Lectins: Specificities and Dynamics

*Peter D’Adamo*
“Give me neither poverty nor riches; feed me with food convenient for me.”

-Proverbs 30:8.
What is a lectin?

1. According to an old definition, ‘Lectins are multivalent carbohydrate-binding proteins or glycoproteins except for enzymes and antibodies.’

2. However, such a narrow definition no longer seems relevant, because a significant number of exceptions are evident.

3. For today, a more flexible interpretation would be ‘lectins are proteins which specifically bind (or cross link) carbohydrates.’
Lectins, Mitogens and Agglutinins

Background

WHY ARE THERE LECTINS?

The most accepted theory is that plant lectins serve as a sort of primitive immune system, protecting young seeds and beans from parasites and fungi much like our own antibodies protect us.

This theory does explain why lectins are found most commonly in seeds and other ‘growing things’.
Lectins, Mitogens and Agglutinins

Lectin Specificity

1. Lectins are often classified based on their saccharide-specificity. Though this conventional method is familiar and practically useful, it is not necessarily relevant for refined specificity.

2. Lectins in the same category (e.g., galactose-specific lectins) show considerably different sugar-binding preferences.

3. Moreover, an increasing number of lectins which never show high affinity to simple saccharides have been found.
Key Concept:

GLYCOCONJUGATES & GLYCOSYLATION

1. Glycoconjugates are simply molecules which have a carbohydrate bonded to a fat or protein.

2. When bonded to a fat, glycoconjugates are called ‘glycolipids’. When carbohydrates are bonded to proteins, they are called ‘glycoproteins’.

3. Although some ABO antigens are glycolipids, the vast majority are glycoproteins.

4. About 11% of the human body is composed of glycoconjugates.
Factors Influencing Membrane Glycosylation in the Gut

1. Animal Species
2. Blood Group Specificity
3. Age
4. Site in the Intestines
5. Position Along the Crypt/Villus Axis
6. Diet
7. Pathology
8. State of Differentiation and Maturation
9. Bacterial Status

Trends Glycosci. Glycotechnol. 8: 149-165
### Lectins, Mitogens and Agglutinins

<table>
<thead>
<tr>
<th>Blood Group</th>
<th>Primary Glycoside</th>
<th>Secondary Glycosides</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>N-acetyl galactosamine</td>
<td>Mannose (3,4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glycophorin A (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Galactosyl-A glycolipids (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>“Band 3” (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alpha -N-acetylgalactosaminyl (Tn antigen)</td>
</tr>
<tr>
<td>B</td>
<td>Galactose</td>
<td>N-neuramininic acid (8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glycophorin B(1)</td>
</tr>
<tr>
<td>O</td>
<td>Fucose</td>
<td>N-acetyl glucosamine (5,6,7)</td>
</tr>
<tr>
<td>AB</td>
<td>Both A and B</td>
<td>Both A and B</td>
</tr>
</tbody>
</table>

Lectins, Mitogens and Agglutinins

Lectins in the world/lectins in the diet

50% of over 2000 plants investigated contained lectins.

36 of 88 lectins identified in common foodstuffs showed some ABO specificity.
**Backgrounder: Altered glycosylation in disease**

**GALACTOSE-DEFICIENT ANTIBODIES**

In several auto-immune diseases, most strikingly RA and IgA nephropathies, the galactosyl groups normally used as the hinge of the Fc region of the IgG and IgA molecule is instead replaced with n-acetyl glucosamine or mannose.
Key Concept:

ALTERED GLYCOSYLATION IN PROINFLAMMATORY STATES AND LECTIN SENSITIVITY

• Immunoglobulins with affinity for the Fc region of IgG molecules are found in rheumatoid arthritis and linked with the severity of the disease.
  • Agalactosylated glycoforms of immunoglobulin G may induce association with the mannose-binding lectin and contribute to the pathology.
  • This may well be the ‘smoking gun’ linking wheat consumption to increased RA symptomatology.
  • Altered glycosylation also affects immune complex formation.
    • The abnormality of lectin binding seen in IgA nephropathy is typical of Henoch-Schonlein purpura with nephritis.
Freed’s Lectin Hypothesis

1. “Of the various rheumatogenic foods, wheat and other grains top the list. Avoidance of these is frequently the only dietary maneuver required, especially in early cases.”

