Transgenic rice expressing Allium sativum leaf lectin with enhanced resistance against sap-sucking insect pests. Planta. 2006 May; 223(6):1329-43.
122. Peumans WJ, Stinissen HM, Carlier AR. Isolation and partial characterization of wheat-germ-agglutinin-like lectins from rye (Secale cereale) and barley (Hordeum vulgare) embryos. Biochem J. 1982 Apr 1; 203(1):239-43.
123. Alencar VB, Assreuy AM, Alencar NM, Meireles AV, Mota MR, Aragão KS, Cajazeiras JB, Nagano CS, Brito GA, Silva LI, Pinto VP, Sampaio AH, Debray H, Cavada BS, Ribeiro RA. Lectin of Pisum arvense seeds induces in-vivo and in-vitro neutrophil migration. J Pharm Pharmacol. 2005 Mar; 57(3):375-81.
124. Muñoz A, Alonso B, Alvarez O, Llovo J. Lectin typing of five medically important Candida species. Mycoses. 2003 Apr; 46(3-4):85-9.
125. Lima JE, Sampaio AL, Henriques MG, Barja-Fidalgo C. Lymphocyte activation and cytokine production by Pisum sativum agglutinin (PSA) in vivo and in vitro. Immunopharmacology. 1999 Feb; 41(2):147-55.
126. Haas H, Falcone FH, Schramm G, Haisch K, Gibbs BF, Klaucke J, Pöppelmann M, Becker WM, Gabius HJ, Schlaak M. Dietary lectins can induce in vitro release of IL-4 and IL-13 from human basophils. Eur J Immunol. 1999 Mar; 29(3):918-27.
127. Sarker AB, Koirala TR, Murakami I, Jeon HJ. Bauhinia purpurea and Pisum sativum lectin binding in human breast. Indian J Pathol Microbiol. 1995 Jul; 38(3):261-5.
128. Yin ZF, Tu ZX, Cui ZF, Wu MC. Alpha-fetoprotein reaction to Pisum sativum agglutinin in differentiation of benign liver diseases from hepatocellular carcinoma. Chin Med J (Engl). 1993 Aug; 106(8):615-8.
129. Lu LM. Study of the Pisum sativum agglutinin receptors and DNA ploidy during carcinogenesis in esophageal epithelium by flow cytometry. Zhonghua Bing Li Xue Za Zhi. 1992 Dec; 21(6):355-7.
130. Mendoza C, Carreras A, Moos J, Tesarik J. Distinction between true acrosome reaction and degenerative acrosome loss by a one-step staining method using Pisum sativum agglutinin. J Reprod Fertil. 1992 Aug; 95(3):755-63.
131. Fabian RH, Coulter JD. Transneuronal transport of lectins. Brain Res. 1985 Sep 30; 344(1):41-8.
132. Taylor CE, Stashak PW, Caldes G, Prescott B, Fowlkes BJ, Baker PJ. Lectin-induced modulation of the antibody response to type III pneumococcal polysaccharide. Cell Immunol. 1984 Jan; 83(1):26-33.
133. Grant G, More LJ, McKenzie NH, Stewart JC, Pusztai A. A survey of the nutritional and hemagglutination properties of legume seeds generally available in the UK. Br J Nutr. 1983 Sep; 50(2):207-14.
134. Kanai T, Amakawa M, Kato R, Shimizu K, Nakamura K, Ito K, Hama Y, Fujimori M, Amano J. Evaluation of a new method for the diagnosis of alterations of Lens culinaris agglutinin binding of thyroglobulin molecules in thyroid carcinoma. Clin Chem Lab Med. 2009; 47(10):1285-90.
135. Sterling RK, Jeffers L, Gordon F, Venook AP, Reddy KR, Satomura S, Kanke F, Schwartz ME, Sherman M. Utility of Lens culinaris agglutinin-reactive fraction of alpha-fetoprotein and des-gamma-carboxy prothrombin, alone or in combination, as biomarkers for hepatocellular carcinoma. Clin Gastroenterol Hepatol. 2009 Jan; 7(1):104-13.
136. Toyoda H, Kumada T, Kaneoka Y, Osaki Y, Kimura T, Arimoto A, Oka H, Yamazaki O, Manabe T, Urano F, Chung H, Kudo M, Matsunaga T. Prognostic value of pretreatment levels of tumor markers for hepatocellular carcinoma on survival after curative treatment of patients with HCC. J Hepatol. 2008 Aug; 49(2):223-32.
137. Leerapun A, Suravarapu SV, Bida JP, Clark RJ, Sanders EL, Mettler TA, Stadheim LM, Aderca I, Moser CD, Nagorney DM, LaRusso NF, de Groen PC, Menon KV, Lazaridis KN, Gores GJ, Charlton MR, Roberts RO, Therneau TM, Katzmann JA, Roberts LR. The utility of Lens culinaris agglutinin-reactive alpha-fetoprotein in the diagnosis of hepatocellular carcinoma: evaluation in a United States referral population. Clin Gastroenterol Hepatol. 2007 Mar;5(3):394-402
138. Dalla Pellegrina C, Perbellini O, Scupoli MT, Tomelleri C, Zanetti C, Zoccatelli G, Fusi M, Peruffo A, Rizzi C, Chignola R. Effects of wheat germ agglutinin on human gastrointestinal epithelium: insights from an experimental model of immune/epithelial cell interaction. Toxicol Appl Pharmacol. 2009 Jun 1;237(2):146-53

139. Ebert C, Nebe B, Walzel H, Weber H, Jonas L. Inhibitory effect of the lectin wheat germ agglutinin (WGA) on the proliferation of AR42J cells. Acta Histochem. 2009; 111(4):335-42.
140. Dalla Pellegrina C, Rizzi C, Mosconi S, Zoccatelli G, Peruffo A, Chignola R. Plant lectins as carriers for oral drugs: is wheat germ agglutinin a suitable candidate? Toxicol Appl Pharmacol. 2005 Sep 1; 207(2):170-8.
141. Ohno Y, Naganuma T, Ogawa T, Muramoto K. Effect of lectins on the transport of food ingredients in Caco-2 cell cultures. Biofactors. 2004; 21(1-4):399-401.
142. Valentiner U, Fabian S, Schumacher U, Leathem AJ. The influence of dietary lectins on the cell proliferation of human breast cancer cell lines in vitro. Anticancer Res. 2003 Mar-Apr; 23(2B):1197-206.
143. Miyoshi N, Koyama Y, Katsuno Y, Hayakawa S, Mita T, Ohta T, Kaji K, Isemura M. Apoptosis induction associated with cell cycle dysregulation by rice bran agglutinin. J Biochem. 2001 Dec; 130(6):799-805.
144. Wyczólkowska J, Weyer A, Dastych J. Inhibitory effect of wheat germ agglutinin on mouse mast cell adhesion to fibronectin. Int Arch Allergy Immunol. 2000 Jul; 122(3):216-23.
145. Ovelgönne JH, Koninkx JF, Pusztai A, Bardocz S, Kok W, Ewen SW, Hendriks HG, van Dijk JE. Decreased levels of heat shock proteins in gut epithelial cells after exposure to plant lectins. Gut. 2000 May; 46(5):679-87.
146. Schwarz RE, Wojciechowicz DC, Picon AI, Schwarz MA, Paty PB. Wheat germ agglutinin-mediated toxicity in pancreatic cancer cells. Br J Cancer. 1999 Aug; 80(11):1754-62.
147. Pusztai A, Ewen SW, Grant G, Brown DS, Stewart JC, Peumans WJ, Van Damme EJ, Bardocz S. Antinutritive effects of wheat-germ agglutinin and other N-acetylglucosamine-specific lectins. Br J Nutr. 1993 Jul; 70(1):313-21.
148. Hwang SB, Wang S. Wheat germ agglutinin potentiates specific binding of platelet-activating factor to human platelet membranes and induces platelet-activating factor synthesis in intact platelets. Mol Pharmacol. 1991 Jun; 39(6):788-97.
149. Inazu T, Taniguchi T, Ohta S, Miyabo S, Yamamura H. The lectin wheat germ agglutinin induces rapid protein-tyrosine phosphorylation in human platelets. Biochem Biophys Res Commun. 1991 Feb 14; 174(3):1154-8.
150. Kilpatrick DC, McCurrach PM. Wheat germ agglutinin is mitogenic, nonmitogenic, and anti-mitogenic for human lymphocytes. Scand J Immunol. 1987 Apr; 25(4):343-8.
151. Landreth GE, Williams LK, McCutchen C. Wheat germ agglutinin blocks the biological effects of nerve growth factor. J Cell Biol. 1985 Nov;101(5 Pt 1):1690-4
152. Kolberg J, Sollid L. Lectin activity of gluten identified as wheat germ agglutinin. Biochem Biophys Res Commun. 1985 Jul 31; 130(2):867-72.
153. Kahn HJ, Baumal R. Differences in lectin binding in tissue sections of human and murine malignant tumors and their metastases. Am J Pathol. 1985 Jun; 119(3):420-9.
154. Higashihara M, Takahata K, Ohashi T, Kariya T, Kume S, Oka H. The platelet activation induced by wheat germ agglutinin. FEBS Lett. 1985 Apr 22; 183(2):433-8.
155. Toribio ML, de la Hera A, Pereira P, de Landázuri MO. Modulation of cytolytic T cell function by lectins. J Immunol. 1985 Apr;134(4):2179-84
156. Reed JC, Robb RJ, Greene WC, Nowell PC. Effect of wheat germ agglutinin on the interleukin pathway of human T lymphocyte activation. J Immunol. 1985 Jan; 134(1):314-23.
157. Hammarstrom S and Kabat EA. Purification and characterization of a blood group A reactive hemagglutinin from the snail Helix pomatia and a study of its combining site. Biochem 8: 2696–2705 (1969
158. Brooks SA, Leathem AJC, Campeljohn RS, Gregory W. Markers of prognosis in breast cancer – the relationship between HPA binding and histological grade, SPF, and ploidy. Breast Cancer Res Treat 25: 247–256 (1993)
159. Brooks SA. The involvement of Helix pomatia lectin (HPA) binding N- acetylgalactosamine glycans in cancer progression. Histology and Histopathology 15(1): 143–158 (2000)
160. Fenlon S, Ellis IO, Bell J, Todd JH, Elston CW, Blamey RW. Helix pomatia and Ulex europaeus lectin binding in human breast carcinoma. J Pathol 152: 169–176 (1987)
161. Leathem AJ and Brooks SA (1987) Predictive value of lectin binding on breast cancer recurrence and survival. Lancet i: 1054–1056
162. Macartney JC. Lectin histochemistry of galactose and N-acetylgalactosamine glycoconjugates in normal gastric mucosa and gastric cancer and the relationship with ABO blood group status. J Pathol. 1986; 150: 135–144.
163. Yoshida Y, Okamura T, Shirakusa T. An immunohistochemical study of Helix pomatia agglutinin binding on carcinomas of the esophagus. Surg Gynecol Obstet 177: 299–302 (1993)
164. Ikeda Y, Mori M, Adachi Y, Matsushima T, Sugimachi K. Prognostic value of the histochemical expression of Helix pomatia agglutinin in advanced colorectal cancer. Dis Colon Rectum 37: 181–184 (1994)
165. Sasano H, Rojas M, Silverberg SG. Analysis of lectin binding in benign and malignant thyroid tissue. Arch Pathol Lab Med 113: 186–189 (1989)
166. Shiraishi T, Atsumi S, Yatani R. Comparative study of prostatic carcinoma bone metastasis amongst Japanese in Japan and Japanese, Americans and Whites in Hawaii. Adv Exp Med Biol 324: 7–16 (1992)
167. Brooks SA, Hall DM, Buley I. GalNAc glycoprotein expression by breast cell lines, primary breast cancer and normal breast epithelial membrane. Br J Cancer. 2001 Sep 28; 85(7):1014-22.

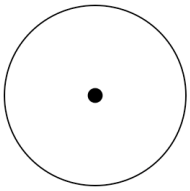
168. Gomes MM, Suzuki H, Brooks MT, Tomana M, Moldoveanu Z, Mestecky J, Julian BA, Novak J, Herr AB. Recognition of galactose-deficient O-glycans in the hinge region of IgA1 by N-acetylgalactosamine-specific snail lectins: a comparative binding study. Biochemistry. 2010 May 27
169. Springer GF. Tn epitope (N-acetyl-D-galactosamine alpha-O-serine/threonine) density in primary breast carcinoma: a functional predictor of aggressiveness. Mol Immunol. 1989 Jan; 26(1):1-5.
170. Leathem AJ, Brooks SA. Predictive value of lectin binding on breast-cancer recurrence and survival. Lancet. 1987 May 9; 1(8541):1054-6.
171. Brooks SA. The involvement of Helix pomatia lectin (HPA) binding N-acetylgalactosamine glycans in cancer progression. Histol Histopathol. 2000 Jan; 15(1):143-58.
172. Kritchevsky D, Tepper SA, Klurfeld DM. Lectin may contribute to the atherogenicity of peanut oil. Lipids. 1998 Aug; 33(8):821-3.
173. Thomsen MK, Hansen GH, Danielsen EM. Galectin-2 at the enterocyte brush border of the small intestine. Mol Membr Biol. 2009 Aug; 26(5):347-55.
174. Hernandez JD, Klein J, Van Dyken SJ, Marth JD, Baum LG. T-cell activation results in microheterogeneous changes in glycosylation of CD45. Int Immunol. 2007 Jul; 19(7):847-56.
175. Taraszewska A, Matyja E. Lectin binding pattern in meningiomas of various histological subtypes. Folia Neuropathol. 2007; 45(1):9-18.
176. Suzuki O, Nozawa Y, Abe M. Loss of L-PHA-, PNA-, or ConA-reactive oligosaccharides is associated with a poor prognosis in human Burkitt's lymphoma. Oncol Rep. 2007 Apr; 17(4):775-9.
177. Mukai T, Kaneko S, Matsumoto M, Ohori H. Binding of Bifidobacterium bifidum and Lactobacillus reuteri to the carbohydrate moieties of intestinal glycolipids recognized by peanut agglutinin. Int J Food Microbiol. 2004 Feb 1; 90(3):357-62.
178. Shiba T, Aiba Y, Ishikawa H, Ushiyama A, Takagi A, Mine T, Koga Y. The suppressive effect of bifidobacteria on Bacteroides vulgatus, a putative pathogenic microbe in inflammatory bowel disease. Microbiol Immunol. 2003; 47(6):371-8.
179. Kelsall A, FitzGerald AJ, Howard CV, Evans RC, Singh R, Rhodes JM, Goodlad RA. Dietary lectins can stimulate pancreatic growth in the rat. Int J Exp Pathol. 2002 Aug; 83(4):203-8.
180. Suzuki H, Kawaguchi T, Higuchi M, Shio Y, Fujiu K, Kanno R, Ohishi A, Motoki R, Gotoh M. Expression of peanut agglutinin-binding carbohydrates correlates with nodal involvement in human lung adenocarcinoma. Cancer Lett. 2002 Dec 10; 187(1-2):215-21.
181. Klein PJ, Vierbuchen M, Fischer J, Schulz KD, Farrar G, Uhlenbruck G. The significance of lectin receptors for the evaluation of hormone dependence in breast cancer. J Steroid Biochem. 1983 Jul; 19(1C):839-44.
182. Urbanski SJ, Levine ME, Borgs P, Bruce WR, Krepinsky JJ. Colonic mucus in human fetal development. Oncodev Biol Med. 1983; 4(5):363-70.
183. Vierbuchen M, Klein PJ, Rösel S, Fischer J. Peanut agglutinin (PNA) binding sites: a useful marker for hormonal dependence in experimental breast cancer. Cancer Detect Prev. 1983; 6(1-2):207-14.
184. Coon JS, Weinstein RS, Summers JL. Blood group precursor T-antigen expression in human urinary bladder carcinoma. Am J Clin Pathol. 1982 Jun; 77(6):692-9.
185. Reisner Y, Biniaminov M, Rosenthal E, Sharon N, Ramot B. Interaction of peanut agglutinin with normal human lymphocytes and with leukemic cells. Proc Natl Acad Sci U S A. 1979 Jan;76(1):447-51
186. London J, Berrih S, Bach JF. Peanut agglutinin. I. A new tool for studying T lymphocyte subpopulations. J Immunol. 1978 Aug; 121(2):438-43.
187. Berthier-Vergnes O, Réano A, Doré JF. Lectin binding glycoproteins in human melanoma cell lines with high or low tumorigenicity. Int J Cancer. 1986 May 15; 37(5):747-51.
188. Chastonay P, Hurlimann J, Gardiol D. Biological tissue markers in benign and malignant disease of the human prostate. Virchows Arch A Pathol Anat Histopathol. 1986; 410(3):221-9.
189. Yonezawa S, Nakamura T, Tanaka S, Kuroki K, Sato E. Lectin histochemistry in a case of ulcerative colitis complicating rectal carcinoma. Acta Pathol Jpn. 1985 Nov; 35(6):1571-9.
190. Tamaki K, Hino H, Ohara K, Furue M. Lectin-binding sites in Paget's disease. Br J Dermatol. 1985 Jul; 113(1):17-24.
191. Cooper HS, Reuter VE. Peanut lectin-binding sites in polyps of the colon and rectum. Adenomas, hyperplastic polyps, and adenomas with in situ carcinoma. Lab Invest. 1983 Dec; 49(6):655-61.
192. Shinozuka T, Takei S, Yanagida J, Watanabe H, Ohkuma S. Binding of lectins to "young" and "old" human erythrocytes. Blut. 1988 Sep; 57(3):117-23.
193. Skutelsky E, Hoenig S, Griffel B, Alroy J. The distribution of lectin receptor sites in human breast lesions. Pathol Res Pract. 1988 Aug; 183(4):469-75.
194. Stanley MW, Kiang DT, Sibley RK. Peanut lectin binding in breast carcinoma. Lack of correlation with estrogen receptor content. Cancer. 1986 Nov 1; 58(9):2046-51.
195. Rak JW, Basolo F, Elliott JW, Russo J, Miller FR. Cell surface glycosylation changes accompanying immortalization and transformation of normal human mammary epithelial cells. Cancer Lett. 1991 Apr; 57(1):27-36.
196. Gan RL. Peanut lectin-binding sites in gastric carcinoma and the adjacent mucosa. Zhonghua Bing Li Xue Za Zhi. 1990 Jun; 19(2):109-11.
197. Rhodes EG, Flynn M. Peanut agglutinin shows specificity for bone marrow plasma cells. Br J Haematol. 1989 Feb; 71(2):183-7.
198. Rameshkumar K, Cooper R, Jalihal A, Nirmala V. Peanut agglutinin binding by gastric mucosal epithelial cells in Helicobacter pylori associated gastritis. Indian J Med Res. 1994 Jul;100:26-30

