INHIBITION OF SELECTIN BINDING

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority benefit of U.S. provisional application No. 60/012,894, filed Mar. 1, 1996, pending, which is hereby incorporated herein by reference in its entirety.

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TECHNICAL FIELD

This invention relates generally to the field of therapeutic compounds designed to interfere between the binding of carbohydrate ligands and their receptors on cell surface. More specifically, it provides products and methods for inhibiting cell migration and activation via P- and L-selectin, using polymerized glycoliposomes.

BACKGROUND

The adhesion of circulating neutrophils to endothelial cells is one of the important events occurring in the process of inflammation. Neutrophil recruitment to tissues is initiated by an adhesion cascade. Through this process, cells roll and eventually attach firmly to the endothelium. The factors that contribute to the high binding strength of this interaction are not fully understood, but is thought to involve interaction between selectins on one cell with carbohydrate ligands on another cell. By interfering with the binding between these components, it may be possible to counter pathological sequelae related to cell migration.

A number of adhesion molecules mediate the interaction of neutrophils and other leukocytes to the endothelium. Amongst them are the ICAMs, VCAM, CD 11, CD 18, the integrin $\alpha4\beta1$, and several receptors now known collectively as selecting. Each of these molecules is part of a ligand-receptor pair, one of which is expressed on each of the two interacting cells. For a general review, the reader is referred to Bevilacqua (Annu. Rev. Immunol. 11:767, 1993). In various combinations, these and other molecules support leukocyte adhesion to the vessel wall and extravasation, and may also participate in activation of cell effector functions. Expression of many of these molecules is up-regulated by soluble factors such as cytokines, thereby acting to increase the recruitment of leukocytes to an affected area.

Amongst the plurality of adhesion molecules that have been described, three have been collected together in a category known as selecting. One was formerly known as ELAM-1, and was identified using inhibitory monoclonal antibodies against cytokine-activated endothelial cells. Another was formerly designated as PADGEM, GMP-140, or CD61. It was originally identified on platelets, and is now known as P-selectin. A third identified on lymphocytes was formerly designated as mLHR, Leu8, TQ-1, gp90^{MEL}, Lam-1, or Lecam-1, and is now known as L-selectin. The selectins were grouped together on the basis of a structural similarity, before very much was known about their binding specificity. All are single chain polypeptides having a carbohydrate binding domain near the N-terminus, an EGF repeat, and anywhere between 2 to 9 modules of ~60 amino acids each sharing homology with complement binding proteins. For general reviews, the reader is referred to Lasky (Annu. Rev. Biochem. 64:113, 1995) and Kansas (Blood 88:3259, 1996).

The three selectins differ from each other in a number of important respects. As depicted in FIG. **2A**, the selectins have different ligand counterparts in the adhesion process. Each selectin is regulated differently, and participates in a different manner in the process of inflammation or immunity. There is also an increasing appreciation for differences in the ligand binding requirements between the selectins.

E-selectin has garnered a significant amount of recent research interest because of its role in inflammation. The migration of inflammatory mediator cells to an inflammatory site is thought to be mediated in part by adhesion of the cells to vascular endothelial cells. Studies in vitro have suggested that E-selectin participates in the adhesion of not only neutrophils, but also eosinophils, monocytes and a subpopulation of memory T-cells to endothelium that has been activated by endotoxin, IL-1, or TNF. Expression of E-selectin by endothelial monolayer increases by about 10-fold and peaks at about 4 hours after stimulation with IL-1, subsiding to near basal levels within 24 hours. The biological role of E-selectin is thought to be a strong binding of cells bearing a suitable E-selectin ligand, over a time-course of 20 min to 1 hour, particularly during the course of local inflammation.

Phillips et al. (Science 250:1130, 1990) first identified the binding target of E-selectin as the oligosaccharide sialyl Lewis X (sLe^x) (NeuAcα2,3Galβ1,4(fucαl,3)GlcNac-), a terminal structure found on cell surface glycoprotein of neutrophils. This has become the prototype carbohydrate ligand for the selectin class. This and related oligosaccharides are the subject of U.S. Pat. No. 5,576,305 and PCT application WO 92/07572.

The sLe^x unit has been assembled into various polymeric structures in an attempt to improve its weak binding to selecting. For example, U.S. Pat. No. 5,470,843 and DeFrees et al. (J. Am. Chem. Soc. 117:66, 1995) disclose bivalent sialyl X saccharides. U.S. Pat. No. 5,470,843 discloses a carbohydrate-containing polymer having a synthetic polymer backbone with 10–20 sLe^x, sLe^a, or GlcNac linked via a bifinctional spacer.

DeFrees et al. (J. Am. Chem. Soc. 118:6101, 1996) describe a sLe^x preparation made with conventional phospholipid liposome technology. The liposomes contain phosphatidyl choline, cholesterol, phospholipid conjugated with methoxypolyethylene glycol, and phospholipid conjugated with sLe^x through a polyethylene glycol spacer. Data is presented showing that this composition is 5×10^3 fold more potent than the sLe^x monomer in inhibiting the binding of E-selectin to cells. Murohara et al. (Cardiovasc. Res. 30:965, 1995) tested sLe^x phospholiposomes in a myocardial reperfusion model, and found that a dose of 400 μg/kg body weight reduced the proportional size of the area of risk and necrosis.

P-selectin is a transmembrane glycoprotein of ~140 kDa, substantially larger than E-selectin. It was originally described on platelets, in which it may be found in α- and