2. Proposes that ingested wheat lectin (and other dietary lectins) enter the bloodstream from the intestine and bind to the connective tissues, making them abnormally stiff.
   - Connective tissue and skin proteoglycans have considerable quantities of amino sugar and bind wheat lectin strongly.

http://elfstrom.com/arthritis/rheumaticpatches.html
Key Concept:

GLUCOSE FLUX LEADS TO ALTERED GLYCOSYLATION AND ENHANCED LECTIN SENSITIVITY


Chronically increased glucose flux results in enhanced O-GlcNAcylation of GLUT4 and/or GLUT4-associated proteins and may contribute to insulin resistance.

Consumption of a diet containing kidney bean caused an increase in the level of mRNAs for the insulin receptor (327%) and GLUT-4 (185%) in the gastrocnemius muscle. (Int J Biochem 1992 Jun;24(6):897-902)
Insulin mimicry by dietary lectins

When insulin is replaced by a lectin, the result is an activated insulin receptor with no negative feedback inhibition.
CELL SURFACE GLYCOSYLATION IN MALIGNANT STATES

Aberrant glycosylation is a hallmark of malignancy. Some products are truly tumor-specific antigens that have not been found in normal tissue.

• PSA, CA15-3, CA-125 etc.

Other antigens are present in normal tissue, but over expressed on tumor cells

• ABH, Sialyl Lewis X

Others are displayed during fetal development, disappear in the adult, only to arise again during malignant transformation.

• CEA
Mucins are major glycoprotein components of mucus, covering the luminal surfaces of epithelial respiratory, gastrointestinal and reproductive tracts.

Aberrations in mucins and other cell surface carbohydrates are a universal characteristic of the malignant transformation of cells.

Are responsible for altered cell adhesion or metastasis and to the avoidance of immunological defense.

Comprise peripheral structures representing blood group antigens as well as core structures such as Tn and T and their sialylated counterparts, sialyl Tn and sialyl T.
Dietary lectins can alter the proliferation of colonic cells. A normal healthy cell is said to be well differentiated, meaning that its characteristic features and structures are intact. Differentiation is regulated by adhesion molecules which, being glycosylated, 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.

VFA (broad bean lectin) stimulated an undifferentiated colon cancer cell line to differentiate into gland-like structures. The adhesion molecule epCAM is involved in this.

Agaricus bisporus (mushroom) lectin was active as well.

Dietary or therapeutic VFA may slow progression of colon cancer.
Lectins and Gut Permiability

1. Lectins which react with sialic acid in secreted glycoproteins, such as those from the bark of the elder tree (*Sambucus nigra*) can overstimulate and exhaust the capacity of goblet cells to synthesise mucin.

2. 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.

3. Endocytosis of lectins is more extensive in the colon where bacterial counts are high.

4. Lectins can directly interact with and agglutinate some resident bacteria of the digestive tract and then selectively remove these from the lumen.
Lectins, Mitogens and Agglutinins

Systemic Effects of Dietary Lectins

1. Lectins are good mimics of the effects of endogenous growth factors, hormones and cytokines in all types of cells.
2. Lectins can bind to the neuroendocrine cells of the gut and stimulate the secretion of gut peptide hormones into the systemic circulation.
3. Lectins can be transmitted through the gut wall into the blood circulation and mimic the effects of endocrine hormones. The organs most often affected are the pancreas, skeletal muscle, liver, kidneys and thymus.
4. Lectins can modulate IgE responses to other antigens.