199. Ryder SD, Parker N, Ecclestone D, Haqqani MT, Rhodes JM. Peanut lectin stimulates proliferation in colonic explants from patients with inflammatory bowel disease and colon polyps. Gastroenterology. 1994 Jan; 106(1):117-24.
200. Hamada S, Furumoto H, Kamada M, Hirao T, Aono T. High expression rate of Tn antigen in metastatic lesions of uterine cervical cancers. Cancer Lett. 1993 Nov 1; 74(3):167-73.
201. Mazumdar S, SenGupta SK, Param R, Sinha SN. Binding pattern of eight different lectins in healthy subjects and patients with dysplastic and malignant lesions of the oral cavity. Int J Oral Maxillofac Surg. 1993 Oct; 22(5):301-5.
202. Levi G, Teichberg VI. Isolation and physicochemical characterization of electrolectin, a beta-D-galactoside binding lectin from the electric organ of Electrophorus electricus. J Biol Chem. 1981 Jun 10; 256(11):5735-40.
203. Podleski TR, Greenberg I. Distribution and activity of endogenous lectin during myogenesis as measured with antilectin antibody. Proc Natl Acad Sci U S A. 1980 Feb; 77(2):1054-8.
204. Gilboa-Garber N. Towards anti-Pseudomonas aeruginosa adhesion therapy. Adv Exp Med Biol. 1996; 408:39-50.
205. Sharon N and Lis H. *Lectins*. Second Edition. Kluwer Academic Publishers. 2003
206. Ewart RB, Kornfeld S, Kipnis DM. Effect of lectins on hormone release from isolated rat islets of Langerhans. Diabetes. 1975 Aug; 24(8):705-14.
207. Gray AM, Flatt PR. Insulin-releasing and insulin-like activity of Agaricus campestris (mushroom). J Endocrinol. 1998 May; 157(2):259-66.
208. Yu L, Fernig DG, Smith JA, Milton JD, Rhodes JM. Reversible inhibition of proliferation of epithelial cell lines by Agaricus bisporus (edible mushroom) lectin. Cancer Res. 1993 Oct 1; 53(19):4627-32.
209. Jordinson M, El-Hariry I, Calnan D, Calam J, Pignatelli M. Vicia faba agglutinin, the lectin present in broad beans, stimulates differentiation of undifferentiated colon cancer cells. Gut. 1999 May; 44(5):709-14.
210. Allen AK, Desai NN, Neuberger A. Vicia faba lectin. Methods Enzymol. 1978; 50:335-9.
211. Rodríguez-Juan C, Pérez-Blas M, Suárez-García E, López-Suárez JC, Múzquiz M, Cuadrado C, Martín-Villa JM. Lens culinaris, Phaseolus vulgaris, and Vicia faba lectins specifically trigger IL-8 production by the human colon carcinoma cell line CACO-2. Cytokine. 2000 Aug; 12(8):1284-7.
212. Datta PK, Basu PS, Datta TK. Enhancement of lectin-erythrocyte agglutination by gums. Biochim Biophys Acta. 1988 Nov 2; 957(1):164-7.
213. Gebauer G, Schimpl A, Rüdiger H. Lectin-binding proteins as potent mitogens for B-lymphocytes from nu/nu mice. Eur J Immunol. 1982 Jun; 12(6):491-5.
214. Yu LG, Milton JD, Fernig DG, Rhodes JM. Opposite effects on human colon cancer cell proliferation of two dietary Thomsen-Friedenreich antigen-binding lectins. J Cell Physiol. 2001 Feb; 186(2):282-7.
215. Carrizo ME, Capaldi S, Perduca M, Irazoqui FJ, Nores GA, Monaco HL. The antineoplastic lectin of the common edible mushroom (Agaricus bisporus) has two binding sites, each specific for a different configuration at a single epimeric hydroxyl. J Biol Chem. 2005 Mar 18;280(11):10614-23
216. Henić E, Thiel S, Mårdh PA. Mannan-binding lectin in women with a history of recurrent vulvovaginal candidiasis. Eur J Obstet Gynecol Reprod Biol. 2010 Feb; 148(2):163-5.
217. Lima-Neto RG, Beltrão EI, Oliveira PC, Neves RP. Adherence of Candida albicans and Candida parapsilosis to epithelial cells correlates with fungal cell surface carbohydrates. Mycoses. 2009 Sep 7.
218. Donders GG, Babula O, Bellen G, Linhares IM, Witkin SS. Mannose-binding lectin gene polymorphism and resistance to therapy in women with recurrent vulvovaginal candidiasis. BJOG. 2008 Sep; 115(10):1225-31.
219. Kohatsu L, Hsu DK, Jegalian AG, Liu FT, Baum LG. Galectin-3 induces death of Candida species expressing specific beta-1,2-linked mannans. J Immunol. 2006 Oct 1; 177(7):4718-26.
220. Filler SG. Candida-host cell receptor-ligand interactions. Curr Opin Microbiol. 2006 Aug;9(4):333-9
221. Liu F, Liao Q, Liu Z. Mannose-binding lectin and vulvovaginal candidiasis. Int J Gynaecol Obstet. 2006 Jan; 92(1):43-7.
222. Newman SL, Holly A. Candida albicans is phagocytosed, killed, and processed for antigen presentation by human dendritic cells. Infect Immun. 2001 Nov; 69(11):6813-22.
223. Cormack BP, Ghori N, Falkow S. An adhesin of the yeast pathogen Candida glabrata mediating adherence to human epithelial cells. Science. 1999 Jul 23; 285(5427):578-82.
224. Vardar-Unlü G, McSharry C, Douglas LJ. Fucose-specific adhesins on germ tubes of Candida albicans. FEMS Immunol Med Microbiol. 1998 Jan; 20(1):55-67.
225. Filler SG, Pfunder AS, Spellberg BJ, Spellberg JP, Edwards JE Jr. Candida albicans stimulates cytokine production and leukocyte adhesion molecule expression by endothelial cells. Infect Immun. 1996 Jul; 64(7):2609-17.
226. Eue I, Kauschke E, Mohrig W, Cooper EL. Isolation and characterization of earthworm hemolysins and agglutinins. Dev Comp Immunol. 1998 Jan-Feb; 22(1):13-25.
227. Camby I, Le Mercier M, Lefranc F, Kiss R. Galectin-1: a small protein with major functions. Glycobiology. 2006 Nov; 16(11):137R-157R.
228. Liu FT, Rabinovich GA. Galectins: regulators of acute and chronic inflammation. Ann N Y Acad Sci. 2010 Jan; 1183:158-82.
229. de Boer RA, Yu L, van Veldhuisen DJ.Galectin-3 in cardiac remodeling and heart failure. Curr Heart Fail Rep. 2010 Mar;7(1):1-8

230. Cummings RD and Liu FT. Galectins. In: Varki A, Cummings R, Esko J, Freeze H, Stanley P, Bertozzi C, Hart G, and Etzler M. *Essentials of Glycobiology*, 2nd edition, Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2009
231. Gallegos B, Pérez-Campos E, Martinez R, Leyva P, Martinez M, Hernández R, Pina S, Hernández C, Zenteno E, Hernández P.O-glycosylation expression in fibroadenoma. Prep Biochem Biotechnol. 2010; 40(1):1-12.
232. Lee MC, Turcinov D, Damjanov I. Lectins as markers for eosinophilic leukocytes. Histochemistry. 1987; 86(3):269-73.
233. Hayes CE, Goldstein IJ. An alpha-D-galactosyl-binding lectin from Bandeiraea simplicifolia seeds. Isolation by affinity chromatography and characterization. J Biol Chem. 1974 Mar 25;249(6):1904-14
234. Murphy LA, Goldstein IJ. Five alpha-D-galactopyranosyl-binding isolectins from Bandeiraea simplicifolia seeds. J Biol Chem. 1977 Jul 10;252(13):4739-42.
235. Eckhardt AE, Malone BN, Goldstein IJ. Inhibition of Ehrlich ascites tumor cell growth by Griffonia simplicifolia I lectin in vivo. Cancer Res. 1982 Aug;42(8):2977-9.
236. Chen YF, Boland CR, Kraus ER, Goldstein IJ. The lectin Griffonia simplicifolia I-A4 (GS I-A4) specifically recognizes terminal alpha-linked N-acetylgalactosaminyl groups and is cytotoxic to the human colon cancer cell lines LS174t and SW1116. Int J Cancer. 1994 May 15;57(4):561-7.
237. Siraganian PA, Siraganian RP. Basophil activation by concanavalin A: characteristics of the reaction. J Immunol. 1974 Jun;112(6):2117-25
238. Katelaris PH, Naeem A, Farthing MJ. Attachment of Giardia lamblia trophozoites to a cultured human intestinal cell line. Gut. 1995 Oct;37(4):512-8.
239. Grant G. et al. Nutritional and hemagglutinating properties of several tropical seeds. J Agric Sci. 1995; 124, 437-445.
240. Larue-Achagiotis C, Picard M, Louis-Sylvestre J. Feeding behavior in rats on a complete diet containing Concanavalin A. Reprod Nutr Dev. 1992;32(4):343-50.
241. Ohno Y, Naganuma T, Ogawa T, Muramoto K. Effect of lectins on the transport of food factors in caco-2 cell monolayers. J Agric Food Chem. 2006 Jan 25;54(2):548-53.
242. Nagler A, Morecki S, Slavin S. The use of soybean agglutinin (SBA) for bone marrow (BM) purging and hematopoietic progenitor cell enrichment in clinical bone-marrow transplantation. Mol Biotechnol. 1999 Apr;11(2):181-94.
243. Masaoka H, Shibata K, Yamaguchi H. Topological and functional characterization of the N-glycans of soybean (Glycine max) agglutinin. J Biochem. 1999 Jul;126(1):212-7.
244. Jordinson M, Beales IL, Calam J. Soybean agglutinin stimulated cholecystokinin release from cultured rabbit jejunal cells requires calcium influx via L-type calcium channels. Peptides. 1998;19(9):1541-7.
245. Benjamin CF, Figueiredo RC, Henriques MG, Barja-Fidalgo C. Inflammatory and anti-inflammatory effects of soybean agglutinin. Braz J Med Biol Res. 1997 Jul;30(7):873-81.
246. Gordon SR, Wood M. Soybean (Glycine max) agglutinin binds to corneal endothelial cells during wound repair and alters their microfilament pattern. Cell Mol Biol (Noisy-le-grand). 1997 May;43(3):329-36.
247. Terashima S, Takano Y, Ohori T, Kanno T, Kimura T, Motoki R, Kawaguchi T. Soybean agglutinin binding as a useful prognostic indicator in stomach cancer. Surg Today. 1997;27(4):293-7.
248. Li N. The phytohemagglutinins and soybean agglutinin in diagnosis of endometrial adenocarcinoma. Zhonghua Fu Chan Ke Za Zhi. 1993 Jun;28(6):349-50, 381.
249. Loy TS, Kyle J, Bickel JT. Binding of soybean agglutinin lectin to prostatic hyperplasia and adenocarcinoma. Cancer. 1989 Apr 15;63(8):1583-6.
250. Aoki D, Kawakami H, Nozawa S, Udagawa Y, Iizuka R, Hirano H. Differences in lectin binding patterns of normal human endometrium between proliferative and secretory phases. Histochemistry. 1989;92(3):177-84.
251. Morecki S, Margel S, Slavin S. Removal of breast cancer cells by soybean agglutinin in an experimental model for purging human marrow. Cancer Res. 1988 Aug 15; 48(16):4573-7.
252. Ben-Bassat H, Weksler-Zangen S, Shlomai Z, Prokocimer M. Interaction of soybean agglutinin with human leukemia-lymphoma lines at various stages of differentiation. Leuk Res. 1987; 11(7):589-95.
253. Drickamer K, Dodd RB. C-type lectin-like domains in Caenorhabditis elegans: predictions from the complete genome sequence. Glycobiology. 1999; 9, 1357-1369.
254. Pereira ME, Gruezo F, Kabat EA. Purification and characterization of lectin II from Ulex europaeus seeds and an immunochemical study of its combining site. Arch Biochem Biophys. 1979 May; 194(2):511-25.
255. Roychowdhury DF, Tseng A Jr, Fu KK, Weinburg V, Weidner N. New prognostic factors in nasopharyngeal carcinoma. Tumor angiogenesis and C-erbB2 expression. Cancer. 1996 Apr 15; 77(8):1419-26.
256. Sobral AP, Rego MJ, Cavalacanti CL, Carvalho LB Jr, Beltrão EI. ConA and UEA-I lectin histochemistry of parotid gland mucoepidermoid carcinoma. J Oral Sci. 2010; 52(1):49-54.
257. Domino SE, Hurd EA, Thomsson KA, Karnak DM, Holmén Larsson JM, Thomsson E, Bäckström M, Hansson GC. Cervical mucins carry alpha(1,2)fucosylated glycans that partly protect from experimental vaginal candidiasis. Glycoconj J. 2009 Dec; 26(9):1125-34.
258. Neumann H, Wex T, Mönkemüller K, Vieth M, Fry LC, Malfertheiner P. Lectin UEA-I-binding proteins are specifically increased in the squamous epithelium of patients with Barrett's esophagus. Digestion. 2008; 78(4):201-7.
259. Gao X, Chen J, Tao W, Zhu J, Zhang Q, Chen H, Jiang X. UEA I-bearing nanoparticles for brain delivery following intranasal administration. Int J Pharm. 2007 Aug 1; 340(1-2):207-15.

260. Franz S, Frey B, Sheriff A, Gaipl US, Beer A, Voll RE, Kalden JR, Herrmann M. Lectins detect changes of the glycosylation status of plasma membrane constituents during late apoptosis. Cytometry A. 2006 Apr; 69(4):230-9.
261. Meier B, Schirmacher P, Klesper B. Expression of lectin binding sites as a prognostic parameter in mouth mucosa carcinomas. Mund Kiefer Gesichtschir. 2001 Mar; 5(2):130-5.
262. Fujitani N, Liu Y, Toda S, Shirouzu K, Okamura T, Kimura H. Expression of H type 1 antigen of ABO histo-blood group in normal colon and aberrant expressions of H type 2 and H type 3/4 antigens in colon cancer. Glycoconj J. 2000 May; 17(5):331-8.
263. Lazaris AC, Chatzigianni EB, Paraskevakou H, Tseleni-Balafouta S, Davaris PS. Lectin histochemistry as a predictor of dysplasia grade in colorectal adenomas. Pathol Oncol Res. 2000; 6(4):265-71.
264. Sakamoto K, Hirose H, Onizuka A, Hayashi M, Futamura N, Kawamura Y, Ezaki T. Quantitative study of changes in intestinal morphology and mucus gel on total parenteral nutrition in rats. J Surg Res. 2000 Dec;94(2):99-106
265. Gornik I, Maravić G, Dumić J, Flögel M, Lauc G. Fucosylation of IgG heavy chains is increased in rheumatoid arthritis. Clin Biochem. 1999 Nov; 32(8):605-8.
266. Yatabe K, Hiraguri M, Sueishi M, Takeuchi M, Nonaka I, Kawai M. The significance of Ulex europaeus agglutinin I lectin binding fibers in various muscular diseases. Rinsho Shinkeigaku. 1998 May; 38(5):395-401.
267. Flögel M, Lauc G, Gornik I, Macek B. Fucosylation and galactosylation of IgG heavy chains differ between acute and remission phases of juvenile chronic arthritis. Clin Chem Lab Med. 1998 Feb; 36(2):99-102.
268. Gebert A, Cetin Y. Expression of fucose residues in enteroendocrine cells. Histochem Cell Biol. 1998 Feb; 109(2):161-5.
269. Erhan Y, Alkanat MB, Akyildiz M, Icoz G, Yilmaz R, Erhan Y. Ulex Europaeus Agglutinin (UEA-I) lectin binding in breast carcinoma and its relationship to prognostic factors. Ann Saudi Med. 1996 Sep; 16(5):509-11.
270. Barre A, Van Damme EJ, Peumans WJ, Rougé P. Structure-function relationship of monocot mannose-binding lectins. Plant Physiol. 1996 Dec; 112(4):1531-40.
271. Van Damme EJ, Smeets K, Engelborghs I, Aelbers H, Balzarini J, Pusztai A, van Leuven F, Goldstein IJ, Peumans WJ. Cloning and characterization of the lectin cDNA clones from onion, shallot, and leek. Plant Mol Biol. 1993 Oct; 23(2):365-76.
272. Peumans WJ, Smeets K, Van Nerum K, Van Leuven F, Van Damme EJ. Lectin and alliinase are the predominant proteins in nectar from leek (Allium porrum L.) flowers. Planta. 1997; 201(3):298-302.
273. Chandra NR, Dam TK, Surolia A, Vijayan M. Crystallization and preliminary crystallographic studies on the mannose-specific lectin from garlic. Acta Crystallogr D Biol Crystallogr. 1997 Nov 1; 53(Pt 6):787-8.
274. Jiratchariyakul W, Wiwat C, Vongsakul M, Somanabandhu A, Leelamanit W, Fujii I, Suwannaroj N, Ebizuka Y. HIV inhibitor from Thai bitter gourd. Planta Med. 2001 Jun; 67(4):350-3.
275. Huang L, Adachi T, Shimizu Y, Goto Y, Toyama J, Tanaka H, Akashi R, Sawaguchi A, Iwata H, Haga T. Characterization of lectin isolated from Momordica charantia seed as a B cell activator. Immunol Lett. 2008 Dec 22; 121(2):148-56.
276. Sultan NA, Swamy MJ. Energetics of carbohydrate binding to Momordica charantia (bitter gourd) lectin: an isothermal titration calorimetric study. Arch Biochem Biophys. 2005 May 1; 437(1):115-25.
277. Puri M, Kaur I, Kanwar RK, Gupta RC, Chauhan A, Kanwar JR. Ribosome inactivating proteins (RIPs) from Momordica charantia for anti-viral therapy. Curr Mol Med. 2009 Dec; 9(9):1080-94.
278. http://www.dadamo.com/lecster2/Lecster-read.pl?show=445
279. Azzi A, Wang T, Zhu DW, Zou YS, Liu WY, Lin SX. Crystal structure of native Cinnamomum isoform III and its comparison with other ribosome inactivating proteins. Proteins. 2009 Jan; 74(1):250-5.
280. Sůla K, Paluska E, Nouza K, Daněk PF. Lentil lectin inhibits cells producing graft-versus-host reaction but does not suppress hematopoietic stem cells in mice. Immunobiology. 1986 Oct; 173(1):35-40.
281. Kornfeld K, Reitman ML, Kornfeld R. The carbohydrate-binding specificity of pea and lentil lectins. Fucose is an important determinant. J Biol Chem. 1981 Jul 10; 256(13):6633-40.
282. Tanwandee T, Setthasin S, Charatcharoenwitthaya P, Chainuvati S, Leelakusolvong S, Pausawasdi N, Srikureja W, Pongprasobchai S, Manatsathit S, Kachintorn U, Ekpo P, Senawong S. Clinical utility of lens culinaris agglutinin-reactive alpha-fetoprotein in the diagnosis of hepatocellular carcinoma: evaluation in a Thai referral population. J Med Assoc Thai. 2009 Mar; 92 Suppl 2:S49-56.
283. Li YL, Ma HB, Guo JX, Shi JB, Xu J, Liu AX, Yang LH, Li BA, Mao YL. The studies on early warning of Lens culinaris-reactive alpha-Fetoprotein (AFP-L3) detecting in primary hepatocellular carcinoma. Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi. 2008 Oct; 22(5):336-8.
284. Shimizu K, Nakamura K, Kobatake S, Satomura S, Maruyama M, Kameko F, Tajiri J, Kato R. The clinical utility of Lens culinaris agglutinin-reactive thyroglobulin ratio in serum for distinguishing benign from malignant conditions of the thyroid. Clin Chim Acta. 2007 Apr; 379(1-2):101-4.
285. Yano Y, Yamashita F, Kuwaki K, Fukumori K, Kato O, Kiyomatsu K, Sakai T, Yamamoto H, Yamasaki F, Ando E, Sata M. Partial spontaneous regression of hepatocellular carcinoma: a case with high concentrations of serum lens culinaris agglutinin-reactive alpha-fetoprotein. Kurume Med J. 2005; 52(3):97-103.
286. Kubo S, Matsukawa M. Tumor markers of hepatocellular carcinoma. Gan To Kagaku Ryoho. 2004 Dec; 31(13):2203-6.
287. Runza VL, Schwaeble W, Männel DN. Ficolins: novel pattern recognition molecules of the innate immune response. Immunobiology. 2008; 213(3-4):297-306.

