



Selectins and Glycosylation in Inflammation

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CARBOHYDRATES AND INFLAMMATION.

Leukocytes move through the micro circulation under considerable shear flow. Arresting these leukocytes at sites of inflammation involves multiple interactions with individual members of several families of cell adhesion molecules⁽¹⁾. The families of cell adhesion molecules involved in leukocyte adhesion and extravasation include the integrins (the 2 integrin sub-family found exclusively on leukocytes, and VLA-4, a β 1 integrin), the selectins, and adhesion molecules which are members of the immunoglobulin superfamily (principally ICAM-1, ICAM-2 and V-CAM).

Based on various in vitro studies, including those using intra-vital microscopy, a model is emerging in which leukocyte extravasation occurs in the following way. Leukocytes in the micro circulation are constantly coming into contact with the endothelial cell lining and are swept off this lining by the normal shear flow, unless the endothelial cells of the post-capillary venules (in the systemic micro circulation) express specific receptor/ligand pairs for leukocyte adhesion. In this case, the leukocytes roll with decreasing speed along the endothelium until they eventually come to rest, flatten against the endothelium, and migrate through it. The various cell adhesion molecules are thought to be involved at different stages of this process and it is the successful involvement of different receptor-ligand pairs acting in sequence, that ultimately leads to leukocyte extravasation. In broad outline, the selectins are involved in the initial adhesion between circulating leukocytes and inflamed endothelium, and serve to “capture” leukocytes from the circulation and initiate the “rolling” process⁽²⁾. Selectin-mediated rolling alone does not seem to be sufficient to bring leukocytes to rest. A second set of interactions involving one or more of the β 2-integrins is necessary to strengthen the adhesion of the leukocyte sufficiently to “flatten” it against the endothelium, and to prevent it being washed off.

E-, P-, AND L-SELECTIN.

The selectins constitute a conserved gene family sharing a common molecular architecture and also a common function during inflammation⁽³⁾. There are three known members of the selectin family, namely E-selectin (previously known as ELAM-1), P-selectin (previously known as PADGEM or GMP-140) and L-selectin (previously known as gp90 Mel14 or LAM-1). Each selectin has an amino-terminal carbohydrate recognition domain (CRD) with considerable homology to C-type lectins, followed by a single epidermal growth factor like domain, a variable number of complement-regulatory domains in series, a single transmembrane polypeptide (there is no evidence for any

selectin of a GPI-membrane anchor), and a carboxy-terminal cytoplasmic domain. Each selectin interacts with its cognate ligand through the amino-terminal CRD in a calcium-dependent way.

E-SELECTIN.

E-selectin is a 95-115 kDa glycoprotein whose expression is induced on endothelium by activation with certain cytokines, including IL-1, TNF, and also by LPS. Its expression requires *de novo* protein synthesis, and levels at the endothelial cell surface reach a maximum about 4-6 hours after cytokine stimulation and then decline to basal levels after about 24 hours. Its carbohydrate ligand(s) are constitutively expressed on certain cell surface glycoproteins, and possibly also glycolipids, on neutrophils, monocytes and certain memory T-lymphocytes. E-selectin is now clearly implicated in the rolling of these leukocytes along inflamed endothelium.

P-SELECTIN.

P-selectin is a 140 kDa glycoprotein located in the secretory granules of platelets and endothelial cells. P-selectin expression at the cell surface is not induced by cytokines but occurs rapidly (minutes) after stimulation of endothelial cells by thrombin or histamine through degranulation. P-selectin mediates adhesion of neutrophils to thrombin- or histamine- activated endothelial cells or platelets, as shown by blockage of this adhesion by anti- P-selectin monoclonal antibodies. The carbohydrate ligands for P-selectin appear to be constitutively expressed on certain cell surface glycoproteins, but not glycolipids, on neutrophils and platelets. By mediating the initial adhesion of neutrophils to endothelium, P-selectin facilitates the further adhesion of neutrophils through PAF (platelet activating factor) β 2 integrin-mediated events.

