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Carbohydrate specificities of the murine DC-SIGN homologue mSIGNR1

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Abstract

C-type lectins are important receptors expressed by antigen presenting cells that are involved in cellular communications as well as in pathogen uptake. An important C-type lectin family is represented by DC-SIGN and its homologues in human and mouse. Here we have investigated the carbohydrate specificity of cellular mSIGNR1 and compared it with DC-SIGN and L-SIGN. mSIGNR1 has a similar specificity as human DC-SIGN for high mannose-containing ligands present on both cellular and pathogen ligands. However, the DC-SIGN molecules differ in their recognition of Lewis antigens; mSIGNR1 interacts not only with $Le^{x/y}$ and $Le^{a/b}$ antigens similar to DC-SIGN, but also with sialylated Le^{x}, a ligand for selectins. The differential recognition of Lewis antigens suggests differences between mSIGNR1 and DC-SIGN in the recognition of cellular ligands and pathogens that express Lewis epitopes.

Keywords: C-type lectins; Lewis antigens; Macrophages; Pathogen

Introduction

Antigen presenting cells such as macrophages and dendritic cells (DCs) express C-type lectins that are involved in pathogen capture, processing and antigen presentation to induce immune responses against these pathogens (Drickamer, 1999). However, it is becoming clear that several lethal pathogens have evolved to subvert the function of some C-type lectins to escape immune surveillance (van Kooyk and Geijtenbeek, 2003). An important C-type lectin family is represented by DC-SIGN and its homologues in both human and mouse.

The "SIGN" family of CLR consists of DC-SIGN and L-SIGN in human and the five murine homologues mDC-SIGN, mSIGNR1 to mSIGNR4. Human DC-SIGN is expressed by DCs (Geijtenbeek et al., 2000a; van Kooyk and Geijtenbeek, 2003), whereas L-SIGN is expressed on specialized liver and lymph node endothelial cells that possess antigen presentation capacity (Bashirova et al., 2001; Engering et al., 2004; Soilleux et al., 2000). In mouse, only mDC-SIGN is expressed by DCs (O'Keeffe et al., 2002), whereas the SIGNR1-4 molecules display a differential tissue distribution (Kang

Abbreviations: Le^{x}, Lewis x-Galβ1, 4(Fucα1, 3)GlcNAc; Le^{a}, Lewis a -Galβ1, 3(Fucα1, 4)GlcNAc; Le^{y}, Lewis y-Fucα1, 2Galβ1, 4(Fucα1, 3)GlcNAc; Le^{b}, Lewis b-Fucα1, 2Gal$\alpha\beta$1, 3(Fucα1, 4)GlcNAc; sialyl Le^{x}, NeuAcα1, 2Galβ1, 4(Fucα1, 3)GlcNAc; sialyl Le^{a}, NeuAc2, 3Galβ1, 3(Fucα1, 4)GlcNAc; sulfo Le^{x}, (SO_4)3Galβ1, 4(Fucα1, 3)GlcNAc; sulfo Le^{a}, (SO_4)3Galβ1, 3(Fucα1, 4)GlcNAc

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et al., 2003). The most extensively studied of these murine homologues is mSIGNR1, which is expressed on marginal zone macrophages (MZM) (Geijtenbeek et al., 2002; Kang et al., 2003) and peritoneal macrophages (Taylor et al., 2004).

DC-SIGN has been demonstrated to interact with various viruses such as HIV-1 (Geijtenbeek et al., 2000b), CMV (Halary et al., 2002), and Dengue virus (Tassaneetrithep et al., 2003) and microbes including *Mycobacterium tuberculosis* (Geijtenbeek et al., 2003; Tailleux et al., 2003) and Leishmania parasites (Colmenares et al., 2002). DC-SIGN has been shown to mediate internalization of ligands for antigen presentation (Engering et al., 2002). Strikingly, HIV-1 exploits this feature of DC-SIGN to gain access to $CD4^+$ T cells that are the primary target cells of infection (Geijtenbeek et al., 2000b). Similarly, other viruses have been shown to use DC-SIGN to enhance viral transmission to target cells (van Kooyk and Geijtenbeek, 2003), whereas *M. tuberculosis* and *Helicobacter pylori* target DC-SIGN to modulate DC function (Bergman et al., 2004; Geijtenbeek et al., 2003). Thus, several pathogens target DC-SIGN to escape immune surveillance. Identification of the carbohydrate specificity of DC-SIGN for high-mannose glycans and non-sialylated fucose-containing Lewis antigens, such as Le^a, Le^b, Le^x, and Le^y (Appelmelk et al., 2003; Mitchell et al., 2001; van Die et al., 2003), has led to the identification of both pathogens and self-antigens that are recognized by this receptor. The high degree of homology between the carbohydrate recognition domains of DC-SIGN and L-SIGN is reflected by their similar ligand specificity. L-SIGN, similar to DC-SIGN, recognizes high-mannose glycans, and binds high-mannose-containing ligands such as HIV-1 and HCV (Bashirova et al., 2001; Lozach et al., 2003). However, L-SIGN does not bind to the fucose-containing Lewis antigens in contrast to DC-SIGN (van Liempt et al., 2004).