288. Jönsson T, Olsson S, Ahrén B, Bøg-Hansen TC, Dole A, Lindeberg S. Agrarian diet and diseases of affluence—do evolutionary novel dietary lectins cause leptin resistance? BMC Endocr Disord. 2005 Dec 10; 5:10.
289. Hummelshoj T. et al. Coding and non-coding polymorphisms in the lectin pathway activator L-ficolin gene in 188 Dutch blood bank donors. Mol Immunol. 2005; 43:851–855.
290. Atkinson AP. et al. L-ficolin in children with recurrent respiratory infections. Clin Exp Immunol. 2004; 138:517–520.
291. Gadjeva M. Takahashi K. Thiel S, Mannan-binding lectin-a soluble pattern recognition molecule. Mol Immunol 2004; 4J:113-21.
292. Kilpatrick DC. Introduction to mannan-binding lectin. Biochem Soc Trans 2003; (Pt 4):745-7.
293. Jack DL, Klein NJ. Turner MW. Mannose-binding lectin: targeting the microbial world for complement attack and opsonophagocytosis. Immunol Rev. 2002; 18O: 86-99.
294. Gupta K, Gupta RK, Hajela K. Disease associations of mannose-binding lectin & potential of replacement therapy. Indian J Med Res. 2008 May; 127(5):431-40.
295. Summerfield JA, Sumiya M, Levin M, Turner MW. Association of mutations in mannose binding protein gene with childhood infection in consecutive hospital series. BMJ 1997; 314: 1229-1232
296. Garred P, Pressler T, Madsen HO, Frederiksen B, Svejgaard A, Hoiby N, Schwartz M, Koch C. Association of mannose-binding lectin gene heterogeneity with severity of lung disease and survival in cystic fibrosis. J Clin Invest 1999; 104: 431-437
297. Neth O, Hann I, Turner MW, Klein NJ. Deficiency of mannose-binding lectin and burden of infection in children with malignancy: a prospective study. Lancet 2001; 358: 614-618
298. Peterslund NA, Koch C, Jensenius JC, Thiel S. Association between deficiency of mannose-binding lectin and severe infections after chemotherapy. Lancet 2001; 358: 637-638
299. Mullighan CG, Bardy PG. Mannose-binding lectin and infection following allogeneic hematopoietic stem cell transplantation. Leuk Lymphoma 2004; 45: 247-256
300. Bouwman LH, Roos A, Terpstra OT, de Knijff P, van Hoek B, Verspaget HW, Berger SP, Daha MR, Frolich M, van der Slik AR, Doxiadis II, Roep BO, Schaapherder AF. Mannose binding lectin gene polymorphisms confer a major risk for severe infections after liver transplantation. Gastroenterology 2005; 129: 408-414
301. Koch A, Melbye M, Sorensen P, Homoe P, Madsen HO, Molbak K, Hansen CH, Andersen LH, Hahn GW, Garred P. Acute respiratory tract infections and mannose-binding lectin insufficiency during early childhood. JAMA 2001; 285: 1316-1321
302. Garred P, Pressler T, Lanng S, Madsen HO, Moser C, Laursen I, Balstrup F, Koch C, Koch C. Mannose-binding lectin (MBL) therapy in an MBL-deficient patient with severe cystic fibrosis lung disease. Pediatr Pulmonol 2002; 33: 201-207.
303. Boniotto M, Braida L, Baldas V, Not T, Ventura A, Vatta S, Radillo O, Tedesco F, Percopo S, Montico M, Amoroso A, Crovella S. Evidence of a correlation between mannose binding lectin and celiac disease: a model for other autoimmune diseases. J Mol Med 2005; 83: 308-315
304. Lee YH, Witte T, Momot T, Schmidt RE, Kaufman KM, Harley JB, Sestak AL. The mannose-binding lectin gene polymorphisms and systemic lupus erythematosus: two case-control studies and a meta-analysis. Arthritis Rheum 2005; 52: 3966-3974
305. Worthley DL, Bardy PG, Gordon DL, Mullighan CG. Mannose-binding lectin and maladies of the bowel and liver. World J Gastroenterol. 2006 Oct 28; 12(40):6420-8.
306. Boniotto M, Braida L, Spano A, Pirulli D, Baldas V, Trevisiol C, Not T, Tommasini A, Amoroso A, Crovella S. Variant mannose-binding lectin alleles are associated with celiac disease. Immunogenetics 2002; 54: 596-598
307. Iltanen S, Maki M, Collin P, Mustalahti K, Kaukinen K, Partanen J, Hulkkonen J, Hurme M, Aittoniemi J. The association between mannan-binding lectin gene alleles and celiac disease. Am J Gastroenterol 2003; 98: 2808-2809
308. Garner OB, Baum LG. Galectin-glycan lattices regulate cell-surface glycoprotein organization and signaling. Biochem Soc Trans. 2008 Dec; 36(Pt 6):1472-7.
309. Barthel SR, Gavino JD, Descheny L, Dimitroff CJ. Targeting selectins and selectin ligands in inflammation and cancer. Expert Opin Ther Targets. 2007 Nov; 11(11):1473-91.
310. Gout S. et al. Death receptor-3, a new E-selectin counter-receptor that confers migration and survival advantages to colon carcinoma cells by triggering p38 and ERK MAPK activation. Cancer Res. 2006; 66(18):9117–9124.
311. Barbalic M, Dupuis J, Dehghan A, Bis JC, Hoogeveen RC, Schnabel RB, Nambi V, Bretler M, Smith NL, Peters A, Lu C, Tracy RP, Aleksic N, Heeriga J, Keaney JF Jr, Rice K, Lip GY, Vasan RS, Glazer NL, Larson MG, Uitterlinden AG, Yamamoto J, Durda P, Haritunians T, Psaty BM, Boerwinkle E, Hofman A, Koenig W, Jenny NS, Witteman JC, Ballantyne C, Benjamin EJ. Large-scale genomic studies reveal central role of ABO in sP-selectin and sICAM-1 levels. Hum Mol Genet. 2010 May 1; 19(9):1863-72.
312. Paterson AD, Lopes-Virella MF, Waggott D, Boright AP, Hosseini SM, Carter RE, Shen E, Mirea L, Bharaj B, Sun L, Bull SB. Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group. Genome-wide association identifies the ABO blood group as a major locus associated with serum levels of soluble E-selectin. Arterioscler Thromb Vasc Biol. 2009 Nov; 29(11):1958-67.
313. Qi L, Cornelis MC, Kraft P, Jensen M, van Dam RM, Sun Q, Girman CJ, Laurie CC, Mirel DB, Hunter DJ, Rimm E, Hu FB. Genetic variants in ABO blood group region, plasma soluble E-selectin levels and risk of type 2 diabetes. Hum Mol Genet. 2010 May 1; 19(9):1856-62.



Lectins and glycoconjugates in oncology

> *"Some cancers contain an A-like substance even when they occur in persons who are not A or AB. These observations suggest that in the tissues, both normal and neoplastic, of all persons, there are blood group A-like antigens present at a biochemical level, which are usually inaccessible to the immune system."*
>
> *—Arthur E. Mourant*

Stephen Paget (1855-1926), an English surgeon, the son of the distinguished surgeon and pathologist Sir James Page, introduced this concept, referred to as the "seed and soil hypothesis," in 1889. (43) According to the theory, the "seed'—the metastatic cell—needs to be compatible with the "soil'—the host tissue—for successful growth to occur (42, 44). In his paper, Paget presents and analyzes 735 fatal cases of breast cancer, complete with autopsy, as well as many other cancer cases from the literature and argues that the distribution of metastases cannot be due to chance. He concludes that, although "the best work in pathology of cancer is done by those who… are studying the nature of the seed (the cancer cell)," the "observations of the properties of the soil (the secondary organ) may also be useful." Despite this early observation, the nature of the interactions controlling both the efficiency of the metastatic process and its tissue specificity remains a poorly understood aspect of cancer progression even today.

The progression of cancer is a multi-step process. Over 80% of malignant tumors are carcinomas that originate in epithelial tissues, whence they invade via the basal membrane into the connective tissue. At some point, subpopulations of cells may detach from the primary tumor and spread via the bloodstream and the lymphatic system. Some of them give rise to metastases in distant organs. Like normal cells during embryogenesis, tumor cells undergo activation and rapid growth, adhere to a variety of other cell types and cell matrices, and invade tissues. Embryonic development and cellular activation in vertebrates are typically accompanied by changes in cellular glycosylation profiles.

Metastases account for the majority of patients" deaths due to cancer, and thus understanding the metastatic process is of critical importance. The metastatic cascade is a very inefficient process; only one in about a thousand cells that leave the primary tumor goes on to form a macroscopic secondary tumor. This property is referred to as "metastatic inefficiency."
(42)

Hematogenous spread of tumor cells and metastasis formation in secondary organs are insidious aspects of cancer. The two major concepts describing cancer metastasis are based either on the adhesion of cancer cells to the blood vessel endothelia ("seed and soil hypothesis") or on the homotypic cancer cell aggregation ("mechanical trapping theory") as a key component of the metastatic cascade. (41) Metastatic tumors also show preferential growth in different organs with a distribution that cannot be explained by blood flow patterns alone. Hence, the efficiency of the metastatic process depends on specific interactions between the invading cancer cells and the local organ tissues. (42)

ABERRENT GLYCOSYLATION AND MALIGNANCY

Tumor development is usually associated with changes in cell surface carbohydrates. These are often divided into changes related to terminal carbohydrate structures, which include incomplete synthesis and modification of normally existing carbohydrates, and changes in the carbohydrate core structure. Cell glycosylation depends on the expression and function of various glycosyltransferases and glycosidases. Numerous data demonstrate that malignant transformation is associated with various and complex alterations in the glycosylation process. In the first step of this sequence, a cell within a colony or solid tissue is instructed to disrupt cadherin-based intercellular junctions and acquire a fibroblastoid, motile phenotype, initiating detachment from the primary site of accretion.

Invasive growth, for either normal development or metastasis, is a complex morphogenetic program in which proliferative responses are integrated by apparently independent events such as migration, survival, matrix degradation, and induction of cell polarity. During this phase, invading cells must induce a constant and dynamic remodeling of integrin-mediated adhesive contacts with the ECM, which provides a mechanical support for cell migration and prevents the induction of apoptosis. Cell depolarization and invasion are followed by stimulation of cell growth, which allows new regions of the extracellular environment to become populated with cells, setting the stage for the restoration of normal tissue complexity. (72)

Plasminogen-related growth factors

The temporal and spatial control of the invasive growth process can be largely attributed to two molecules that are structurally related to plasminogen, the *plasminogen-related growth factor-1 (PRGF-1)* and *plasminogen-related growth factor-1 (PRGF-2)* and possibly to a phylogenetically related family of ligand-receptor pairs represented by the *semaphorins* and *plexins*.

PRGF-1, also known as scatter factor (SF), is a potent survival and regeneration factor mesenchymal-derived effector of dissociation and cell motility --the scattering referred to by the name “scatter factor.” Its role in organ reconstruction depends on both potentiation of cell growth and modulation of complex architectural events that are instrumental for the re-establishment of normal tissue patterning. Indeed, PRGF-1 promotes remodeling of epithelial cells cultured in three-dimensional collagen gels (73), induces the formation of branching tubular structures in mammary gland (74) and metanephric organ cultures (75), and contributes to lung (76), tooth (77), and hair follicle (78) maturation. PRGF-1 stimulates several facets of invasive growth in virtually every tissue of the body. It acts as a potent angiogenic factor (79); it controls bone formation and resorption (80) as well as cartilage remodeling (81); it promotes amplification and differentiation of multipotent and erythroid precursors, their motility through the bone marrow stroma, and their dissemination into the bloodstream. (82)

The second member of the family, PRGF-2, was initially named macrophage-stimulating protein (MSP) due its ability to make resident peritoneal macrophages responsive to chemoattractants. Like PRGF-1, PRGF-2 stimulates growth, motility, and branching morphogenesis of liver progenitor cells as well as proliferation and scattering of keratinocytes. (83) In addition, PRGF-2 can participate in the development of liver, lung, gut, kidney, and specific parts of the nervous system, including spinal ganglia and the nucleus of the hypoglossal nerve. (84)

Increase in N-glycans

After the saccharide part of the dolichol-P-P-glycan has been transferred to a protein, a process of glucose trimming occurs in ER. This serves as a quality control step and insures proper folding of the glycosylated protein. If the folding succeeds, the glycan-bearing polypeptide is transported to cis-Golgi where a number of the mannose residues that constituted the mature precursor are removed by mannosidases. The number of removed mannoses may vary depending on the final destination and function of the glycoprotein. Usually, only three sugar units of this type are left, which allows branches to be added to the glycan structure

Key transferases in the formation process of branched glycan structures are the mannosyl *N*-Acetylglucosaminyltransferases. Several enzymes providing this functionality are known, each with its distinct and restricted specificity. Mgat1 (*MGAT1*) transferase is the first member of this family to gain access to the core N-glycan structure after terminal mannose trimming. Association between multi-antennary N-glycans and cancer was proposed over two decades ago (140) and has been since confirmed as one of the typical changes during carcinogenesis. (141) Traditionally, the most-studied transferase, in this context, has been Mgat.

Mgat adds the first β1,2-*N*-acetylglucosamine to the α1,3-mannose. Further, two mannose units may be removed from the α1,6-mannose by mannosidases encoded by the *MAN2A1* and *MAN2A2* genes. This trimming allows up to three more antennas to be added. Transferases responsible for addition of these branches are enzymes encoded by genes *MGAT2*, *MGAT4* and *MGAT5*, in that order. However, all four antennas are not necessarily synthesized. Due to a regulatory mechanism, this process may be permanently terminated, thus reducing the number of branches. *MGAT3* codes for a member of the same mannosyl *N*-Acetylglucosaminyltransferase family, which by facilitating the addition of a so-called bisecting (β1,4-GlcNAc) branch, may prevent further branching by Mgat4 and Mgat5. All of the mentioned genes were found to have altered expression levels in breast carcinomas in relation to normal breast tissue. *MAN2A1*, *MGAT2*, *MGAT4A,* and *MGAT5B* were upregulated, while *MAN2A2* and *MGAT3* showed lower mRNA levels in malignant samples

Increased transcription of *MGAT5* can be induced by various mechanisms, including viral and chemical carcinogenesis. This increase results in the enhanced expression of UDP-GlcNAc:N-glycan GlcNAc transferase V (GlcNAcT-V). Cell lines with increased GlcNAcT-V expression show an increased frequency of metastasis in animal models, and spontaneous reversals that result in the loss of enzyme activity lose this metastatic phenotype. Clinical specimens of some human tumors show increased staining with the plant lectin L-phytohemagglutinin (L-PHA), which preferentially recognizes branched N-glycans bearing the β1–6 branched GlcNAcT-V product. Most convincingly, Mgat5-deficient mice show a striking reduction in the growth and metastasis of breast tumors induced by a viral oncogene. Conversely, metabolic inhibition of N-glycan processing by the plant alkaloid swainsonine (which blocks α-mannosidase II, thereby preventing complete processing of N-glycans and abrogating addition of the β1–6 branch) gives some reversal of tumorigenic behavior. (92)

Higher prevalence of branched N-glycans may have several implications. The β1,6 branch initiated by the Mgat5 transferase may often be elongated by a polylactosamine chain of varying length. It has been well established that such structures are present on epidermal growth factor (EGFR) and transforming growth factor-β (TGFβ) receptors and may in combination with the galectin family of glycan-binding proteins protect these receptors against endocytosis and thus facilitate their retention at the cell surface. (142) This property

of N-glycans might therefore be crucial for modulation of cytokine signaling involving these receptors which in turn are key factors in epithelial–mesenchymal transition.

N-glycans might also be of importance in regulation of integrin and cadherin-induced signaling. In this regard, the β1,6 branch has been shown to have a crucial role in subunit association and ligand binding of α5β1 integrin. Higher prevalence of N-glycans presenting this branch seemed to induce migration of cells on fibronectin and, in combination with galectin 3, cause focal adhesion remodeling, as well as downstream activation of the kinases FAK and PI3K. In line with these observations, overexpression of the transcript of the MGAT3 gene is responsible for addition of a bisecting branch and thus competitively inhibiting Mgat5, resulted in suppressed cell migration. Similar interactions have also been demonstrated for another integrin, α3β1, where Mgat5 promoted cell migration on laminin 5 while Mgat3 negated this effect.

In addition to modulation of EGFR, TβR, and integrin, homotypic cadherin adhesion is influenced by N-glycosylation. In this case, Mgat3 appears to play the central role. Previous studies have demonstrated that there is a mutual regulation between the two: MGAT3 expression is induced under dense conditions closely resembling the state of cells in a terminally differentiated tissue, while cells transfected with MGAT3 showed greater adhesion through E-cadherin. Loss of E-cadherin is one of the well-established changes characterizing epithelial–mesenchymal transition. (141-143)

Mucins

Cell-surface mucins and mucin-like glycoproteins have roles in cell adhesion. A number of diseases are associated with abnormal mucin gene expression and abnormal mucin carbohydrate structures and properties. These include cancer, inflammatory bowel disease, lung disease, and cystic fibrosis. Overexpression of mucins in carcinomas has been described for many years. Most epithelial mucin polypeptides belong to the MUC family. In the normal polarized epithelium, mucins are expressed exclusively on the apical domain, toward the lumen of a hollow organ. Likewise, soluble mucins are secreted exclusively into the lumen. In malignant epithelial cells. However, mucins are expressed on all aspects of the cells, and soluble mucins can then enter the extracellular space and body fluids.