L-SELECTIN.

L-selectin is a glycoprotein which is constitutively expressed on all leukocytes. Both L-selectin and its murine homolog, gp90 Mel14 are involved in the normal recirculation of lymphocytes - each mediates the interaction between circulating lymphocytes and vascular ligands (often referred to as “addressins”) on the high endothelial venules of lymphoid organs⁽⁵⁾. In addition to its role as a lymphocyte homing receptor, L-selectin is also involved in the adhesion of circulating leukocytes to non-lymphoid tissue, including endothelium, during inflammation. L-selectin is shed from the leukocyte surface following leukocyte activation⁽⁴⁾, and this may be important in retaining activated leukocytes at sites of inflammation.

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SELECTIN-LIGAND INTERACTIONS.

A variety of studies⁽⁶⁻⁸⁾, including (a) use of soluble carbohydrates as competitive ligands, (b) use of anti-carbohydrate antibodies, (c) exoglycosidase treatments of leukocytes and cytokine-activated endothelial cells, and (d) induction of selectin-dependent adhesion by transfection with a fucosyl transferase, have all led to the consensus view that each selectin recognizes *in vivo* a carbohydrate ligand. In particular, all three selectins can recognize the blood group determinant sialyl-Lewis^X (sLe^X)(NeuNAc α 2-3Gal β 1-4(Fuc α 1-3)GlcNAc) and its isomer sialyl-Lewis^a (sLe^a)(NeuNAc α 2-3Gal β 1-3(Fuc α 1-4)GlcNAc). Either or both of these carbohydrates most likely constitutes part of the endogenous ligand(s) for E- and P-selectin. In the case of L-selectin, which is capable of recognizing sLex, the endogenous ligand is thought not to be this but rather a sulfated, fucosylated O-linked oligosaccharide present on the vascular addressin GlyCAM-1, whose structure is not yet fully defined. In the case of P-selectin binding of either sLe^X or sLe^a and recognition of a protein determinant on the glycoprotein ligand for P-selectin has been suggested. Recently, it has been proposed that both E- and P-selectin can bind the 3-O-sulfated form of Le^X and Le^a ⁽⁹⁾, a calcium-independent recognition of both sulphated Lex and sulfated Le^a can occur. The physiological relevance of the interaction of E- and P-selectin with sulfated ligands awaits further study.

The molecular details of the interactions between selectins and carbohydrate ligands have been intensively studied in order to facilitate rational design of antagonists. The molecular details of selectin-carbohydrate interaction have principally been investigated by two approaches⁽¹⁰⁾. First, the minimum conserved structural features in the solution conformations of carbohydrates which are able to bind E- and/or P-selectin have been delineated. Second, analogs with controlled positional modifications to sLe^X and sLe^a have been made and their competitive ability to reduce sLe^X dependent E- and P-selectin adhesion has been correlated to the structural modification(s).

Recently, a crystal structure to 2Å⁽¹¹⁾ has been solved for the CRD of E-selectin, and by interpreting this together with site-directed mutagenesis studies, key amino acids involved in carbohydrate binding were proposed. A co-crystal with carbohydrate is awaited. The binding site of the E-selectin appears to be rather shallow, with the ligand binding via multiple hydrogen bonds to the site.

While the selectins are exciting new molecular targets, the clinical utility of carbohydrate-based selectin antagonists is still unproven. It is probable that individual selectins will be more or less involved, in each inflammatory condition, depending on the particular leukocytes implicated and the

site and nature of the inflammatory lesion, and it is therefore possible that control over several clinical conditions could be achieved using selectin antagonists. Available pre-clinical data in animal models does show that carbohydrate ligands of human selectins can dramatically reduce P-selectin dependent deep lung injury induced by cobra venom factor in a rodent model⁽¹²⁾, and can protect against E-selectin dependent acute lung injury induced by deposition of IgG-based immune complexes⁽¹³⁾.

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