*Trends Glycosci.Glycotechnol.* **8:** 149-165
Ricin toxicity

- Oral, respiratory and injection most likely forms of contact.
- Inhalation perhaps most dangerous producing diffuse necrotizing pneumonia of the airways, with interstitial and alveolar inflammation and edema.
- Animal studies have shown that either active immunization or passive prophylaxis or therapy (if the therapy is given within a few hours) is extremely effective against intravenous or intraperitoneal intoxication with ricin.
- More than 150 agents, including cellular membrane effectors, calcium channel–blocking agents, antioxidants, effectors of endocytosis, nucleoside derivatives, antibacterials, ricin analogs, effectors of cellular metabolism, and competitors for binding have been tested. Of these only 3 were shown to even moderately effective in vitro.
- D-galactose blocks ricin adhesion in vitro (best natural sources probably milk, arabinogalactan and citrus pectin; also in figs, sugar beets, gums, seaweed, flaxseeds)
Lectins, Mitogens and Agglutinins

Lectins as mitogens

- Mitogens are substances capable of producing a non-specific, polyclonal activation of lymphocytes. Most of them are lectins or plant glycoproteins such as Phytohaemagglutinin (PHA), Concanavalin-A (Con-A) and Pokeweed Mitogen (PWM). From this kind of work, it appears that cross-linking of surface receptors is a crucial event in B-cell activation.

- Urtica dioica contains a lectin which is a T cell mitogen distinguishable from classical T cell lectin mitogens by its ability to discriminate a particular population of CD4+ and CD8+ T cells as well as its capacity to induce an original pattern of T cell activation and cytokine production.

**Concanavalin A (con-A) activates T cells**

**Phytohemagglutinin (PHA) activates T cells**

**Pokeweed mitogen (PWM) activates B & T cells**

*Each bind different glycoprotein.*
Mitogen therapy for biological warfare/terrorist attacks and viral hemorrhagic fever control.

Wimer BM.

“Working full time in devising protective measures that might help control the damaging effects of terrorist attacks, Alibek has placed strong emphasis on stimulating nonspecific immunities of victims mainly with interleukins and other cytokines. A more productive alternative would be giving mitogens such as PHA and PWM to reinforce vaccine and antibiotic actions, at the same time stimulating protective immune, myelopoietic, and lymphopoietic responses.”

“….. Functioning in a different mode, PWM has the advantage of much greater potency, and can be given either intravenously or orally, since these appear to be equally effective routes of administration.”
Lectins and Allergy

1. Most lectins given orally are immunogenic. Pooled human blood typically contains high titre anti-banana lectin (BanLec-1) IgG4.

2. Lectins can modulate IgE responses to other antigens [hG3 also does this, and there is some evidence that CD23 (the low-affinity IgE receptor) is in itself a C-type lectin.]

3. Dietary lectins can induce the release of IL-4 and IL-13 from human basophils. Dietary lectins are known to prime TH2 helper lymphocytes.

4. Most humans carry antibodies to common dietary lectins, including WGA, PNA, SBA. Interestingly the antibodies are still active against denatured lectins, and do not block their agglutinating properties.

5. Several Galectins (hG3, epsilon BP [CBP 35] bind to IgE receptors

Cell Mol Life Sci 2002 Apr;59(4):648-64
Trends Glycosci.Glycotechnol.8:149-165
FEBS Lett 1996 Nov 18;397(2-3):139-42
Lectins and Platelets

1. Virtually all platelets carry ABH antigens, the relative amount of which is determined by ABO type (O>B>AB>A) and secretor status.

2. Thrombospondin (TSP) is a glycoprotein secreted from the alpha-granules of platelets upon activation. In the presence of divalent cations, the secreted protein binds to the surface of the activated platelets and is responsible for the endogenous lectin-like activity associated with activated platelets. Like hG3 it plays a critical role in endothelial adhesion to the cellular matrix.