Secreted/ gel-forming mucins

- *MUC5AC* is overexpressed in lung diseases such as Sjögren's syndrome, asthma, bronchitis, COPD, or cystic fibrosis. (1) A decrease in MUC5AC expression is associated with decreased survival in colorectal carcinoma patients. (2)
- *MUC2* downregulation is detected in colorectal cancer (3) gastric cancer (4,5) adenomas, mucinous carcinomas, and ovarian tumors. (6)
- *MUC6* is expressed in gastric mucosa and mucopeptic cells located at the neck region of the body. It is also expressed in gastric and duodenal mucous glands, pancreatobiliary and endocervical epithelial cells, and in the stomach of 8-week-old embryos. Aberrant expression of MUC6 is reported in gastric carcinomas. (7) In addition, MUC6 expression is detected at significant levels in well-differentiated cholangiocarcinomas, (8) breast cancer, (9) and in colonic sessile serrated adenoma. (10)

Soluble mucins

- *MUC7* functions as an antimicrobial agent in the oral cavity by interacting with microorganisms. It agglutinates HIV-1 (11) and inhibits the HIV infection. (12) MUC7 also possesses anti-candidal activity. (13) The MUC7 gene is also expressed in bladder cancer during the malignant transformation of bladder urothelium in preinvasive carcinoma in situ, but no MUC7 gene expression is detected in superficial, non-invasive bladder tumors. (14)
- *MUC8* is upregulated in nasal polyp epithelium and it may play an important role in the pathogenesis of chronic sinusitis with polyps. (15)

Transmembrane mucins

- *MUC1* has been extensively studied in relation to their pathological implication in the disease process. (16) MUC1 was first identified in human milk, and it is expressed by almost all glandular epithelial surfaces of respiratory, female reproductive tract, gastrointestinal tract, middle ear, salivary gland, mammary gland, and normal pancreatic intralobular ducts. (17) MUC1 expression is dramatically increased in breast, ovarian, lung, pancreatic, prostate, and colorectal cancers. In secretory epithelial cells, MUC1 is normally expressed on the apical borders, but in tumor cells, its expression is spread throughout the cell surface. One major role of the MUC1 protein is to act as an anti-adhesive protein to maintain the luminal integrity of the lining epithelium, thus aiding in the metastasis of tumor cells. (18,19)
- *MUC4* is expressed in various normal tissues such as the respiratory tract, lung, salivary glands, stomach, colon, eye, vagina, ectocervix, uterus, and prostate. An abnormal expression of MUC4 has been observed in various inflammatory diseases and cancers, such as dysplasia and adenocarcinoma of the esophagus, (20) Crohn's disease, (21,22) gall bladder carcinomas, (23) pancreatic ductal adenocarcinomas, (17) epithelial ovarian carcinomas, (24) prostate cancer, (17) breast cancer, (25) and other inflammatory diseases of airways such as cystic fibrosis and chronic obstructive pulmonary disease. (17)
- MUC11 is expressed more widely in gastrointestinal, respiratory, reproductive, urinary tracts and is detected more in the liver and thymus, when compared with MUC12 and MUC3. Its expression is commonly downregulated in colorectal cancer. (26)
- *MUC13* encodes a cell surface membrane-anchored mucin expressed in the normal gastrointestinal, respiratory tracts, trachea, middle ear, and kidney. It is aberrantly expressed in colorectal, esophageal, gastric, pancreatic, and lung cancers. In colorectal cancers, the highest expression of MUC13 was observed in poorly differentiated tumors. (26)
- *MUC16* encodes a transmembrane-bound molecule also known as CA125. MUC16 is mainly expressed in the ocular surface, respiratory tract, and female reproductive tract epithelia and middle ear. Its overexpression is correlated with the progression of ovarian cancer. (27)
- MUC17 is expressed in the gastrointestinal tract showing the highest expression in the duodenum and transverse. It is also expressed in the stomach, fetal kidney, and conjunctival epithelium. (17) The MUC17 is overexpressed in pancreatic cancer cells when compared with the normal pancreas and pancreatitis tissues. (28)
- MUC21 expression was examined in various normal tissues by PCR screening using human epiglycanin/MUC21 specific primers. It was

demonstrated that expression was remarkably high in lung, thymus, colon, normal bronchi, bronchioles, and bronchial glands, large intestine, and testis. (29)

Galectins in cancer

Galectins are an important family of β-galactoside-binding lectins that may be involved in a wider spectrum of processes of key importance in carcinogenesis. Besides their impact on growth factor receptor modulation, such processes include tumor immune escape by T-cell specific induction of apoptosis, mediated by for example galectins-1, -3, and -9 encoded by genes LGALS1, 2, 3, and 9.

Many types of tumors, including melanomas, astrocytomas, and bladder and ovarian tumors overexpress various galectins, and their heightened expression usually correlates with clinical aggressiveness of the tumor and the progression to a metastatic phenotype. The immunosuppressive and apoptotic effects of galectin-1 can contribute to tumor survival, as revealed by knockdown studies, where decreased galectin-1 expression is associated with decreased tumor survival, due to increased survival of IFN-γ-producing Th1 cells and heightened T-cell-mediated tumor rejection. Recent studies using galectin-1 knockout cells have shown that expression of galectin-1 in tumor cell endothelium is essential for tumor angiogenesis. Thus, galectins are likely to play important roles in tumor progression and metastasis through indirect effects in regulating tumor immune responses and direct effects in tumor angiogenesis. Overexpression of galectin-3 correlates well with neoplastic transformation and tumor progression toward metastasis, and expression of galectin-3 may be a histological tumor marker. Some studies even suggest that blocking galectin-3 function may limit tumor metastasis. (123)

However, the biological mechanisms behind these processes are yet to be fully understood and it is therefore in many cases uncertain whether these functions are due to interaction with N-glycans. In the studied cohorts, mRNA transcripts of galectins 2, 8 and 9 (genes *LGALS2*, *LGALS8,* and *LGALS9*, respectively) were expressed significantly higher, while 3, 4, 7, and 12 (*LGALS3*, *LGALS4*, *LGALS7,* and *LGALS12*) were found to have lower levels of expression in carcinomas compared to healthy tissue. (143)

Tumor-associated carbohydrate antigens

Many malignant cells (such as those found in breast and stomach cancer) develop a tumor marker called the Thomsen-Friedenreich (T) antigen. This antigen is encrypted in normal healthy cells; much like a rock is covered over by water at high tide. T antigen only becomes “unsuppressed” as a cell moves towards malignancy; much like the covered rock in our example becomes uncovered as the tide moves out.

It is so rare to find the Tn antigen in healthy tissue that most people produce antibodies (TFA agglutinins) to it —probably in response to cross-induction by the gut flora. ABO blood group appears to influence the amount and activity of these antibodies against T and Tn antigens. Although derived from the MN blood group antigens, Tn antigen shows structural homology to the A antigen. Antibodies against Tn antigen cross-react with A glycolipids. Since Tn antigen and A glycolipids share terminal GalNAc, Tn antigen was concluded to be an A-like antigen in a broad sense. “A-like” is actually considered an antigenic entity, a substance not A and not Tn, but capable of sharing antigenic sensibility with them. (46) The fact that blood-group-A gastric cancer patients have the greatest and

most uniform suppression of the level of TFA agglutinins, irrespective of age, cancer stage or tumor morphology, and lower levels of anti-B isohemagglutinins (47) strongly supports the notion.

One common consequence with the O-glycans is the expression of Tn and T antigens. Like T-antigen, Tn antigen is also usually "hidden" because of the action of beta1,3-galactosyltransferases, which elongate the Tn monosaccharide to the T disaccharide. However, in certain malignancies, autoimmune disorders and idiopathic Tn syndrome (permanent mixed-field polyagglutinability), the Tn antigen is exposed. (30) Because such structures occur infrequently, in normal tissues, it is thought that they may provoke immune responses in the patient. An abnormal feature of carcinoma mucins is incomplete glycosylation. These alterations produce what are known as *tumor-associated carbohydrate antigens (TACA).* Two of the most common TACA's are the Tn and Sialyl-Tn (STn) antigens, which more than 80% of human carcinomas express. Tn and STn also occur on multiple secreted and surface glycoproteins and mucins. Indeed, a correlation exists between the expression of the T (Galβ1–3GalNAc-α1-O-Ser/Thr) and Tn (GalNAc-α1-O-Ser/Thr) antigens, the spontaneous expression of antibodies directed against them, and the prognosis of patients with carcinomas.

Pancarcinoma antigen

It has been estimated that T antigen (or its precursor Tn antigen) is expressed and uncovered in about 90% of all cancers, earning it the appellation "pancarcinoma associated antigen." (45) As a rule, the orderly expression of T antigens on a cancer cell usually indicates a cancer with a relatively favorable outlook. However, a prevalence of Tn antigens (a less well-developed T antigen) on a cancer cell usually denotes a highly aggressive, metastatic cancer irrespective of the organ involved, or the form of cancer.

The extreme form of under-glycosylation results in expression of "naked" mucin polypeptides. Clinical trials are under way to deliberately provoke or enhance these immune responses by injecting patients with synthetic peptide antigens, sometimes bearing Tn or Sialyl-Tn (Siaα2-6GalNAc-α1-O-Ser/Thr) structures. As for the mechanism causing excessive sialyl-Tn to appear on tumor cells, current evidence does not support the notion that simple overexpression of an ST6GalNAc Golgi enzyme can produce this structure.

This appears to be a mutation of the gene encoding Cosmc, a chaperone required for formation of the active T-synthase. (31) Tn and STn appear to result from somatic mutations in the *Cosmc* (*C1GALT1C1*). Diverse neoplastic lesions, including colon cancer and melanoma-derived cells lines, express both Tn and STn antigen due to loss-of-function mutations in *Cosmc*. (32) As the gene for Cosmc is on the X chromosome (Xq24), a single mutation would be sufficient to eliminate expression. In this scenario, Sialyl-Tn accumulation would then result as a side effect of the loss of ability to make the core-1 O-glycan and its extensions, along with expression of the specific ST6GalNAc sialyltransferases. In most cases, the gene (*C1GALT1*) that codes for enzyme UDP-Gal:GalNAc-beta1,3-Gal transferase (core 1 beta3-GalT, or T-synthase) that transfers galactose to GalNAc-alpha1-O-Ser/Thr, in a beta1,3 linkage is absent, silenced, or inactive. More generally, its function is dependent on the expression the molecular chaperone (Cosmc) required for its proper folding.

All human tumor cells examined, including human tumor cell lines and two human cervical cancer specimens, which express the Tn and STn antigens, harbor mutations in *Cosmc* that result in a loss of function of Cosmc, and thus, consequent loss of T-synthase activity.

Given the frequency of sialyl-Tn accumulation by cancer cells, it is likely that its expression confers some yet unknown advantage to the tumor cells. The expression of T and Tn antigens, in particular the T antigen, may promote interactions with galectins mediating adhesive properties of cancer cell. Highly metastatic cells (MDA-MB-435) expressing high levels of both galectin-3 and T antigen demonstrated significantly increased adhesion to monolayers of endothelial cells compared with their non-metastatic counterpart. (40) The expression of the Tn antigen correlates with metastatic potential and poor prognosis in many cancers (33,34) including cervical (35), lung adenocarcinomas (36) colorectal carcinomas (37) breast carcinomas (38) and gastric carcinomas. (39)

At present, three lectins are known that exhibit reactivity with the T antigen, peanut agglutinin (PNA) (120), the *Artocarpus integrifolia* lectin (Jacalin) (121) and the recently characterized amaranthin (122).

Georg F. Springer, MD, spent over 20 years harnessing the potential of the immune system to combat cancer. Although his treatments were outside of the mainstream, he was anything but (coming from a very traditional medical and research background). Originally, a pioneer in work with blood group antigens, Springer dedicated his life and his unique expertise to breast cancer after his wife died from this disease. His work eventually led him to the development of what is known as "Springer's Vaccine" and his reported five and ten-year survival rates for stage II, III, and IV breast cancer with this novel T (Thomson-Friedenreich) and Tn antigen therapy are nothing short of amazing when compared to standard treatments.

The autoimmunogenicity of carcinoma T/Tn antigen led Georg Springer more than two decades ago to begin intradermal vaccination of patients with advanced breast carcinoma of stages IV-IIb, predominately after modified radical mastectomy and sometimes lumpectomy plus axillary dissection always followed by adjuvant radio/chemotherapy. The vaccine consists of human group O red blood cell membrane-derived, HLA-free T/Tn antigen, containing as adjuvant $Ca_3(PO_4)_2$ plus a trace of phosphoglycolipid A hyperantigen, i.e., *S. typhi* vaccine (USP), which itself has T and Tn specificities. (130)

His protocol entailed giving his vaccine to patients subcutaneous (under the skin), initially at 6-week intervals, eventually extending the gap to 12 weeks. For people receiving chemotherapy, he would wait 3 to 4 weeks after cessation of the last round of chemotherapy prior to beginning his treatment. In the case of radiation, he would wait 1 to 3 months after the last dose of radiation prior to initiating treatment. He recommended that his patients receive this vaccine “ad infinitum.” His results were quite encouraging, in particular with his stage III and IV patients. (131)

Springer passed away in the spring of 1998 and the vaccine he used with such great results is, as far as we know, currently unavailable. The *S. typhi* vaccine, a component of his vaccine, however, is readily available.

Stomach cancer cells express the A-like Thomsen-Friedenreich (T) antigen. Lower natural anti-Thomsen-Friedenreich immune response is associated with blood group A phenotype. This tendency is quite strong in group A individuals with stomach cancer, who demonstrate the greatest and uniform suppression of the level of TFA agglutinins, irrespective of age, cancer stage, or tumor morphology. (135)

MNS blood groups

Tn antigen (GalNAc directly linked to serine or threonine) is formed by incomplete synthesis of mucin-type carbohydrates including MN blood group antigens. (136)

"A-Like" antigen and "ligand-like" complex (LLC)

A blood group "A-like" has been linked to a generally recognized "cancer-proneness" in that blood group. The A-like cancer antigen may not be the true A antigen, but rather, what one researcher termed as the "Tumor-associated, A cross-reacting antigens" occurring in a wide variety of human adenocarcinomas of hosts belonging to all ABO blood groups. (48)

A loss of A and B antigens is observed in most types of carcinomas, such as carcinomas from the buccal epithelium, stomach, proximal colon, pancreas, larynx, lung, endometrium, ovary, prostate, urinary bladder, and breast. However, these antigens appear on carcinomas derived from some tissues where they are normally not present, such as the colorectal epithelium, liver parenchyma, and thyroid. The loss of A and B antigens is associated with a poor prognosis in carcinomas of the lung, urinary bladder, and head and neck. Inversely, in the case of colorectal carcinomas, their presence is a sign of unfavorable outcome (93). In addition, a higher incidence of various types of carcinomas is observed for blood group A and B individuals compared with blood group O individuals (94-102).

Breast cancer researchers have given this aberrant glycosylation moiety the moniker "ligand-like complex" (LLC) which by virtue of altered antigenicity both allows for metastatic egress from the regional lymph nodes or detachment from the extracellular matrix and thus is associated with poor-prognosis cancers. (49) LLC may well be the "A-like," pancarcinoma, cross-reacting antigen. GalNAc-binding lectin from the Roman Snail, *Helix pomatia* agglutinin (HPA), appears to identify this oligosaccharide (50), and separate reports indicate that "Springer's Vaccine" (human group O red blood cell membrane derived T/Tn antigen containing traces of phosphoglycolipid A hyperantigen) has had significant effects as an immune modulator in breast carcinoma of even advanced stage. (51)

The epidermal growth factor receptor (EGF-R) bears an antigenic determinant that is closely related to the human blood group A carbohydrate structure. An increase in the number of high affinity EGF binding sites was observed in donors with blood group A1-erythrocytes as compared to red cells taken from donors with blood groups O and B. (52)

It is now well documented that the blood group A antigen can also bind to EGF receptors as well. Therefore, it is likely that free A antigen in blood groups A and AB (especially if they are secretors) can find their way onto these excess EGF receptors and act to simulate cell growth. Like von Willebrand and Factor VIII, excessive activation of the EGF receptor results in cancer cells that become more mobile and able to develop new and additional blood supplies (angiogenesis). (53)

The association of group A with gastric carcinoma is quite old, first demonstrated by Aird, Bentall, and Fraser Roberts in 1953. (54) It has been hypothesized that the mechanisms behind the association between blood group A and gastric carcinoma is that the carcinoma cells produce an antigen immunologically related to blood group A, which particularly in O individuals may have a protective effect by preventing the growth and spread of the tumor. (55) It appears that the progression of stomach cells to stomach cancer involves a necessary mutation at the ABO gene, the result of which is the production of A antigen, even if this is not the person's blood group. Von Willebrand factor (vWF) promotes platelet

adhesion during thrombus formation and elevated vWF levels have been detected in various cancers. Levels of vWF are typically 30% higher in normal subjects of type A blood. The lower VWF values observed in O group individuals are attributable to a shorter VWF survival and half-life. (89)

Deletion, reduction or inappropriate expression of blood group A or B antigen in tumors of A or B individuals is clearly correlated with the degree of malignancy and metastatic potential. (56) The most significant variations are summarized below:

Tissue, organ	Normal appearance of BGA's	In Malignancy	Reference
Colon (4)	Present	Absent	Lab Invest 1987 Oct;57 (4):421-8
Bladder (1)	Absent	Present	Hinyokika Kiyo 1989 Aug;35 (8):1311-21
Prostate	Present	Absent	Br J Urol 1987 May;59 (5):430-5
Liver (2)	Absent	Present	Zhonghua Bing Li Xue Za Zhi 1992 Feb;21(1):24-6
Squamous	Present	Absent	Am J Clin Pathol 1991 Jun;95 (6):844-9
Endometrium (3)	Absent	Present	Cancer 1987 Dec 15;60 (12):2985-93
Stomach	Present	Absent	Pathol Res Pract 1988 Aug;183 (4):476-80
Thyroid (4)	Absent	Present	Langenbecks Arch Chir 1995;380 (5):269-72
Esophagus	Present	Absent	Cancer 1991 Jun 15;67 (12):3042-50

1. BGA's better than all other tumor markers
2. BGA's effective at prediction hepatitis transformation to malignancy
3. Vast majority of BGAs secreted are H antigen
4. Inversely correlated

Figure 6.5.1 Variation in blood group antigen expression in normal and neoplastic tissues (58)

A and B histo-blood group antigens are present on carcinoma cells at the early stages of carcinogenesis and tend to disappear at later stages. The group A antigen may render malignant cells resistant to apoptosis; in animal studies, the faster tumor growth of the A antigen-positive cells in immunocompetent animals was due to their higher ability to escape immune control, and this was associated with their higher degree of resistance to apoptosis. (57) These changes provide a selective advantage for tumor cells during their progression to more invasive and metastatic forms.

