B7. Role of Cell Surface Carbohydrates

Cell surface carbohydrates present information-rich binding sites for other molecules and act as "receptors" for biological agents as diverse as viruses, bacteria, toxins, and other cells. This is illustrated well by studying the properties of circulating immune cells. The cells must often pass through the walls of capillaries as they hone in on a site of infection. (Cancer cells do this as well as they escape the boundaries of the organ in which they developed and pass through the blood vessels and into new tissues in the process of forming metastases.) Immune cells must first bind to endothelial cells (a monolayer of cells that line the lumen of the blood vessels) before they can pass through the vessel walls. Proteins called selectins our found on cells that can pass through vessels and on endothelial cells. There are 3 types:

1. L-selectins: found on leukocytes ("white" blood cells that are circulating immune cells)
2. P-selectins: found on activated platelets (which can aggregate to form a type of blood clot) and activated endothelial cells. Activation occurs during the inflammatory response which can lead to the quick movement of pre-formed selectins stored within the cytoplasm to the membrane. In addition, their expression can be induced.
3. E-selectins: found on activated endothelial cells only after the cells have been induced to form them by certain immune hormones called cytokines releases by immune cells during an inflammatory response.

These selectins are transmembrane proteins with an extracellular CHO binding domain, an EGF-like (epidermal growth factor like) domain, varying numbers of C (complement regulatory) domains, and a transmembrane domain. The extracellular CHO binding domain is found in proteins in all organisms. Proteins that bind carbohydrate motifs are called lectins.

### Lectins and CHO ligands

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<th>Lectin Family/Lectin</th>
<th>Abbreviation</th>
<th>Ligand(s)</th>
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In animals, lectins facilitate cell-cell interactions by forming multiple, but weak interactions between the protein and many sugars on the ligand to which it binds.

The selectins are also part of a class of molecules called adhesion molecules. As mentioned for the selectins, adhesion molecules contain

- an extracellular CHO binding domain (the lectin domain), which mediates binding to adjacent cells or to the extracellular matrix; The P, L, and E selectins can bind a tetrasaccharide containing Sia-Gal-GalNAc-Fuc (called sialyl-Lewisx) on selectin ligand proteins and glycolipids.
- a transmembrane domain;
- and a cytoplasmic domain which often interacts with the cytoskeleton within the cell.

The selectins recognize Ser-linked CHO residues (a tetrasaccharide containing sialic acid, galactose, GalNAc and fucose) displayed on transmembrane glycoproteins called selectin ligands. L selectins bind to endothelial cell ligands while P and E selectins bind to ligands on leukocytes. These interactions slow the leukocyte down as it rolls along the surface of the endothelial cells. These interactions involve protein-CHO binding.

This initial binding mediated by selectin-CHO interactions activate the expression of another adhesion molecule on the leukocyte, integrin, a heterodimer with an a and b chain. These cause strong leukocyte-endothelial cell interactions,
leading to ultimate movement of the leukocytes through the vessel wall. Other classes of adhesion molecules (in addition to selectins and integrins) are cadherins (calcium-dependent adhesion molecules), and the immunoglobulin-like superfamily (ICAM1, ICAM2, VCAM). VCAM (Vascular Adhesion Molecule) binds the integrin expressed on activated lymphocytes, leading to passage of the lymphocyte from the lumen of the vessel into the tissues. Integrins appear to bind proteins in the extracellular matrix through RGD (Arg-Gly-Asp) and also through LDV (Leu-Asp-Val) motifs on the proteins, including fibronectin (RGD), thrombospondin (RGD & LDV), fibrinogen (RGD & LDV), van Willebrand Factor (RGD), vitronectin (RGD). They also bind other matrix proteins with an "alpha domain" including collagen and laminin. Integrin/Adhesion molecule interations involve protein/protein interactions.

Genbacev et al. have recently shown that a fertilized egg (in the blastocyst stage which is ready for implantation in the uterine cell wall) express L-selectin which allows a low affinity (rolling-type) interaction of the fertilized egg with the uterine epithelial cells. These cells expressed the CHO ligands on their surface which bind to the L-selectin on the blastocyst. The CHO ligands are only transiently expressed on the surface of the epithelial cells of the uterus, presumably only when the uterus is primed for implantation. After the initial interaction of the blastocyst and epithelial cells, further expression of integrins on the blastocyst surface might result. Problems in any of these molecular steps could result in infertility.

Figure: Endothelial Cell/Leukocyte Interactions: Selectins, Integrins, and ICAMs

An interesting experiment was recently done by Davis et al. that showed the importance of protein modification (like glycosylation) to binding and biological function. Post-translational modifications represent one of natures way to change protein function. The researchers were able to chemically modify surface features of a protein to produce new functionalities. They did so by using mutagenesis to change surface amino acids to Cys or replacing Mets with nonnatural amino acid analogs that contain azide or alkyne groups. These modified groups could then direct the location of chemical modifying reagents (such as sugars) to these sites. The researchers studied a pair of proteins involved in inflammation, P-selectin, which binds a transmembrane protein P-selectin-glycoprotein ligand-1, that requires two post-translational changes to bind to P-selectin. They picked a protein completely unrelated to PSGL-1, and selectively modified it using this approach so it contain a glycosylated and a sulfated side chain. The unrelated protein bound to P-selectin.
Selectins: L-selectin | P-selectin | E-selectin | Selectin Ligands

Integrins at a glance

Integrins: Great Source of Information!

Receptor for Sialic Acids

Lectins that recognize sialic acids, especially members of the Siglec family (sialic acid-recognizing Ig-superfamily lectins) turn out to be important players in our propensity for disease. As we previously discussed, humans lack a hydroxylase gene necessary for the hydroxylation of Neu5Ac to Neur5Gc which is found in chimps who possess the enzyme. Chimp’s immune systems seems to confer protection from acquiring simian version of AIDS, cirrhosis, and other diseases which humans acquire when they are infected with the human versions of the HIV virus, hepatitis B or C, or other viruses. These disease and others associated with overactive T cells (rheumatoid arthritis, asthma, type-I diabetes) are not common in chimps. It turns out that there is a link between the type of sialic acid and the expression of siglics that influences the difference on our disease propensity. Varki et al have shown that chimps and gorillas show much higher levels of expression of Siglecs on T cells, which are critical regulatory and effector cells in the immune system. When siglecs on T cells are activated, T-cell responses are down regulated. Although HIV virus ultimately kills T helper cells, the virus initially activates them on infection, leading to their proliferation and production of a larger number of cells for the virus to infect.

Influenza virus that has caused some of the greatest pandemics in world history also binds to sialic acid on host cells, through a viral binding protein called hemagglutinin. On binding, conformational changes activate a neuraminidase activity of another viral protein, allowing cleavage of the sialic acid glycosidic bond, and subsequent entry of the virus into the cell.

Contributors

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