Blood 1985 Mar;65(3):615-9
Biochemistry 1985 Jun 18;24(13):3128-34
Lectins, Mitogens and Agglutinins

**Lectin ‘Families’**

**GALECTINS**
Endogenous animal galactose-specific lectins

**LEGUME LECTINS**
Huge number of members.

**ANNEXINS**
Have an affinity for lipids

**CALCIUM DEPENDANT (C-TYPE) ANIMAL LECTINS**
- **Selectins** enhance leukocyte adhesion to endothelial cells through sialyl-LewisX recognition.
- **Collectins**, specific for mannose. They are supposed to be involved in innate immunity

**INVERTEBRATES**
Known to contain various lectins in their body fluids, probably as body-protection factors.
Galectins (Sometimes called ‘S-lectins’)

1. Galectins show wide biological distribution not only in vertebrates but also in invertebrates, including nematodes, insects and sponges.

2. Galectins are involved in development, differentiation, morphogenesis, tumor metastasis, apoptosis, and RNA splicing. However, relatively little is known about the mechanism by which galectins exert these functions.

3. GGL (Gallus Gallus Lectin), Chick Beta-galactoside-Binding Lectin, Chicken Thymic Electrolectin (CTE), Chick Embryonic Skin Lectins are all galectins.

4. Since galectins are galactoside specific, not surprisingly they tend to react with the blood group B antigen.
Human Galectins

C-Reactive Protein

- CRP is an acute phase reactant. CRP is released by the body in response to acute injury, infection, or other inflammatory stimuli.
- Positive association between CRP and coronary artery disease. In a survey of 388 British men aged 50-69, the prevalence of coronary artery disease increased 1.5 fold for each doubling of CRP level.

Human Placenta Lectins

- HPL-Hs bind heparin.

Galectin-VIII (hG8)

- Binds to integrins inhibits cell adhesion and induces apoptosis. It inhibits adhesion of human carcinoma (1299) cells to plates coated with integrin ligands, and induces cell apoptosis.
Human Galectins

**Serum Amyloid P Component (pentraxin)**

- SAP binds to late apoptotic cells and is involved in the phagocytosis of these cells by human monocyte derived macrophages. Drugs capable of displacing SAP may help amyloidosis and diseases associated with local amyloid including Alzheimer's disease and type 2 diabetes.

**Galectin VII, hGal-7**

- Galectin-7 is specifically expressed in stratified epithelia and notably in epidermis. Galectin-7 overexpression is associated with the apoptotic process in UVB-induced sunburn keratinocytes.

**epsilon BP (CBP 35)**

- The IgE-binding protein, epsilon BP (CBP 35) is a galectin that mediates the high affinity binding of IgE to substrate, most noteworthy on pancreatic islet cells. Cells of blood groups A and B are more strongly bound by epsilon BP than those of blood group O.
Galectin III, hGal-3

1. Elevated levels in a wide range of neoplasms, and expression was shown to be associated in some tumor cell systems with metastases.

2. Galectin-3 has been shown to be involved in cell adhesion and activation of immune cells and plays a significant role in cell to extracellular matrix interactions. High levels of galectin-3 in metastatic cancer cells suggest an impact on metastasis formation.

3. Galectin 3 and the blood group precursor and tumor-associated carbohydrate T antigen play a leading role in docking breast and prostate cancer cells onto endothelium. Importantly, T antigen-bearing glycoproteins are also capable of mobilizing galectin-3 to the surface of endothelial cells, thus priming them for harboring metastatic cancer cells.

4. Mediates genistein-induced G(2)/M arrest and inhibits apoptosis.

5. Has a binding capacity for various glycoconjugates including IgE.
Apoptosis ('Programmed cell death')

Programmed cell death is needed to destroy cells that represent a threat to the integrity of the organism.

1. The withdrawal of positive signals; that is, signals needed for continued survival (Growth factors, Interleukins)

2. The receipt of negative signals (Tumor necrosis factor - alpha [TNF-a ])

Anoikia (from the Greek meaning ‘without home’)

Cells require attachment to a substrate to grow; when denied anchorage or when attachment is prevented, they undergo a particular type of apoptosis known as ‘anoikia.’ Strategies aimed at blocking the effects.