The loss or suppression of the red cell antigen has been observed in a number of patients with leukemia. (62) These patients had normal phenotypes before the onset of the leukemia and in the course of their disease the A antigen became extremely weak. The decrease in strength of the A antigen was accompanied by an increased reactivity with anti-H reagents, so that the cells reacted like O cells. The saliva of the patients who were secretors contained normal amounts of A antigen, indicating that the alteration occurred in hematopoietic cells. Selective loss of the A antigen in a type A patient has also been observed, and there is one report of loss of the B antigen in an AB patient. (64) Somatic mutation, caused by the disease or the treatment, has been suggested as the cause of these alterations. (63,64)

Acquisition of a "B-like" antigen has been noted in patients with carcinoma of the colon or rectum and in a few patients with other malignant processes or infections. (65,66) Their sera contained anti-B that did not react with their own red cells. Many bacteria possess lipopolysaccharides with blood group B activity, (62) and these substances adhere firmly to red cells in vitro. (68) It was proposed that altered permeability of the diseased areas permit the bacteria substances to enter the blood and adsorb to erythrocytes, and this phenomenon has been observed in the course of enteritis caused by *Escherichia coli*. (69) However, Marsh (70) reported that certain bacterial filtrates contained enzymes capable of causing the appearance of B antigen in A or O red cells, and this mechanism may also have a role.

The blood group-related carbohydrate structures Le(x), sialyl-Le(x), ABH, and Le(y) are examples of terminal carbohydrate structures that are related to tumor prognosis. These structures are of increasing interest since they may function as adhesion molecules or motility factors. (59)

Lewis blood groups can modulate the expression of several tumor-associated antigens including DU-PAN9 and CA 19-9, and some researchers have suggested that taking into account Lewis secretor status in order to establish reference ranges might actually be a way to increase their clinical utility.

Accurately predicting the relevance of some tumor markers for diagnosis of cancer appears to be dependent on both secretor status and Lewis blood group. As an example, some researchers have suggested that taking into account aspects of Lewis and/or Secretor status in order to establish reference ranges might actually be a way to increase the clinical utility of the CA 19-9 tumor marker. (136)

Lewis Phenotype	CA19-9 *	DU-PAN-9
Lewis (a+b-)	Highest levels	Lower levels
Lewis (a-b+)	High levels	Lower levels
Lewis (a-b-)	Zero to very low levels	Highest levels
Individuals having homozygous inactive Se alleles (sese) and homozygous active Le alleles (LeLe), exhibited the highest mean CA19-9 value. All of the Lewis Negative individuals (lele genotype) had completely negative CA19-9 values, irrespective of the Se genotype. (60)		

Figure 6.5.2 CA19-9 and DU-PAN-9 expression in colorectal cancer correlated to Lewis type.

There is a substantial difference in levels of this tumor marker are under the control of Secretor and Lewis genetics. Individuals having homozygous inactive Se alleles (se/se) and homozygous active Le alleles (Le/Le), exhibited the highest mean CA19-9 value. All of the Lewis negative individuals (Le (a- b-) consisting of a le/le genotype) had completely negative CA19-9 values, irrespective of the Se genotype.

On the other hand, Lewis negative individuals showed a higher mean DU-PAN-2 value than did the Le-positive individuals. Among patients with colorectal cancer, the Le-negative

patients (le/le) with colorectal cancer showed undetectable CA19-9 values, i.e., less than 1.0 unit/ml, but many of them exhibited highly positive DU-PAN-2 values. In contrast, many of the Le-positive patients (Le/Le or Le/le) had positive CA19-9 values, whereas very few of them exhibited positive DU-PAN-2 values. (137)

Sialic acids

In 1963, Joseph Aub, a researcher at Massachusetts General Hospital, discovered by chance that there were many surface differences between normal cells and cancer cells, an idea that was thought at the time to be so strange, as in the words of one biographer, "to border on lunacy." (103) Aub believed that these differences enabled cancer cells to multiply when normal cell would not, detach from their primary site, and spread throughout the body.

Aub originally worked with enzymes, attempting to digest certain portions of the cancer cell's surface to see if there were any differences. Then, as with many medical discoveries, luck intervened. Of all the enzymes he used, only one derived from wheat germ showed any effect, agglutinating the cancer cells. When he replaced this enzyme with an identical one from hog pancreas, again, nothing happened. Obviously, something in the wheat germ (other than the enzyme Aub was looking at) was agglutinating the cancer cells. In fact, when he heated the wheat germ extract and destroyed the enzyme, it continued to destroy cancer cells. Aub and his colleagues soon found that the wheat germ enzyme was contaminated with a small protein that was responsible for the agglutinating activity. Aub had discovered a lectin in wheat germ that agglutinated the cancer cells.

This classic observation of increased wheat germ agglutinin (WGA) binding to animal tumor cells is likely explained by an overall increase in cell-surface sialic acid (SA) content, which in turn reduces attachment of metastatic tumor cells to the matrix, and may help protect them from recognition by the alternative pathway of complement activation. The metastatic potential of tumor cells has been extensively correlated with increases in sialylation of cell surface glycoproteins. An important cause of this increased sialylation is the increased branching of complex N-linked oligosaccharides in highly metastatic cells. There is some evidence that the overexpression of Siaα2–6Galβ1–4GlcNAc units on N-glycans may enhance β1-integrin action. As discussed above, sialyl-Tn expression may well be a side effect of decreased O-glycan extension, and it is currently a target for immunotherapy. Inhibitors of N-linked glycan processing, such as swainsonine, reduce the level of sialylated, complex N-glycans and these inhibitors can reverse some of the growth and metastatic properties of tumor cells in vivo and vitro.

SA's are critically required for early mammalian development. Cultured cell lines that are grossly deficient in sialylated glycans show generally normal growth patterns. However, apart from the function of polysialic acid (polySia) in allowing "neural plasticity," the exact roles of SA during development remain uncertain. Several examples of SA regulation have been reported in living animals. Certain classes of T lymphocytes have O-acetylated SA whereas others do not. The expression of polysialylation and O-acetylation in neural gangliosides varies with developmental stage and location, and differences in O-acetylation of brain gangliosides have been reported between cold- and warm-blooded species, and between awake and hibernating animals.

A subgroup of I-type lectins, called *Siglecs,* specifically recognizes many structural features of sialic acids, and Siglecs are found on innate immune cells. It is possible that altered sialylation of tumor cells affects interactions with some Siglecs. Such interactions could be potentially beneficial to the tumor cell by sending an inhibitory signal to innate immune

cells. A variety of sialyltransferase-null mice have been produced that show interesting and specific phenotypes, ranging from altered Siglec-2/CD22 function to defects in T-cell maturation and changes in brain development. (71) Because selectins, which are known to mediate cell adhesion and extravasation, can bind SA residues there is a good precedent for the role of SA in the type of adhesion that must accompany metastasis. (104)

Another interesting phenomenon is the aberrant expression of N-Glycolylneuraminic acid (Neu5Gc) in human tumor cells. This sialic acid differs from the usual N-acetylneuraminic acid (Neu5Ac) by the addition of a single oxygen atom. Adult humans do not express significant levels of Neu5Gc on their normal cells, and they mount an immune response to this epitope when infused with Neu5Gc-containing animal serum. The type and linkages of endothelial, plasma protein, and erythrocyte SA can undergo marked changes in responses to inflammatory stimuli.

Glycosphingolipids are abundantly decorated by sialic acid residues where sialyltransferases are of crucial importance. The degradation process of these structures, mediated by sialidases, is equally interesting. Currently three sialidases are known, encoded by *NEU1*, *-2* and *-3*, each of which has a distinct cellular localization and thus differs functionally

Some conflicting findings as to the effects of sialidase over-expression in malignancy have been published. Early work suggested that activity of these enzymes was higher in transformed cells. (151,152) A more recent study demonstrated that high level of the plasma membrane sialidase (NEU3) was linked to protection from apoptosis in colon cancer. (153) It has also been shown that activity of the lysosomal sialidase (NEU1) appears to be enhanced in hepatomas in comparison to normal liver tissue, while the cytosolic sialidase (NEU2) was generally less active in the malignant tissue. (154,155) On the other hand, several studies have shown that sialidases might also possess anti-metastatic properties (156,157), leaving the precise role for sialidases in tumor progression still to be explained.

With the exception of the role of SA in selectin ligands, the precise mechanisms by which these SA changes enhance tumorigenesis or invasive behavior remain uncertain. Increased sialylation may also enhance the masking effect of SA on antigenic sites of tumor cells, which become more like “self” and therefore more invasive. Regardless of the mechanisms involved, certain sialylated molecules are specific markers for some cancers and potential ligands for targeted therapies.

Fucosylation

Cores of N-glycans may be decorated with various sugar moieties along their passage through Golgi. The most common modification of the N-glycan core in mammals is the α1,6-fucosylation of the GlcNAc residue bound to asparagine. Core fucosylation is of special interest due to its ability to modulate growth and development through altering functional properties of integrins. Changes in N-linked glycosylation are known to occur during the development of various diseases. Increased branching of oligosaccharides, in particular fucosylation, has been associated with cancer metastasis; and it has been correlated to tumor progression in human cancers of the breast, colon, and melanomas. Numerous clinicopathological studies have shown a clear correlation between aberrant glycosylation status of primary tumor and invasive/ metastatic potential of human cancer, as reflected by 5- or 10-year survival rates of patients. (135)

Increases in core fucosylation have also been associated with the development of hepatocellular carcinoma (HCC). (128) HCC is the fifth most common cancer in the world and is the third leading cancer killer worldwide Changes in glycosylation, most notably fucosylation of alpha-fetoprotein (AFP), have been associated with the development of hepatocellular carcinoma (HCC). (127)

The reasons why there is an increase in fucosylated glycoforms in the serum of patients with liver cancer is a mystery but may be associated with loss of cell polarity. That is, many epithelial cells normally become polarized, with at least two distinct plasma membrane surfaces. In the case of the intestinal epithelium, proteins are oriented either basolaterally (toward the blood) or apically (toward the lumen). Hepatocytes are unusual in that they polarize in three dimensions rather than as a two dimensional sheet. The basolateral surface is in contact with the blood, while the apical surfaces of the cells form the bile canaliculi. Glycoproteins directed to these surfaces may be selective or even specific for the apical or basolateral surface, and thus maintenance of this polarity depends upon the continuous sorting of newly made proteins and membranes. (128)

Recently, it has been suggested that fucosylation of N-linked glycan within polarized hepatocytes directs glycoproteins to the apical surface and into the bile; and consequently, fucosylated glycoforms are normally rare in the blood and are enriched in the bile [35]. Thus, if cancer cells become "depolarized," it is reasoned that fucosylated glycoforms would rise in abundance in the blood. (129)

FUT8, a gene that codes for α1,6-fucosyltransferase, an enzyme that catalyzes the introduction of α1,6 core fucose to the innermost N-acetylglucosamine residue of the N-glycan, has been implicated in development, the immune system, and tumorigenesis. A1,6-fucosyltransferase and E-cadherin expression levels are significantly elevated in primary colorectal cancer samples. (124) Glycopeptides from 11 human neuroblastoma tumors obtained from 10 different patients were examined, and all contained fucosyl residues linked as Fucα-1-3(4)GlcNAc. (125)

In breast cancer patients, increases in sialylation and fucosylation of glycan structures appeared to be indicative of cancer progression. (133) In breast carcinoma (BCa) cell lines prepared from biopsies obtained from patients at each of the pathological Stages I, II, III and from patients with disseminated liver metastasis; results indicate a correlation between progression of malignancy from PS I to the metastatic stage PS IV and the magnitude of malignancy phenotypes, resistance to the host killer cells, and oligosaccharide profile shift to a higher molecular size with increased sialylation and fucosylation of the carbohydrate moieties. (134) In breast cancer patients, an increase in core fucosylation of N-glycans for the circulating alpha-1-proteinase inhibitor (API) has been suggested previously. (139)

Prostate-specific antigen (PSA) is widely used as a diagnostic marker for prostate cancer (PC) because of its high specificity. However, elevated serum PSA does not occur only in PC but also in benign prostatic hyperplasia (BPH). There is elevated expression of α1,2-fucosylation and β-N-acetylgalactosaminylation of PSA during carcinogenesis. (126)

The appearance of fucosylated haptoglobin has been reported in other diseases such as hepatocellular carcinoma, liver cirrhosis, gastric cancer, and colorectal cancer. In a clinical investigation of 100 cases of colorectal cancer, cases in which it was located near the liver showed a higher positive rate of fucosylated haptoglobin, suggesting that the location of the cancer might also be an important factor for fucosylated haptoglobin if cancer tissues produce such inducible factors. Thus, fucosylated haptoglobin could become a novel tumor marker for PC and complicated mechanisms would be involved in its production. (132)

Sialyl Lewis structures

Lewis antigens are functionally important terminal glycan epitopes, which were first implicated in breast cancer development several decades ago. (145,146) These antigens are common to many types of glycans, including N- and O-linked glycans as well as glycosphingolipids. Lewis antigens and their aberrant expression have been viewed as one of the underlying mechanisms for metastasis in different carcinomas, partially due to the interactions between these epitopes and E-selectin presented by activated endothelial cells. Sialyl Lewis x (SLeX) antigen expression is a common feature of breast carcinomas (147), and transformed cells appear in many cases to interact strongly with stimulated endothelium. (148) Ectopic expression of the non-sialylated Lewis X has also been suggested to play a role in the interaction between breast carcinoma cells and endothelial cells promoting metastases, presumably through a different mechanism involving collectins. (149)

Sialyl Lewis X (SLe^X) carbohydrate antigen acts as an adhesion molecule expressed on the surface of cancer cells and is the most important ligand of the selectins present on endothelial cells. (105) SLe^X expression was correlated to the metastatic potential of breast cancer. SLe^X has been demonstrated to play an important role in the adhesion of human cancer cells to human vascular endothelium, inducing metastasis.

Immunohistochemical studies on tumor specimens have shown that both SLe^X and Sialyl Lewis A structures (SLe^A) are frequently overexpressed in carcinomas, being carried on O-glycans as well as on N-glycans and glycosphingolipids. Indeed, SLe^X and SLe^A (CA19-9) were first identified as tumor antigens. The expression of these antigens by epithelial carcinomas correlates with tumor progression, metastatic spread, poor prognosis in humans, and metastatic potential in mice.

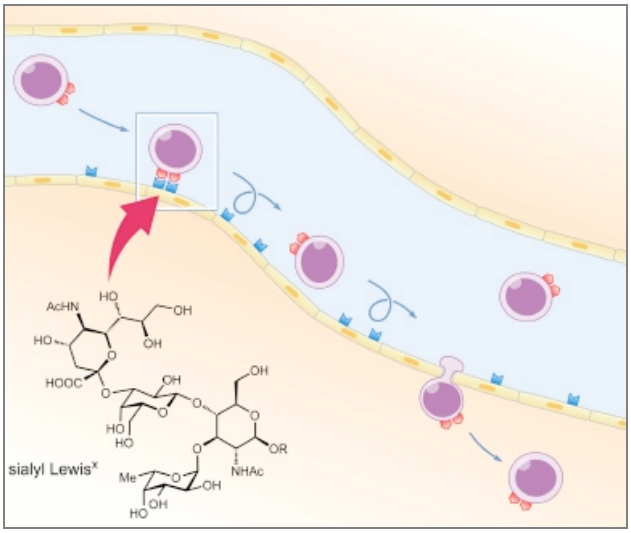


Figure 6.5.3 SLe^X and E-selectin work together to induce leukocyte "rolling."

Tumor cells gain a selective advantage by presenting pathological selectin ligands that mediate interactions with endogenous selectins. The interaction of SLeX on tumor cells and E-selectin was shown to mediate adhesion of tumor cells to endothelial cells. (106) Calcium-dependent selectin ligands on carcinoma cells have been demonstrated, and mucin-like tumor antigens binding to E-selectin were directly demonstrated in the blood of colon carcinoma patients. Likewise, overexpression of E-selectin in the transgenic mouse liver induced redirection of the metastatic patterns of syngeneic carcinomas that normally

colonize the lung. The expression of SLe^X, SLe^A, E-cadherin, and Cathepsin D was moderate in invasive carcinomas without metastases. However, a strong expression of both SLe^X and SLe^A and a very weak expression of E-cadherin were detected in primary carcinoma with lymph node metastases. (107)

Tumor metastasis is also attenuated in mice with P-selectin or L-selectin deficiency. These and other studies indicate that interactions between tumor-derived mucins and selectin molecules play a part in the metastatic cascade of some carcinoma cells. This relationship ties in with the classic observation that cancer cells entering the bloodstream form complex thromboemboli with platelets and leukocytes, which are thought to facilitate arrest at ectopic sites, assist interactions with the endothelium, and help in evasion of the immune system.

Forskolin: A metastatic promoter?

Forskolin (FSK) is a labdane diterpene that is produced by the Indian Coleus plant (*Coleus forskohlii*). FSK is known as an up-regulator of intracellular camp. It was found that FSK stimulated cell growth, increased cAMP in the cells, and enhanced the metastasis-related phenotypes, including adhesion to laminin (Ln) and human umbilical vein epithelial cells (HUVEC), chemotactic migration and invasion. These effects were supposed to result from the increase of the SLeX expression induced by FSK via protein kinase B (PKB). It can be concluded that FSK shows a metastasis-promoting effect ex vivo. (108)

Current data suggest that this phenomenon can be explained by interactions between platelet and endothelial P-selectin and carcinoma mucins. Thus, carcinoma cells show a reduced metastatic rate in P-selectin-deficient mice, which can be explained at least in part by a lack of P-selectin-dependent rosetting of platelets on the tumor cells. With regard to L-selectin, one of the mechanisms appears to involve leukocyte interactions with fucosyltransferase-7 (FucT-7)-dependent endothelial ligands, which are induced at the site of tumor embolization in the vasculature.