The carbohydrate specificity of mSIGNR1 demonstrated specificity for mannose- and fucose-terminating oligosaccharides (Galustian et al., 2004; Koppel et al., 2004). Using a soluble recombinant chimera of SIGNR1 fused to an Fc domain, Galustian et al. (2004) demonstrated that SIGNR1 binds to the Lewis antigens $Le^{a/b}$ and $Le^{x/y}$, similar to DC-SIGN. Although the use of Fc chimeras provides information about the binding characteristics of C-type lectins, studies into their binding specificity should also be performed with the C-type lectins expressed on cells due to their multimerization and possible clustering, which is crucial to the carbohydrate binding specificity. Indeed, mSIGNR1-Fc did not interact with the polysaccharide dextran, whereas cellular-expressed mSIGNR1 interacts with dextran as demonstrated by several groups (Galustian et al., 2004; Geijtenbeek et al., 2002; Kang et al., 2003).

Here, we have investigated and compared the carbohydrate specificity of cellular mSIGNR1 with its human homologues DC-SIGN and L-SIGN. DC-SIGN, L-SIGN and mSIGNR1 have a similar specificity for mannose-containing carbohydrates present on both cellular and pathogen ligands. However, the three receptors differ in their recognition of fucose containing Lewis antigens. mSIGNR1 interacts not only with $Le^{x/y}$ and $Le^{a/b}$ antigens similar to DC-SIGN, but also with sialylated Le^x. The differential recognition of Lewis antigens suggests differences between mSIGNR1 and DC-SIGN in the interaction with Lewis antigen-containing cellular and pathogen ligands.

Materials and methods

Antibodies, reagents and cells

The following antibodies were used: ER-TR9 (anti-SIGNR1 (Geijtenbeek et al., 2002)). AZN-D1 (anti-DC-SIGN), AZN-D2 (anti-L-SIGN/DC-SIGN (Geijtenbeek et al., 2000a)). Raji-1 (previously referred to as THP-1 cells (Wu et al., 2004)) transfectants expressing wild-type DC-SIGN, L-SIGN or mSIGNR1 were generated by transfection with 10 μg pRc/CMV-SIGN plasmid by electroporation as previously described (Bashirova et al., 2001; Geijtenbeek et al., 2002; Geijtenbeek et al., 2000b). ManLAM was obtained from J. T. Belisle, the TB Vaccine Testing and Research Materials Contract NIAID N01-AI-40091, Colorado State University). Purified LPS from *H. pylori* was obtained from M. Monteiro (National Research Council, Ottowa, Canada).

Fluorescent bead adhesion assay

Carboxylate-modified TransFluorSpheres (488/645 nm, 1.0 μm; Molecular Probes, Eugene, OR) were coated with HIV-1 gp120 and ICAM-2 as described (Geijtenbeek et al., 2000b). Mannan was covalently coupled to the beads according to the manufacturer's protocol. Streptavidin was covalently coupled to the beads as described and streptavidin-coated beads were incubated with biotinylated PAA-linked glycoconjugates (50 pmol; Synthesome, Munich, Germany). The fluorescent bead adhesion assay was performed as described (Geijtenbeek et al., 1999). Cells were preincubated for 30 min at 37 °C with inhibitors (mannan, 1 mg/ml or EGTA, 10 mM). Next, ligand-coated fluorescent beads (20 beads/cell) were added to the cells for 45 min at 37 °C, washed and analyzed by flow cytometry (FACScan, Becton Dickinson, Oxnard, CA), by measuring the percentage of cells that had bound fluorescent beads.