1. Blocking the actions of T-antigen and Galectin 3 look like a VERY PROMISING way of inhibiting the endothelium from harboring of metastatic cells.
Lectins, Mitogens and Agglutinins

Lectins that can induce apoptosis

<table>
<thead>
<tr>
<th>Lectin</th>
<th>Cell Line</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice bran lectin</td>
<td>Human leukemic cells</td>
<td><em>J Biochem (Tokyo)</em> 2001 Dec;130(6):799-805</td>
</tr>
<tr>
<td>Pokeweed mitogen</td>
<td>Prime lymphokine-activated killer (LAK) cell-mediated cytotoxicity to induce apoptosis in a variety of tumor systems</td>
<td><em>Zhonghua Zhong Liu Za Zhi</em> 1995 Jul;17(4):245-8</td>
</tr>
<tr>
<td>Urtica dioica lectin</td>
<td>Human T cell apoptosis</td>
<td><em>Res. Immunol.</em> 1995 (146); 249-262</td>
</tr>
</tbody>
</table>
Selectins: Cell Adhesion Molecules

1. Selectins are cell-cell adhesion molecules that are involved in leukocyte-endothelial cell adhesive interaction, which is required for extravasation at target tissue sites.

2. 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.

3. 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. Selectin activity is up regulated in most asthmatic states.

APMIS 2001 Jan;109(1):9-31
Integrins and Cadherins

1. Integrins are receptor proteins which are of crucial importance. They are the main way that cells both bind to and respond to the extra cellular matrix. They are part of a large family of cell adhesion receptors which are involved in cell-extracellular matrix and cell-cell interactions.
   • Integrins can adhere (bind) an array of ligands. Common ligands are for example fibronectin and laminin

2. Cadherins mediate cell adhesion and play a fundamental role in normal development. They participate in the maintenance of proper cell-cell contacts.
   • E-cadherin, a calcium-dependent transmembrane glycoprotein, is an adhesion molecule. The loss of E-cadherin expression may be involved in regional lymph node metastasis and in malignant potential of thyroid neoplasms. See: J Surg Oncol 2001 Mar;76(3):176-80
Collectins (collagen-like lectin)

1. The collectins include three serum proteins, mannan-binding protein (MBP) (also called mannan-binding lectin, MBL), bovine conglutinin and bovine collectin-43 (CL-43), and two lung surfactant proteins (SP-A and SP-D). They play important roles in innate immunity without involvement of antibodies. SP-A contains blood group A antigenic determinants.

2. They bind to non-host carbohydrate structures present on the surface of a range of microorganisms, including bacteria, yeasts, fungi, parasitic protozoa and viruses, and exhibit antibacterial activity through killing mediated by the complement system or by promoting phagocytosis.

3. MBP can activate the complement system through the classical pathway deficiencies of MBP are associated with chronic otitis media in both children and adults. The degree of MBL binding is well correlated with the expression of Lewis A and Lewis B antigens and may explain why Lewis ‘double negatives are often prone to chronic infections.
Opsonins

Opsonins are freely circulating serum molecules which are produced to attach to the surface of microbes, rendering them more attractive to phagocytes.

- Examples of opsonins include IgG antibody and the C3b molecule of the complement system. Each has receptors for both foreign particle and host phagocyte.
- Opsonisation can itself stimulate the local activation of complement, further enhancing the local production of C3b opsonin and phagocytosis.

The Opsonic Index

Is calculated by dividing the number of bacteria in blood per phagocyte when an immune response has been mounted compared to that previously. Each figure is an average for identical unit volumes or smear counts.

- *Veratrum viride* will raise the opsonic index against the Diplococcus Pneumonia, 70 to 109 per cent. (Boericke)
“Lectins are causes in search of diseases.”

DJ Freed, MD

“Lectins are cures in search of healers.”

PJ D’Adamo, ND