Structurally, these epitopes are comprised of a fucosylated Gal-GlcNAc-β1,3/4-Gal backbone, which can be optionally sialylated. Addition of GlcNAc to the inner galactose of the backbone is mediated by several transferases, for example, those encoded by the *B3GNT1*, 2, 3, and 5 genes. Subsequently, either a β1,3 or a β1,4-galactose can be added to this sugar moiety. B-1,3-Galactosyltransferases encoded by genes *B3GALT1*, 2, and 5 predetermine the final structure to be a type 1 Lewis epitope, a group that includes Le^a, SLe^A, and Le^b structures. On the other hand, β-1,4-galactosyltransferases 1, 2, 3, and 4 (*B4GALT1,* 2, 3, 4) will synthesize type 2 Lewis antigens (for example, Lewis X and Y) by transferring a β-1,4-galactose to the GlcNAc saccharide.

All type 1 structures contain an α1,4-Fuc residue on the GlcNAc added by fucosyltransferase 3 (FUT3). The same is the case for type 2 antigens, but the linkage is α1,3 instead (catalyzed by products of *FUT3* through 7 and *FUT9*). The terminal galactose may or may not be modified. Examples of unmodified Lewis antigens are Le^a and Le^X. Alternatively, this galactose may be either sialylated or fucosylated. Sialylation by transferases encoded from *ST3GAL3* and -4 yields SLeA, and from ST3GAL6 results in SLe^X, while addition of fucose forms Le^b and Le^Y epitopes that are both synthesized by fucosyltransferases 1 and 2 (FUT1 and FUT2). (143)

Among this group of terminal glycan structures, several type 2 antigens including Lewis X (also known as SSEA-1 or CD15), sialyl Lewis X and Lewis Y are considered tumor-associated markers. (150) Higher prevalence of SLe^X has been reported in breast cancer cells and appears to correlate with the expression of an α-1,3-fucosyltransferase, FUT6. Higher transcription levels of three of the four relevant β-1,4-galactosyltransferases (*B4GALT1,* 2, 3) were found in malignant tissues. This is in agreement with higher prevalence of type 2 epitopes in breast carcinomas. The fucosylation and sialylation processes appeared to be altered during the malignant transformation, as well with up-regulation of *FUT5, FUT11,* and *ST3GAL4* and down-regulation of *FUT4, FUT9, FUT10, ST3GAL3,* and *ST3GAL6* in breast carcinoma samples. (143)

Chitinase-like proteins

YKL-40, a member of the "mammalian chitinase–like proteins," is expressed and secreted by several types of solid tumors. YKL-40 binds chitin of different lengths in a fashion similar to the family 18 chitinases but has no chitinase activity: the glycoprotein is thought to play a role in the process of inflammation and tissue remodeling. (86) It is coded for by the *CHI3L1* (chitinase 3-like 1) gene. YKL-40 exhibits growth factor activity for cells involved in tissue remodeling processes and may have a role in cancer cell proliferation, survival, and invasiveness, in the inflammatory process around the tumor, angiogenesis, and remodeling of the extracellular matrix. (85) YKL-40 is neither organ- nor tumor-specific. It has been suggested that YKL-40 may play a role in the proliferation and differentiation of malignant cells, protects the cancer cells from undergoing apoptosis, stimulates angiogenesis, has an effect on extracellular tissue remodeling, and stimulates fibroblasts surrounding the tumor, although *in vivo* proof of these hypotheses are yet to be obtained.

Increased serum levels of CHI3L1 parallel disease severity, poorer prognosis, and shorter survival in many human neoplasms, including cancers of the breast, colon, prostate, ovaries, brain, thyroid, lung, and liver. Increased serum CHI3L1 also correlates with disease severity in rheumatoid arthritis, osteoarthritis, liver fibrosis, inflammatory bowel disease, and bacterial septicemia. (85)

If future studies show that YKL-40 has a role in the ability of cancer cells to proliferate, invade, and metastasize, YKL-40 could be an attractive target in the design of anticancer therapy. Any approach that would inhibit the function of YKL-40 (e.g., inhibition of YKL-40 gene expression, protein synthesis and secretion, neutralization of YKL-40 activity, blocking YKL-40 conversion from a latent to an active form, interruption of YKL-40 affinity, or reaction with its receptor) might limit cancer growth and metastases and improve the survival of cancer patients with YKL-40–expressing tumor cells. (87)

Glycosphingolipids (GSL's)

Several gangliosides are relevant to carcinogenesis, perhaps most notably GM3 and GD3. Certain reports have shown that induction of the cell surface expression of the GM3 structure was associated with reversion of malignancy through an integrin- and CD9-dependent mechanism. This ganglioside has also been found to be associated with intermediate filaments although the precise role of this interaction has not been fully elucidated. Some attention has also been devoted to interactions between glycosphingolipids and immune response. In this regard, GM3 and GD3 have been

proposed to reduce cytotoxicity of NK-cells and peripheral blood leukocytes, perhaps by influencing the arachidonic acid cascade. (143)

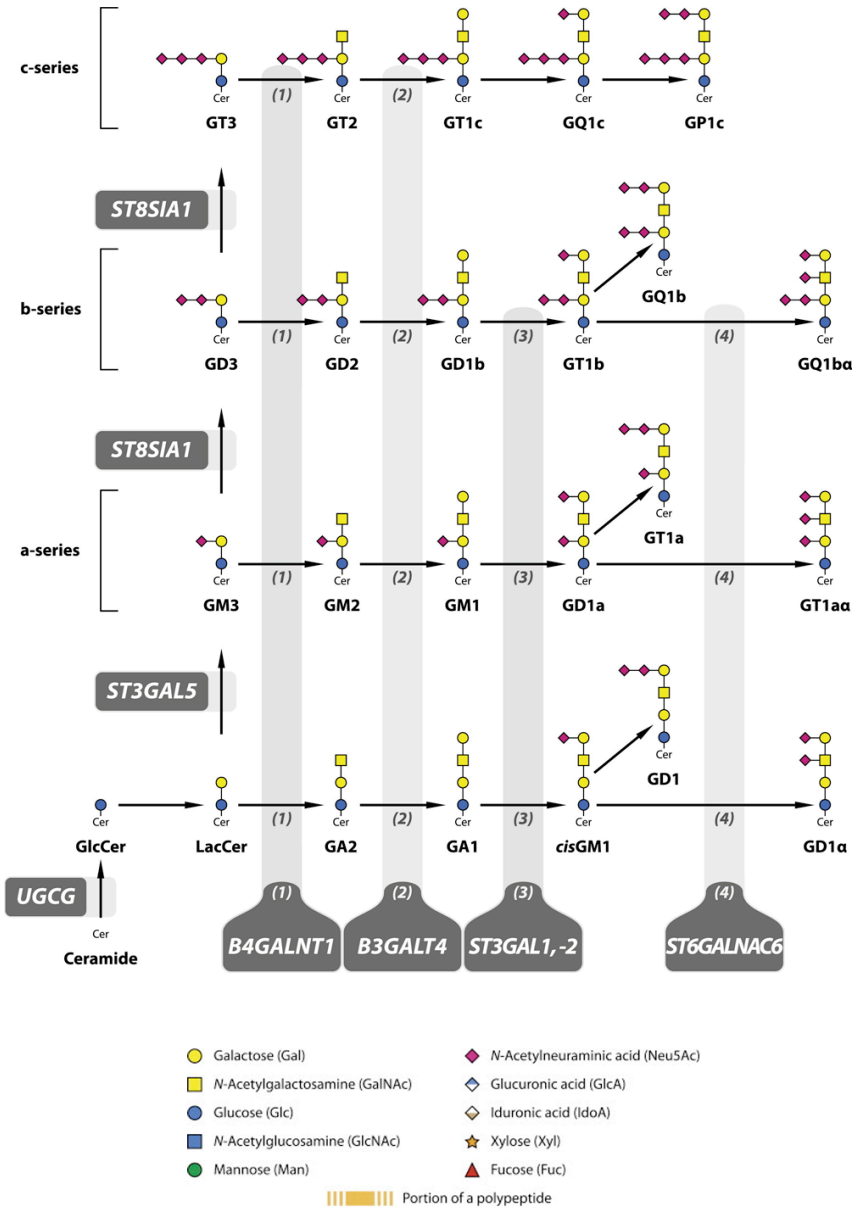


Figure 6.5.4 Ganglio-series glycosphingolipid synthesis pathway. Key genes involved in the pathway are indicated, and four have been denoted numerically to indicate that the sialyltransferases encoded by these genes catalyze several steps of the pathway. Modified from Potapenko IO, Haakensen VD, Lüders T, Helland A, Bukholm I, Sørlie T, Kristensen VN, Lingjaerde OC, Børresen-Dale AL. Glycan gene expression signatures in normal and malignant breast tissue; possible role in diagnosis and progression. Mol Oncol. 2010 Apr; 4(2):98-118.

Ganglio-series glycosphingolipids are in most cases highly sialylated structures built around a Galβ1-3GalNAcβ1-4Galβ1-4GlcβCer core. The first step in the synthesis pathway

is the addition of a glucose monosaccharide residue to a ceramide. A ceramide glucosyltransferase encoded by UGCG is responsible for catalyzing this reaction. As the synthesis progresses, a β4-galactose is added to create lactosylceramide (LacCer). This galactose may hold a branch of one, two, or three sialic acid residues. The three resulting GSL's, and structures derived from these, have been given specific designations: a-, b- and c-series, respectively.

Stepwise addition of these residues is performed by two sialyltransferases. Synthesis of the GM3 structure (a-series precursor) by addition of the innermost α3 sialic acid to LacCer is done by a transferase encoded by the *ST3GAL5* gene. The other transferase (gene ST8SIA1) may add up to two more sialic acids to create GD3 (b-series precursor) and GT3 (c-series precursor). Further, pathways progress in parallel by similar reactions to synthesize a complete core structure. The first step is addition of a GalNAc that results in a GA2, GM2, GD2, or GT2 structure. The transferase catalyzing this step is coded by *B4GALNT1*. Product of the *B3GALT4* gene may subsequently act on the backbone to extend the core further with a galactose (creating GA1, GM1, GD1b, and GT1c).

Both the GalNAc and the terminal galactose residues can be sialylated by transferases encoded by the genes *ST3GAL1/-2* (resulting in cisGM1, GD1a and GT1b structures) and *ST6GALNAC6* (creating GD1α, GT1aα and GQ1bα) in all but the c-series. Several other sialyltransferases are known to mediate this step, including for example *ST6GALNAC3* and *ST6GALNAC5,* although these enzymes have narrower specificity and only catalyze addition of sialic acid to cisGM1, creating GD1α. The latter gene is of interest since it has recently been reported to enhance metastasis of breast carcinomas to the brain. (143)

Proteoglycans (PG)

The association between cancer and venous thromboembolism (VTE) works both ways, with cancer inducing a hypercoagulable state, and the pro-thrombotic changes in turn facilitating cancer growth and metastasis. Cancer cells have been shown to express aberrantly several components involved in coagulation. For example, tissue factor, a key activator of the coagulation cascade, is expressed on endothelial cells, monocytes, and, most importantly, on tumor cells themselves; and it is thought to play a pivotal role in cancer-induced hypercoagulability. (88)

Cancer is also associated with disturbances of the fibrinolytic system. Plasmin, which breaks down fibrin clots, is produced from its precursor molecule plasminogen in response to plasminogen activator or urokinase-type plasminogen activator, and is inhibited by plasminogen activator inhibitor. However, deregulation of these factors is observed in cancer patients, resulting in disruptions to the normal process of clot lysis. Cancer patients, therefore, may possess abnormal expression of a number of factors that are crucial for normal hemostasis, resulting in a general state of hypercoagulability. (90) Low-molecular-weight heparins (LMWH's) may have a role in cancer care and subsequent prophylaxis.

Tumorigenesis is associated with changes in the PG synthesis. Heparan sulfate (HS) PG's are involved in several aspects of cancer biology, including tumor progression, angiogenesis, and metastasis. PG's can have both tumor-promoting and tumor-suppressing activities, depending on the protein core, the GAG attached, the molecules they associate with, localization, the tumor subtype, stages, and degree of tumor differentiation. Perlecan is an angiogenic factor involved in tumor invasiveness. The C-terminal domain V of perlecan, named endorepellin, has however been shown to inhibit angiogenesis. Another angiogenic factor is endostatin, the COOH-terminal domain of the part-time PG collagen XVIII. Glypicans and syndecans may promote local cancer cell growth in some cancer

tissues, but inhibit tissue invasion and metastasis in others. The GAG hyaluronan (HA) promotes cancer growth by providing a loose matrix for migrating tumor cells and mediates adhesion of cancer cells. HSPG degrading enzymes like heparanase, heparitinase, and other enzymes such as hyaluronidase and MMP are also important in tumor metastasis. Several different treatment strategies that target PG's have been developed.

Heparin is a potent inhibitor of P- and L-selectin interactions and the pharmacological effects of heparin on selectin interactions are thought to explain some prior reports of benefits of heparin therapy in cancer. They have the potential to be effective in reducing tumor growth and inhibit the formation of metastases. (91)

Hyaluronan (HA) is glycosaminoglycan defined by the disaccharide unit (GlcNAcβ1–4GlcAβ 1–3)$_n$ that is neither sulfated nor covalently linked to protein. HA is a very large negatively charged polysaccharide composed of the repeating disaccharide (GlcAβ1–3GlcNAcβ1–4). It differs from other glycosaminoglycans in that it is nonsulfated and exists as a free polymer, rather than being covalently linked to a protein. Furthermore, it is synthesized and extruded from the cell directly at the plasma membrane, rather than being processed through the ER-Golgi pathway. It is referred to in older literature as "hyaluronic acid.'

In normal tissues, HA serves at least three functions, which may also contribute to tumor progression. It increases levels of tissue hydration, which can facilitate movement of cells through tissues. Second, it is intrinsic to the assembly of extracellular matrices through specific interactions with other macromolecules, and thus it participates in tumor cell–matrix interactions that facilitate or inhibit tumor cell survival and invasion. Finally, HA interacts with several types of cell-surface receptors, especially CD44 and the receptor for hyaluronan-mediated motility (RHAMM/CD168). HA–CD44 interactions are often crucial to tumor malignancy and are a current target for novel therapies. In carcinomas, hyaluronan is usually enriched in the tumor-associated connective tissue elements and blood vessels. This stroma is usually prominent in breast cancer. HA is involved in melanoma development and extracellular matrix remodeling during melanoma progression. (109)

Activation of HA–CD44 signaling is much more important in cancer progression than actual levels of CD44. In normal adult tissues, HA appears to be relatively inert with respect to cell signaling and behavior. However, during embryonic development, during tissue healing and regeneration, and in various pathological situations, hyaluronan-induced signaling, via interaction with CD44, becomes activated. The consequences of this signaling are dramatic because they are essential to, or promote, cell behaviors such as proliferation, survival, migration, and invasion, which are also key elements of the malignant phenotype. HA – CD44 interaction at the tumor cell surface is required for the constitutive activation of some well-known oncogenes, especially the receptor tyrosine kinase, ErbB2, which is amplified or mutated in a large number of carcinomas. Accordingly, HA–CD44 interaction promotes downstream intracellular pathways that are also hallmarks of cancer, such as the phosphatidylinositol–3-kinase/AKT and mitogen-activated protein kinase (MAPK) pathways.

RHAMM (CD168) is also well known as a HA-binding protein. RHAMM is expressed on the cell surface and in the cytoplasm, as well as in the cytoskeleton and nucleus. In general, the interactions of HA with CD44 and RHAMM are especially important for tumorigenesis and tumor progression by activating downstream signaling molecules, but how this receptor transmits signal to downstream targets is still unclear. Both RHAMM and CD44 mediate hyaluronan signaling and participate in growth factor-regulated signaling. However, they likely regulate signaling by different mechanisms because they are not homologous proteins, and they are compartmentalized differently in the cell. (113)

Controlling the balance between pro-and anti-angiogenic agents is crucial and its deregulation leads to serious disease. The extracellular matrix (ECM) plays an important role in controlling angiogenesis, allowing at least, the distribution of growth factors and the regulation of endothelial cell migration. The breakdown products of HA act strongly to promote angiogenesis. (110) Hyaluronidase (HAase) is a HA-degrading endoglycosidase. Levels of HAase are elevated in many cancers. Hyaluronidase-1 (HYAL1) is the major tumor-derived HAase. HYAL1 knockout mice with breast cancer cell xenografts exhibit markedly inhibited tumor growth and microvessel density. (111) HA is the principal glycosaminoglycan (GAG) in the ECM of the brain and is the critical factor for glioma invasion. (112) HA promotes tumor metastasis and is an accurate diagnostic marker for bladder cancer (114) and squamous cell laryngeal carcinoma. (115)

The HA matrix is also a potential target for anticancer therapies. In early phase clinical trials, patients with breast or head and neck tumors treated with anti-CD44 conjugates experienced stabilized disease. HA has been used as a drug carrier and a ligand on liposomes or nanoparticles to target drugs to CD44 overexpressing cells. Drugs can be attached to HA via the carboxylate on the glucuronic acid residue, the hydroxyl on the N-acetylglucosamine or the reducing end, which are located on a repeating disaccharide. Drugs delivered in HA-modified liposomes exhibited excellent antitumor activity both in vitro and in murine tumor models. By manipulating the interaction of HA with cell surface receptors, either by degrading it with hyaluronidase or by interfering with CD44-HA interactions using soluble CD44 proteins, blocked tumor progression. Finally, cytotoxic drugs or prodrug converting enzymes can be attached to the HA matrix to generate a cytotoxic fence around the tumor. (116)

Attaching butyric acid, a histone deacetylase inhibitor, to HMW-HA targets the drug conjugate to CD44 expressing cells, leads to internalization, and improves *in vivo* delivery by altering the release profile of the drug. Coradini et al., showed, in several human tumor models (breast, lung, melanoma and hepatocellular), that HA-conjugated butyric acid increased apoptosis, inhibited cell growth *in vitro* and decreased tumor burden *in vivo*. Initial studies of HA-conjugated butyric acid showed rapid cellular uptake in a human breast carcinoma cell line *in vitro*. This interaction was blocked by an anti-CD44 antibody. (117)

Among protein tyrosine kinase inhibitors, emodin (3-methyl-1,6,8-trihydroxyanthraquinone) has strong anti-invasive activity for HA-induced glioma invasion. Emodin is one of the main active components contained in the root and rhizome of *Rheum palmatum* L. Emodin has been shown to have a number of biological activities, including antiviral, antimicrobial, immunosuppressive, hepatoprotective, anti-inflammatory and anti-cancer effects. *Rheum palmatum*, known as Turkey rhubarb, Chinese rhubarb, ornamental rhubarb, and East Indian rhubarb, is a plant in the family Polygonaceae. (118,119) The U.S. National Institutes of Health's Medline lists *Rheum palmatum* as one of the active ingredients in the controversial cancer treatment “Essiac.”

