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Chemokines bind to sulfatides as revealed by surface plasmon resonance

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Abstract

Chemokines bind to sulfated cell surface glycosaminoglycans and thereby modulate signaling mediated by G-protein-coupled seven-transmembrane domain chemokine receptors. Similar to glycosaminoglycans, sulfated oligosaccharides are also exposed on the cell surface by sulfatides, a class of glycosphingolipids. We have now identified sulfated glycosphingolipids (sulfatides) as novel binding partners for chemokines. Using surface plasmon resonance (SPR), the binding of proinflammatory and homeostatic chemokines to glycosphingolipids, in particular sulfatides, was investigated. Chemokines were immobilized while glycosphingolipids or additional phospholipids incorporated into liposomes were applied as soluble analytes. A specific affinity of the chemokines MCP-1/CCL2, IL-8/CXCL8, SDF-1α/CXCL12, MIP-1α/CCL3 and MIP-1β/CCL4 to the sulfatides SM4s, SM3, SM2a and SB2, SB1a was detected. No significant interactions with the chemokines were observed for gangliosides, neutral glycosphingolipids or phospholipids. Chemokine receptors have been associated with the detergent-insoluble fraction supposed to contain 'rafts', i.e., glycosphingolipid enriched microdomains of the cell surface. Accordingly, the data suggest that early chemokine receptor signaling may take place in the vicinity of sulfated glycosphingolipids on the cell surface, whereby these sulfatides could modulate the chemokine receptor-mediated cell activation signal.

Keywords: Chemokine; Sulfatide; Surface plasmon resonance; Liposome; Glycosphingolipid; Glycosaminoglycan

Abbreviations: Chemokine short hand designations were IL-8, interleukin 8; MCP-1, monocyte chemo-attractant protein-1; MIP-1, macrophage inflammatory protein-1; SDF-1α, stromal cell-derived factor; EDC, *N*′-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide; GAG(s), glycosaminoglycans; Ganglioside short hand designations were GM3, II^3-*N*-acetyl(or *N*-glycolyl)-neuraminyl-lactosylceramide; GM2, II^3-*N*-acetyl(or *N*-glycolyl)-neuraminyl-gangliotriaosylceramide; GM1a, II^3-*N*-acetyl(or *N*-glycolyl)-neuraminyl-gangliotetraosylceramide; GD1a, II^3,IV^3-bis-*N*-acetyl(or *N*-glycolyl)-neuraminyl-gangliotetraosylceramide; GSL(s), glycosphingolipid(s); glycosphingolipid short hand designations were GlcCer, glucosylceramide; LacCer, lactosylceramide; Gg$_3$Cer, gangliotriaosylceramide; Gg$_4$Cer, gangliotetraosylceramide; NHS, *N*-hydroxysuccinimide; Phospholipid short hand designations were PE, phosphatidylethanolamine; PC, phosphatidylcholine; PI, phosphatidylinositol; PS, phosphatidylserine; SM, sphingomyelin; [RU], response unit; Sugar short hand designations were Fuc, fucose; Gal, galactose; GalNAc, *N*-Acetyl-galactosamine; Glc, glucose; GlcNAc, *N*-acetyl-glucosamine-; GlcA, glucuronic acid; Ins, inositol; Man, mannose; NeuNAc, *N*-acetyl-neuraminic acid; Sulfatides were abbreviated according to Ishizuka [52], i.e., SM4s, galactosylceramide sulfate, GalCer I^3-sulfate; SM3, lactosylceramide sulfate, LacCer II^3-sulfate; SM2a, gangliotriaosylceramide II^3-sulfate; SB1a, gangliotetraosylceramide II^3, IV^3-bis-sulfate