Glycan structures or pathways	Differentially expressed genes
N-glycans	
↑ Precursor synthesis	*ALG3 (↑), ALG8 ↑, ALG10 ↑, ALG14 (↑)*
↑ Core fucosylation	*FUT8 ↑*
↑ Branching	*MGAT4 ↑, MGAT5B ↑, MGAT3 ↓*
O-glycans	
↑/↓ Initiation	*GALNT2 (↑), GALNT3 (↑), GALNT5 (↑), GALNT6 ↑, GALNT7 ↑, GALNT10 (↑), GALNT11 (↓), GALNT12 (↓), GALNTL1 ↓, GALNTL2 (↓), GALNTL4 (↑)*
↑ Sialyl T synthesis	*ST3GAL1 ↑*
Lewis antigens	
↑ Type 2 Lewis antigens	*B4GALT1 (↑), B4GALT2 (↑), B4GALT3 ↑*
↑/↓ Altered sialylation	*ST3GAL3 (↓), ST3GAL4 (↑), ST3GAL6 (↓)*
Glycosphingolipids	
↑ cisGM1, GD1a	*ST3GAL1 ↑*
↓ GD3, GT3, GQ1bα, GT1aα, GD1α	*ST8SIA1 ↓, ST6GALNAC3 ↓, ST6GALNAC6 ↓*
Glycosaminoglycans	
↑ Core tetrasaccharide common to CS/DS and HS chondroitin sulfate	*XYLT2 ↑, B3GALT6 ↑*
↑ Core protein	*VCAN ↑, ACAN (↑)*
↑ Disaccharide	*CHSY1 ↑, CHPF ↑*
↓ Sulfation patterns CS-C and D	*CHST3 ↓*
↑ Sulfation patterns CS-A and E	*GALNAC4S-6ST ↑, CHST11 (↑)*
Keratan sulfate	
↑ Disaccharide	*B3GALT3 ↑, GLB1 ↑*
↑/↓ Sulfation	*CHST1 (↑), CHST2 (↓), CHST4 ↓, CHST6 (↑)*

Figure 6.5.6 Summary of differentially expressed genes/pathways in normal versus malignant breast tissue. The most pronounced alterations in gene expression and the impact they may have on various glycosylation processes and prevalence of certain structures are presented. In each case, relevant genes are listed. Up-regulation in tumors of both data sets in comparison to normal samples is indicated by an upward pointing arrow, ↑, and down-regulation by an arrow pointing down, ↓. From: Potapenko IO, Haakensen VD, Lüders T, Helland A, Bukholm I, Sørlie T, Kristensen VN, Lingjaerde OC, Børresen-Dale AL. Glycan gene expression signatures in normal and malignant breast tissue; possible role in diagnosis and progression. Mol Oncol. 2010 Apr; 4(2):98-118.

1. Rubin BK. Mucus and mucins. Otolaryngol Clin North Am. 2010 Feb; 43(1):27-34, vii-viii.
2. Kocer B, Soran A, Erdogan S, Karabeyoglu M, Yildirim O, Eroglu A, Bozkurt B, Cengiz O. Expression of MUC5AC in colorectal carcinoma and relationship with prognosis. Pathol Int. 2002; 52:470–477.
3. Blank M, Klussmann E, Krüger-Krasagakes S, Schmitt-Gräff A, Stolte M, Bornhoeft G, Stein H, Xing PX, McKenzie IF, Verstijnen CP, et al. Expression of MUC2-mucin in colorectal adenomas and carcinomas of different histological types. Int J Cancer. 1994 Nov 1; 59(3):301-6.
4. Utsunomiya T, Yonezawa S, Sakamoto H, Kitamura H, Hokita S, Aiko T, Tanaka S, Irimura T, Kim YS, Sato E. Expression of MUC1 and MUC2 mucins in gastric carcinomas: its relationship with the prognosis of the patients. Clin Cancer Res. 1998; 4:2605–2614.
5. Dong Y, Walsh MD, Cummings MC, Wright RG, Khoo SK, Parsons PG, McGuckin MA. Expression of MUC1 and MUC2 mucins in epithelial ovarian tumors. J Pathol. 1997; 183:311–317.
6. Audie JP, Janin A, Porchet N, Copin MC, Gosselin B, Aubert JP. Expression of human mucin genes in respiratory, digestive, and reproductive tracts ascertained by in situ hybridization. J Histochem Cytochem. 1993; 41:1479–1485.
7. De Bolós C, Garrido M, Real FX. MUC6 apomucin shows a distinct normal tissue distribution that correlates with Lewis antigen expression in the human stomach. Gastroenterology. 1995; 109:723–734.
8. Park SY, Roh SJ, Kim YN, Kim SZ, Park HS, Jang KY, Chung MJ, Kang MJ, Lee DG, Moon WS. Expression of MUC1, MUC2, MUC5AC, and MUC6 in cholangiocarcinoma: prognostic impact. Oncol Rep. 2009; 22:649–657.
9. Kasashima S, Kawashima A, Zen Y, Ozaki S, Kobayashi M, Tsujibata A, Minato H. Expression of aberrant mucins in lobular carcinoma with histiocytoid feature of the breast. Virchows Arch. 2007 Apr; 450(4):397-403.
10. Owens SR, Chiosea SI, Kuan SF. Selective expression of gastric mucin MUC6 in colonic sessile serrated adenoma but not in hyperplastic polyp aids in morphological diagnosis of serrated polyps. Mod Pathol. 2008; 21:660–669.
11. Bergey EJ, Cho MI, Hammarskjold ML, Rekosh D, Levine MJ, Blumberg BM, Epstein LG. Aggregation of human immunodeficiency virus type 1 by human salivary secretions. Crit Rev Oral Biol Med. 1993; 4:467–474.
12. Nagashunmugam T, Malamud D, Davis C, Abrams WR, Friedman HM. Human submandibular saliva inhibits human immunodeficiency virus type 1 infection by displacing envelope glycoprotein gp120 from the virus. J Infect Dis. 1998; 178:1635–164.
13. Liu B, Rayment SA, Gyurko C, Oppenheim FG, Offner GD, Troxler RF. The recombinant N-terminal region of human salivary mucin MG2 (MUC7) contains a binding domain for oral Streptococci and exhibits candidacidal activity. Biochem J. 2000; 345:557–564.
14. Retz M, Lehmann J, Roder C, Plotz B, Harder J, Eggers J, Pauluschke J, Kalthoff H, Stockle M. Differential mucin MUC7 gene expression in invasive bladder carcinoma in contrast to uniform MUC1 and MUC2 gene expression in both normal urothelium and bladder carcinoma. Cancer Res. 1998; 58:5662–5666.
15. Seong JK, Koo JS, Lee WJ, Kim HN, Park JY, Song KS, Hong JH, Yoon JH. Upregulation of MUC8 and downregulation of MUC5AC by inflammatory mediators in human nasal polyps and cultured nasal epithelium. Acta Otolaryngol. 2002; 122:401–407.
16. Singh AP, Chauhan SC, Bafna S, Johansson SL, Smith LM, Moniaux N, Lin MF, Batra SK. Aberrant expression of transmembrane mucins, MUC1 and MUC4, in human prostate carcinomas. Prostate. 2006 Mar 1; 66(4):421-9.
17. Rachagani S, Torres MP, Moniaux N, Batra SK. Current status of mucins in the diagnosis and therapy of cancer. Biofactors. 2009 Nov-Dec; 35(6):509-27.
18. Kim YS, Gum J, Jr, Brockhausen I. Mucin glycoproteins in neoplasia. Glycoconj J. 1996; 13:693–707.
19. Taylor-Papadimitriou J, Burchell JM, Plunkett T, Graham R, Correa I, Miles D, Smith M. MUC1 and the immunobiology of cancer. J Mammary Gland Biol Neoplasia. 2002; 7:209–221.
20. Arul GS, Moorghen M, Myerscough N, Alderson DA, Spicer RD, Corfield AP. Mucin gene expression in Barrett's esophagus: an in situ hybridization and immunohistochemical study. Gut. 2000; 47:753–761.
21. Buisine MP, Desreumaux P, Debailleul V, Gambiez L, Geboes K, Ectors N, Delescaut MP, Degand P, Aubert JP, Colombel JF, Porchet N. Abnormalities in mucin gene expression in Crohn's disease. Inflamm Bowel Dis. 1999; 5:24–32.
22. Buisine MP, Desreumaux P, Leteurtre E, Copin MC, Colombel JF, Porchet N, Aubert JP. Mucin gene expression in intestinal epithelial cells in Crohn's disease. Gut. 2001; 49:544–551.
23. Buisine MP, Devisme L, Degand P, Dieu MC, Gosselin B, Copin MC, Aubert JP, Porchet N. Developmental mucin gene expression in the gastroduodenal tract and accessory digestive glands. II. Duodenum and liver, gallbladder, and pancreas. J Histochem Cytochem. 2000; 48:1667–1676.

24. Chauhan SC, Singh AP, Ruiz F, Johansson SL, Jain M, Smith LM, Moniaux N, Batra SK. Aberrant expression of MUC4 in ovarian carcinoma: diagnostic significance alone and in combination with MUC1 and MUC16 (CA125). Mod Pathol. 2006;19:1386–1394
25. Rakha EA, Boyce RW, bd El-Rehim D, Kurien T, Green AR, Paish EC, Robertson JF, Ellis IO. Expression of mucins (MUC1, MUC2, MUC3, MUC4, MUC5AC, and MUC6) and their prognostic significance in human breast cancer. Mod Pathol. 2005;18:1295–1304
26. Walsh MD, Young JP, Leggett BA, Williams SH, Jass JR, McGuckin MA. The MUC13 cell surface mucin is highly expressed by human colorectal carcinomas. Hum Pathol. 2007; 38:883–892.
27. Bast RC, Jr, Badgwell D, Lu Z, Marquez R, Rosen D, Liu J, Baggerly KA, Atkinson EN, Skates S, Zhang Z, Lokshin A, Menon U, Jacobs I, Lu K. New tumor markers: CA125 and beyond. Int J Gynecol Cancer. 2005; 15(Suppl 3):274–281.
28. Moniaux N, Andrianifahanana M, Brand RE, Batra SK. Multiple roles of mucins in pancreatic cancer, a lethal and challenging malignancy. Br J Cancer. 2004; 91:1633–1638.
29. Itoh Y, Kamata-Sakurai M, da-Nagai K, Nagai S, Tsuiji M, Ishii-Schrade K, Okada K, Goto A, Fukayama M, Irimura T. Identification and expression of human epiglycanin/MUC21: a novel transmembrane mucin. Glycobiology. 2008;18:74–83
30. http://www.ncbi.nlm.nih.gov/projects/gv/mhc/xslcgi.cgi?cmd=bgmut/systems_info&system=t
31. Ju T, Lanneau GS, Gautam T, Wang Y, Xia B, Stowell SR, Willard MT, Wang W, Xia JY, Zuna RE, Laszik Z, Benbrook DM, Hanigan MH, Cummings RD. Human tumor antigens Tn and sialyl Tn arise from mutations in Cosmc. Cancer Res. 2008 Mar 15; 68(6):1636-46.
32. Mitchell BS, Schumacher U. The use of the lectin Helix pomatia agglutinin (HPA) as a prognostic indicator and as a tool in cancer research. Histol Histopathol 1999; 14:217–26.
33. Kawaguchi T. Cancer metastasis: characterization and identification of the behavior of metastatic tumor cells and the cell adhesion molecules, including carbohydrates. Curr Drug Targets Cardiovasc Haematol Disord. 2005; 5:39–64.
34. Numa F, Tsunaga N, Michioka T, Nawata S, Ogata H, Kato H. Tissue expression of Sialyl Tn antigen in gynecologic tumors. J Obstet Gynaecol 1995; 21:385–9.
35. Inoue M, Ogawa H, Nakanishi K, Tanizawa O, Karino K, Endo J. Clinical value of sialyl Tn antigen in patients with gynecologic tumors. Obstet Gynecol 1990; 75:1032–6.
36. Laack E, Nikbakht H, Peters A, et al. Lectin histochemistry of resected adenocarcinoma of the lung: Helix pomatia agglutinin binding is an independent prognostic factor. Am J Pathol 2002; 160:1001–8.
37. Konno A, Hoshino Y, Terashima S, Motoki R, Kawaguchi T. Carbohydrate expression profile of colorectal cancer cells is relevant to metastatic pattern and prognosis. Clin Exp Metastasis 2002; 19:61–70.
38. Fernandez Madrid F, Tang N, Alansari H, Karvonen RL, Tomkiel JE. Improved approach to identify cancer-associated autoantigens. Autoimmun Rev. 2005; 4:230–5.
39. Kakeji Y, Tsujitani S, Mori M, Maehara Y, Sugimachi K. Helix pomatia agglutinin binding activity is a predictor of survival time for patients with gastric carcinoma. Cancer 1991; 68:2438–42.
40. Khaldoyanidi SK, Glinsky VV, Sikora L, Glinskii AB, Mossine VV, Quinn TP, Glinsky GV, Sriramarao P. MDA-MB-435 human breast carcinoma cell homo- and heterotypic adhesion under flow conditions is mediated in part by Thomsen-Friedenreich antigen-galectin-3 interactions. J Biol Chem. 2003 Feb 7; 278(6):4127-34.
41. Sahai E. Illuminating the metastatic process. Nat Rev Cancer. 2007 Oct; 7(10):737-49.
42. Fidler IJ. The pathogenesis of cancer metastasis: the “seed and soil” hypothesis revisited. Nat Rev Cancer. 2003 Jun; 3(6):453-8.
43. Stephen Paget P. The Distribution of Secondary Growths in Cancer of the Breast. The Lancet, Volume 133, Issue 3421, 23 March 1889
44. Couzin J. Medicine. Tracing the steps of metastasis, cancer's menacing ballet. Science. 2003 Feb 14; 299(5609):1002-6.
45. Springer GF. T and Tn pancarcinoma markers: autoantigenic adhesion molecules in pathogenesis, prebiopsy carcinoma-detection, and long-term breast carcinoma immunotherapy. Crit Rev Oncog. 1995; 6(1):57-85. Review.
46. Garratty G. Blood group antigens as tumor markers, parasitic/bacterial/viral receptors, and their association with immunologically important proteins. Immunol Invest. 1995 Jan-Feb; 24(1-2):213-32.
47. Kurtenkov O, Klaamas K, Miljukhina L. The lower level of natural anti-Thomsen-Friedenreich antigen (TFA) agglutinins in sera of patients with gastric cancer related to ABO(H) blood-group phenotype. Int J Cancer. 1995 Mar 16; 60(6):781-5.
48. Hirohashi S, Clausen H, Yamada T, Shimosato Y, Hakomori S. Blood group A cross-reacting epitope defined by monoclonal antibodies NCC-LU-35 and -81 expressed in cancer of blood group O or B individuals: its identification as Tn antigen. Proc Natl Acad Sci U S A. 1985 Oct; 82(20):7039-43.
49. Brooks SA, Leathem AJ. Prediction of lymph node involvement in breast cancer by detection of altered glycosylation in the primary tumor. Lancet. 1991 Jul 13; 338(8759):71-4.
50. Brooks SA, Hall DM, Buley I. GalNAc glycoprotein expression by breast cell lines, primary breast cancer and normal breast epithelial membrane. Br J Cancer. 2001 Sep 28; 85(7):1014-22.

51. Springer GF. Immunoreactive T and Tn epitopes in cancer diagnosis, prognosis, and immunotherapy. J Mol Med. 1997 Aug; 75(8):594-602. Review.
52. Defize LH, Arndt-Jovin DJ, Jovin TM, Boonstra J, Meisenhelder J, Hunter T, de Hey HT, de Laat SW. Hubrecht Laboratory, Netherlands Institute for Developmental Biology, Utrecht. A431 cell variants lacking the blood group A antigen display increased high affinity epidermal growth factor-receptor number, protein-tyrosine kinase activity, and receptor turnover. J Cell Biol 1988 Sep;107(3):939-49
53. Engelmann B, Schumacher U, Haen E. Physiologisches Institut, Universitat Munchen, West Germany. Epidermal growth factor binding sites on human erythrocytes in donors with different ABO blood groups. Am J Hematol. 1992 Apr; 39(4):239-41.
54. Aird I, Bentall HH, Roberts JA. A relationship between cancer of stomach and the ABO blood groups. Br Med J. 1953 Apr 11; 1(4814):799-801.
55. Beckman L, Angqvist KA. On the mechanism behind the association between ABO blood groups and gastric carcinoma. Hum Hered. 1987; 37(3):140-3.
56. Ichikawa D, Handa K, Hakomori S. Histo-blood group A/B antigen deletion/reduction vs. continuous expression in human tumor cells as correlated with their malignancy. Int J Cancer. 1998 Apr 13; 76(2):284-9.
57. Marionneau S, Le Moullac-Vaidye B, Le Pendu J. Expression of histo-blood group A antigen increases resistance to apoptosis and facilitates escape from immune control of rat colon carcinoma cells. Glycobiology. 2002 Dec; 12(12):851-6.
58. D'Adamo PJ. Non-transfusion significance of ABO blood group. In: *Textbook of Natural Medicine*. Pizzorno J, Murray M. Editors. Churchill Livingstone. 2006
59. Dabelsteen E. Cell surface carbohydrates as prognostic markers in human carcinomas. J Pathol. 1996 Aug; 179(4):358-69.
60. D'Adamo PJ, Kelly GS. Metabolic and Immunologic Consequences of ABH Secretor and Lewis Subtype Status. Altern Med Rev 2001; 6(4):390-405.
61. Mourant AE. *Blood Groups and Disease*. Oxford Press, UK (1977).
62. van Loghem JJ, Dorfmcier H, van der Hart M. Two A antigens with abnormal serologic properties. Vox Sang. 2:16-24, 1957.
63. Hosoi E. Biological and clinical aspects of ABO blood group system. J Med Invest. 2008 Aug; 55(3-4):174-82.
64. Richards AG. Loss of blood-group antigen in chronic lymphatic leukemia. Lancet. 1962; 2:178.
65. Hoogstraten B, Rosenfield RE, Wasserman LR. Change of ABO blood group in patient with leukemia. Transfusion. 1961; 1:32-35.
66. Richards, AG. Loss of blood-group antigen in chronic lymphatic leukemia. Lancet 2:178, 1962.
67. Cameron C, et al. Acquisition of B-like antigen by red blood cells. Brit M J. 1959; 2:29-32.
68. Springer GF and Horton RE. Erythrocyte sensitization by blood group-specific bacterial antigens. J Gen Physiol. 1964; 47:1229-1250.
69. Young VM, Gillem HC, Akeroyd JH. Sensitization of infant red cells by bacterial polysaccharides of Escherichia coli during enteritis. J Pediat. 1962 Feb; 60:172-176.
70. Marsh WT. Pseudo B antigen: study of its development. Vox Sang. 1960 Sep; 5:387-97.
71. Varki A, Schauer R. Sialic Acids. In: Varki A, Cummings R, Esko J, Freeze H, Stanley P, Bertozzi C, Hart G, and Etzler M. *Essentials of Glycobiology*, 2nd edition, Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2009
72. Comoglio PM, Trusolino L. Invasive growth: from development to metastasis. J Clin Invest. 2002 Apr; 109(7):857-62.
73. Montesano R, Matsumoto K, Nakamura T, Orci L. Identification of a fibroblast-derived epithelial morphogen as hepatocyte growth factor. Cell. 1991; 67:901–908.
74. Yang Y, et al. Sequential requirement of hepatocyte growth factor and neuregulin in the morphogenesis and differentiation of the mammary gland. J Cell Biol. 1995; 131:215–226.
75. Woolf AS, et al. Roles of hepatocyte growth factor/scatter factor and the met receptor in the early development of the metanephros. J Cell Biol. 1995; 128:171–184.
76. Yanagita K, et al. Hepatocyte growth factor may act as a pulmotrophic factor on lung regeneration after acute lung injury. J Biol Chem. 1993; 268:21212–21217.
77. Tabata MJ, et al. Hepatocyte growth factor is involved in the morphogenesis of tooth germ in murine molars. Development. 1996; 122:1243–1251.
78. Lindner G, et al. Involvement of hepatocyte growth factor/scatter factor and met receptor signaling in hair follicle morphogenesis and cycling. FASEB J. 2000; 14:319–332.
79. Bussolino F, et al. Hepatocyte growth factor is a potent angiogenic factor which stimulates endothelial cell motility and growth. J Cell Biol. 1992; 119:629–640.
80. Grano M, et al. Hepatocyte growth factor is a coupling factor for osteoclasts and osteoblasts in vitro. Proc Natl Acad Sci USA. 1996; 93:7644–7648.
81. Takebayashi T, et al. Hepatocyte growth factor/scatter factor modulates cell motility, proliferation, and proteoglycan synthesis of chondrocytes. J Cell Biol. 1995; 129:1411–1419.
82. Galimi F, et al. Hepatocyte growth factor induces proliferation and differentiation of multipotent and erythroid hemopoietic progenitors. J Cell Biol. 1994; 127:1743–1754.
83. Medico E, et al. The tyrosine kinase receptors Ron and Sea control "scattering" and morphogenesis of liver progenitor cells in vitro. Mol Biol Cell. 1996; 7:495–504.