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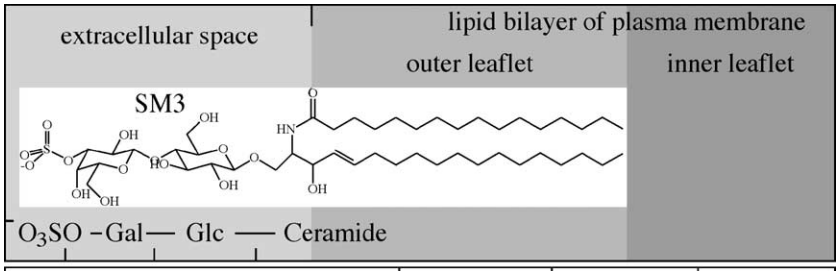
1. Introduction

Chemokines are small chemotactic cytokines that induce the adhesion, migration and activation of diverse cell types. The chemokine subfamilies are grouped according to the orientation of specific critical cysteine residues and are designated as CXC (e.g., interleukin-8/CXCL8), CC (e.g., RANTES/CCL5), C (lymphotactin/CL1), or CX_3CL (fractalkine/CX_3CL-1) chemokines. Chemokines are ligands for a superfamily of G-protein-coupled seven-transmembrane receptors which mediate their biological activities. These receptors are found within the detergent-insoluble fraction at 4 °C thought to be enriched with molecules from lipid rafts [1]. Lipid rafts are discussed to act as signaling platforms on the cell surface.

Chemokines possess two major noncovalent binding epitopes: a high affinity site responsible for specific ligand/receptor interactions and a lower affinity site, also called the heparin- or glycosaminoglycan-binding domain, responsible for the presentation of chemokines on the surface of endothelial cells and extracellular matrix. Some studies have shown that glycosaminoglycan (GAG) binding is not essential for the in vitro activity of chemokines, although it can assist the recruitment of the chemokine to the cell surface [2]. However, formal evidence has recently demonstrated that the interaction is relevant for chemokine activity in vivo [3]. Binding of chemokines to heparan sulfate proteoglycans on the surface of endothelial cells also seems to be crucial for the recruitment of leukocytes to inflammatory sites [3–5]. Glycosaminoglycans (GAGs) are long, unbranched polysaccharide chains composed of repeating disaccharide units. In most cases the amino-sugar is sulfated, and with the exception of hyaluronic acid and heparin, all GAGs are covalently bound to proteins in the form of proteoglycans. The amino acid residues responsible for GAG-binding capacity have been characterized for certain chemokines [3,6–13] and reveal different patterns that suggest specificity in the GAG interaction.

Sulfated saccharide structures are also found on cell-surface glycosphingolipids. These sulfatides (the general name for all sulfated glycosphingolipids, see Fig. 1 for structures) have been shown to bind diverse proteins including hepatocyte growth factor [14], thrombospondin [15], laminin [16], brevican [17], selectins [18,19], as well as properdin and factor H, regulators of the alternative pathway of complement activation [20]. The specific expression of sulfatides on granulocytes, erythrocytes, platelets and certain tumor cells has also been demonstrated [21–27]. According to Mamelak et al. [28],



Structure	R_2	R_1	GSL
Glcβ1-1Cer 3 R		OH OSO_3^- NeuNAc	GalCer SM4s GM4
Galβ1-4Glcβ1-1Cer 3 R		OH OSO_3^- NeuNAc	LacCer SM3 GM3
GalNAcβ1-4Galβ1-4Glcβ1-1Cer 3 3 R_2 R_1	OH OH OH OSO_3^-	OH OSO_3^- NeuNAc OSO_3^-	Gg_3Cer SM2a GM2 SB2
Galβ1-3GalNAcβ1-4Galβ1-4Glcβ1-1Cer 3 3 R_2 R_1	OH OH OH OSO_3^- NeuNAc	OH OSO_3^- NeuNAc OSO_3^- NeuNAc	Gg_4Cer SM1a GM1a SB1a GD1a

Fig. 1. Glycosphingolipid structures. Glycosphingolipids (GSLs) are components of the plasma membrane. Neutral GSLs consist of a lipophilic ceramide-anchor and a polar carbohydrate-head group. Acidic GSLs contain either sulfate(s) (sulfatides) or sialic acid(s) (gangliosides) in addition, or both.