84. Gaudino G, et al. The proto-oncogene RON is involved in development of epithelial, bone and neuroendocrine tissues. Oncogene. 1995; 11:2627–2637.
85. Bi J, Lau SH, Lv ZL, Xie D, Li W, Lai YR, Zhong JM, Wu HQ, Su Q, He YL, Zhan WH, Wen JM, Guan XY. Overexpression of YKL-40 is an independent prognostic marker in gastric cancer. Hum Pathol. 2009 Dec;40(12):1790-
86. Coffman FD. Chitinase 3-Like-1 (CHI3L1): a putative disease marker at the interface of proteomics and glycomics. Crit Rev Clin Lab Sci. 2008; 45(6):531-62.
87. Johansen JS, Jensen BV, Roslind A, Nielsen D, Price PA. Serum YKL-40, a new prognostic biomarker in cancer patients? Cancer Epidemiol Biomarkers Prev. 2006 Feb; 15(2):194-202.
88. Robert F. The potential benefits of low-molecular-weight heparins in cancer patients. J Hematol Oncol. 2010 Jan 14; 3:3.
89. Gallinaro L, Cattini MG, Sztukowska M, Padrini R, Sartorello F, Pontara E, Bertomoro A, Daidone V, Pagnan A, Casonato A. A shorter von Willebrand factor survival in O blood group subjects explains how ABO determinants influence plasma von Willebrand factor. Blood. 2008 Apr 1; 111(7):3540-5.
90. Rickles FR, Falanga A. Molecular basis for the relationship between thrombosis and cancer. Thromb Res. 2001 Jun 15; 102(6):V215-24.
91. Fjeldstad K, Kolset SO. Decreasing the metastatic potential in cancers—targeting the heparan sulfate proteoglycans. Curr Drug Targets. 2005 Sep; 6(6):665-82.
92. Varki A, Kannagi R, Toole P. Glycosylation Changes in Cancer. In: Varki A, Cummings R, Esko J, Freeze H, Stanley P, Bertozzi C, Hart G, and Etzler M. *Essentials of Glycobiology*, 2nd edition, Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2009
93. Le Pendu J, Marionneau S, Cailleau-Thomas A, Rocher J, Le Moullac-Vaydie B, Clément M. ABH and Lewis histo-blood group antigens in cancer. APMIS. 2001; 109, 9–31.
94. Annese V, Minervini M, Gabbrielli A, Gambassi G, Manna R. ABO blood groups and cancer of the pancreas. Int J Pancreatol. 1990; 6:81–88.
95. Bjorkholm E. Blood group distribution in women with ovarian cancer. Int J Epidemiol. 1984; 13:15–17.
96. Henderson J, Seagroat V, Goldacre M. Ovarian cancer and ABO blood groups. J. Epidemiol. Comm. Health, 1993; 47:287–289.
97. Kaur I, Singh IP, Bhasin MK. Blood groups in relation to carcinoma of cervix uteri. Hum Hered. 1992; 42:324–326.
98. Mourant AE, Kopec AC, Domaniewska-Sobczak K. Blood groups and diseases. A study of associations of diseases with blood groups and other polymorphisms. Oxford: Oxford University Press. 1978.
99. Slater G, Itzkowitz S, Azar SS, Aufses AH. Clinicopathological correlations of ABO and Rhesus blood group in colorectal cancer. Dis Colon Rectum. 1993; 36:5–7.
100. Su M, Lu SM, Tian DP, Zhao H, Li XY, Li DR, Zheng ZC. Relationship between ABO blood groups and carcinoma of the esophagus and cardia in Cháoshàn inhabitants of China. World J Gastroenterol. 2001; 7:657–661.
101. Vioque J, Walker AM. Pancreatic cancer and ABO blood group: a study of cases and control. Med Clin. 1991; 96, 761–764.
102. You WC, Ma JL, Liu W, Gail MH, Chang YS, Zhang L, Hu YR, Fraumeni JF, Xu GW. Blood group and family cancer history in relation to precancerous gastric lesions. Int J Epidemiol. 2000; 29:405–407.
103. Sharon N, Lis H. *Lectins*. Second Edition, Kluwer Academic Publishers, 2003.
104. Taylor M, Drickamer K. *Introduction to Glycobiology*. 2nd Edition Oxford University Press 2006
105. Phillips ML, Nudelman E, Gaeta FC, Perez M, Singhal AK, Hakomori S, Paulson JC. ELAM-1 mediates cell adhesion by recognition of a carbohydrate ligand, sialyl-Lex. Science. 1990 Nov 23; 250(4984):1130-2.
106. Walz G, Aruffo A, Kolanus W, Bevilacqua M, Seed B. Recognition by ELAM-1 of the sialyl-Lex determinant on myeloid and tumor cells. Science. 1990 Nov 23; 250(4984):1132-5.
107. Jeschke U, Mylonas I, Shabani N, Kunert-Keil C, Schindlbeck C, Gerber B, Friese K. Expression of sialyl Lewis X, sialyl Lewis A, E-Cadherin and Cathepsin D in human breast cancer: immunohistochemical analysis in mammary carcinoma in situ, invasive carcinomas and their lymph node metastasis. Anticancer Res. 2005 May-Jun; 25(3A):1615-22.
108. Wu SL, Ma J, Qi HL, Zhang Y, Zhang XY, Chen HL. Forskolin up-regulates metastasis-related phenotypes and molecules via protein kinase B, but not PI-3K, in H7721 human hepatocarcinoma cell line. Mol Cell Biochem. 2003 Dec; 254(1-2):193-202.
109. Perrotta R, Bevelacqua Y, Malaguarnera G, Paladina I, Giordano M, Malaguarnera M. Serum markers of cutaneous melanoma. Front Biosci (Elite Ed). 2010 Jun 1; 2:1115-22.
110. Lataillade JJ, Albanese P, Uzan G. Implication of hyaluronic acid in normal and pathological angiogenesis. Application for cellular engineering. Ann Dermatol Venereol. 2010 Apr; 137 Suppl 1:S15-22.
111. Tan JX, Wang XY, Li HY, Su XL, Wang L, Ran L, Zheng K, Ren GS. HYAL1 over-expression is correlated with the malignant behavior of human breast cancer. Int J Cancer. 2010 May 13.
112. Park JB, Kwak HJ, Lee SH. Role of hyaluronan in glioma invasion. Cell Adh Migr. 2008 Jul; 2(3):202-7.

113. Turley EA, Noble PW, Bourguignon LY. Signaling properties of hyaluronan receptors. J Biol Chem. 2002 Feb 15; 277(7):4589-92.
114. Ohyama C. Glycosylation in bladder cancer. Int J Clin Oncol. 2008 Aug;13(4):308-13
115. Vynios DH, Theocharis DA, Papageorgakopoulou N, Papadas TA, Mastronikolis NS, Goumas PD, Stylianou M, Skandalis SS. Biochemical changes of extracellular proteoglycans in squamous cell laryngeal carcinoma. Connect Tissue Res. 2008; 49(3):239-43.
116. Platt VM, Szoka FC Jr. Anticancer therapeutics: targeting macromolecules and nanocarriers to hyaluronan or CD44, a hyaluronan receptor. Mol Pharm. 2008 Jul-Aug; 5(4):474-86.
117. Coradini D, Pellizzaro C, Miglierini G, Daidone MG, Perbellini A. Hyaluronic acid as drug delivery for sodium butyrate: improvement of the anti-proliferative activity on a breast-cancer cell line. Int J Cancer. 1999 May 5; 81(3):411-6.
118. Koyama M, Kelly TR, Watanabe KA. Novel type of potential anticancer agents derived from chrysophanol and emodin. Some structure-activity relationship studies. J Med Chem. 1988 Feb; 31(2):283-4.
119. Huang Q, Shen HM, Ong CN. Inhibitory effect of emodin on tumor invasion through suppression of activator protein-1 and nuclear factor-kappa-B. Biochem Pharmacol. 2004 Jul 15; 68(2):361-71.
120. Uhlenbruck G, Pardoe GI, Bird GW. On the specificity of lectins with a broad agglutination spectrum. II. Studies on the nature of the T-antigen and the specific receptors for the lectin of Arachis hypogaea (ground-nut). Z Immunitatsforsch Allerg Klin Immunol. 1969 Nov; 138(5):423-33.
121. Sastry MV, Banarjee P, Patanjali SR, Swamy MJ, Swarnalatha GV, Surolia A. Analysis of saccharide binding to Artocarpus integrifolia lectin reveals specific recognition of T-antigen (beta-D-Gal(1----3)D-GalNAc).J Biol Chem. 1986 Sep 5;261(25):11726-33.
122. Rinderle SJ, Goldstein IJ, Matta KL, Ratcliffe RM. Isolation and characterization of amaranthin, a lectin present in the seeds of Amaranthus caudatus, that recognizes the T- (or cryptic T)-antigen. J Biol Chem. 1989 Sep 25; 264(27):16123-31.
123. Cummings RD, Liu FT. Galectins. In: Varki A, Cummings R, Esko J, Freeze H, Stanley P, Bertozzi C, Hart G, and Etzler M. *Essentials of Glycobiology*, 2nd edition, Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2009
124. Osumi D, Takahashi M, Miyoshi E, Yokoe S, Lee SH, Noda K, Nakamori S, Gu J, Ikeda Y, Kuroki Y, Sengoku K, Ishikawa M, Taniguchi N. Core fucosylation of E-cadherin enhances cell-cell adhesion in human colon carcinoma WiDr cells. Cancer Sci. 2009 May; 100(5):888-95.
125. Woodbury RA, Santer UV, Elkins WL, Glick MC. Similarities in glycosylation of human neuroblastoma tumors and cell lines. Cancer Res. 1986 Jul; 46(7):3692-7.
126. Fukushima K, Satoh T, Baba S, Yamashita K.alpha1,2-Fucosylated and beta-N-Acetylgalactosaminylated prostate-specific antigen as an efficient marker of prostatic cancer. Glycobiology. 2010 Jan; 20(4):452-60.
127. Wang M, Long RE, Comunale MA, Junaidi O, Marrero J, Di Bisceglie AM, Block TM, Mehta AS. Novel fucosylated biomarkers for the early detection of hepatocellular carcinoma. Cancer Epidemiol Biomarkers Prev. 2009 Jun; 18(6):1914-21.
128. Mehta A, Block TM. Fucosylated glycoproteins as markers of liver disease. Dis Markers. 2008; 25(4-5):259-65.
129. Nakagawa T, Uozumi N, Nakano M, Mizuno-Horikawa Y, Okuyama N, Taguchi T, Gu J, Kondo A, Taniguchi N, Miyoshi E. Fucosylation of N-glycans regulates the secretion of hepatic glycoproteins into bile ducts. *J Biol Chem*. 2006; 281(40):29797–29806.
130. Springer GF. Immunoreactive T and Tn epitopes in cancer diagnosis, prognosis, and immunotherapy. J Mol Med. 1997 Aug; 75(8):594-602.
131. Springer GF, Desai PR, Spencer BD, Tegtmeyer H, Carlstedt SC, Scanlon EF. T/Tn antigen vaccine is effective and safe in preventing recurrence of advanced breast carcinoma. Cancer Detect Prev. 1995; 19(4):374-80.
132. Miyoshi E, Nakano M. Fucosylated haptoglobin is a novel marker for pancreatic cancer: detailed analyses of oligosaccharide structures. Proteomics. 2008 Aug; 8(16):3257-62.
133. Kyselova Z, Mechref Y, Kang P, Goetz JA, Dobrolecki LE, Sledge GW, Schnaper L, Hickey RJ, Malkas LH, Novotny MV. Breast cancer diagnosis and prognosis through quantitative measurements of serum glycan profiles. Clin Chem. 2008 Jul; 54(7):1166-75.
134. Hakim AA. A mechanism by which human breast carcinoma cells escape the host immune system. Neoplasma. 1988; 35(6):691-705.
135. Hakomori S. Tumor malignancy defined by aberrant glycosylation and sphingo(glyco)-lipid metabolism. Cancer Res 1996; 56:5309-5318.
136. Kurtenkov O, Klaamas K, Miljukhina L. The lower level of natural anti-Thomsen-Friedenreich antigen (TFA) agglutinins in sera of patients with gastric cancer related to ABO(H) blood-group phenotype. Int J Cancer. 1995 Mar 16; 60(6):781-5.
137. Vestergaard EM, Hein HO, Meyer H, et al. Reference values and biological variation for tumor marker CA 19-9 in serum for different Lewis and secretor genotypes and evaluation of secretor and Lewis genotyping in a Caucasian population. Clin Chem. 1999 Jan; 45(1):54-61.
138. Narimatsu H, Iwasaki H, Nakayama F, et al. Lewis and secretor gene dosages affect CA19-9 and DU-PAN-2 serum levels in normal individuals and colorectal cancer patients. Cancer Res. 1998 Feb 1; 58(3):512-8.

139. Goodarzi MT, Turner GA. Decreased branching, increased fucosylation and changed sialylation of alpha-1-proteinase inhibitor in breast and ovarian cancer. Clin Chim Acta. 1995; 236:161–171.
140. Yamashita K. et al. Comparative study of the oligosaccharides released from baby hamster kidney cells and their polyoma transformant by hydrazinolysis. J Biol Chem. 1984; 259:10834–10840.
141. Dube DH, Bertozzi CR. Glycans in cancer and inflammation – potential for therapeutics and diagnostics, Nat Rev Drug Discov. 2005; 4:477–488.
142. Lau KS, Dennis JW. N-Glycans in cancer progression, Glycobiology. 2008; 18:750–760.
143. Potapenko IO, Haakensen VD, Lüders T, Helland A, Bukholm I, Sørlie T, Kristensen VN, Lingjaerde OC, Børresen-Dale AL. Glycan gene expression signatures in normal and malignant breast tissue; possible role in diagnosis and progression. Mol Oncol. 2010 Apr; 4(2):98-118.
144. Partridge EA, Le RC, Di Guglielmo GM, Pawling J, Cheung P, Granovsky M, Nabi IR, Wrana JL, Dennis JW. Regulation of cytokine receptors by Golgi N-glycan processing and endocytosis. *Science*. 2004; 306:120–124.
145. Fukushi Y, et al. Novel fucolipids accumulating in human adenocarcinoma. II. Selective isolation of hybridoma antibodies that differentially recognize mono-, di-, and trifucosylated type 2 chain, J Biol Chem. 1984; 259:4681–4685.
146. Fukushi Y, et al. Novel fucolipids accumulating in human adenocarcinoma. III. A hybridoma antibody (FH6) defining a human cancer-associated difucoganglioside (VI3NeuAcV3III3Fuc2nLc6), J Biol Chem. 1984; 259:10511–10517.
147. Kannagi R. Carbohydrate-mediated cell adhesion involved in hematogenous metastasis of cancer. Glycoconj J. 1997; 14:577–584.
148. Tozeren A, et al. E-selectin-mediated dynamic interactions of breast- and colon-cancer cells with endothelial–cell monolayers, Int. J. Cancer 60 (1995), pp. 426–431.
149. Elola MT, et al. Lewis x antigen mediates adhesion of human breast carcinoma cells to activated endothelium. Possible involvement of the endothelial scavenger receptor C-type lectin, Breast Cancer Res Treat. 2007; 101:161–174.
150. Soejima M, Koda Y. Molecular mechanisms of Lewis antigen expression. Leg Med. (Tokyo) 2005; 7:266–269.
151. Bosmann HR, Hall TC. Enzyme activity in invasive tumors of human breast and colon. Proc Natl Acad Sci USA. 1974; 71:1833–1837.
152. Schengrund CL, et al. Sialidase activity in transformed cells. J Biol Chem. 1973; 248:4424–4428.
153. Kakugawa Y, et al. Up-regulation of plasma membrane-associated ganglioside sialidase (Neu3) in human colon cancer and its involvement in apoptosis suppression. Proc Natl Acad Sci USA. 2002; 99:10718–10723.
154. Miyagi T, et al. Multiple forms of mammalian sialidase: altered expression in carcinogenesis, Tohoku J Exp Med. 1992; 168:223–229.
155. Miyagi T, et al. Neoplastic alteration of a membrane-associated sialidase of rat liver. Jpn J Cancer Res. 1990; 81:915–919.
156. Miyagi T, et al. Metastatic potential of transformed rat 3Y1 cell lines is inversely correlated with lysosomal-type sialidase activity. FEBS Lett. 1994; 349:255–259.
157. Kato T, et al. Overexpression of lysosomal-type sialidase leads to suppression of metastasis associated with reversion of malignant phenotype in murine B16 melanoma cells. Int J Cancer. 2001; 92:797–804.