Comparative analysis reveals selective recognition of glycans by the dendritic cell receptors DC-SIGN and Langerin

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DC-SIGN (dendritic cell-specific ICAM-3 grabbing non-integrin) and Langerin are homologous C-type lectins expressed as cell-surface receptors on different populations of dendritic cells (DCs). DC-SIGN interacts with glycan structures on HIV-1, facilitating virus survival, transmission and infection, whereas Langerin, which is characteristic of Langerhans cells (LCs), promotes HIV-1 uptake and degradation. Here we describe a comprehensive comparison of the glycan specificities of both proteins by probing a synthetic carbohydrate microarray comprising 275 sugar compounds using the bacterially produced sialyl-Lex derivative with 6-sulfatated derivatives of Le a and Le x. In contrast, Langerin appeared to recognize a different spectrum of glycan structures on HIV-1, facilitating virus survival, transmission and infection, whereas Langerin, which is characteristic of Langerhans cells (LCs), promotes HIV-1 uptake and degradation. Here we describe a comprehensive comparison of the glycan specificities of both proteins by probing a synthetic carbohydrate microarray comprising 275 sugar compounds using the bacterially produced sialyl-Lex derivative with 6-sulfatated derivatives of Le a and Le x. In contrast, Langerin appeared to recognize a different spectrum of glycan structures on HIV-1, facilitating virus survival, transmission and infection, whereas Langerin, which is characteristic of Langerhans cells (LCs), promotes HIV-1 uptake and degradation.

Keywords: Calcium-binding/Carbohydrate recognition/CRD/Glycan-binding/Lectin

Introduction

DC-SIGN (dendritic cell-specific ICAM-3 grabbing non-integrin; CD209) and Langerin (CD207) belong to the (calcium-dependent) C-type lectin superfamily whose members share the C-type lectin-like domain (CTLD), also referred to as carbohydrate-recognition domain (CRD), as a common structural motif (Curtis et al., 1992; Drickamer, 1999; Valladeau et al., 2000). C-type lectins are found both as secreted and as transmembrane proteins. DC-SIGN and Langerin are cell-surface receptors with an N-terminal intracellular domain and a single extracellular CRD at the C-terminus, thus classified as group II C-type lectins according to their domain architecture (Zelensky and Gready, 2005). In contrast to DC-SIGN, which forms a tetramer (Mitchell et al., 2001; Feinberg et al., 2005), Langerin has been shown to be a trimer (Stambach and Taylor, 2003; Thepaut et al., 2009; Feinberg et al., 2010). Thus, the DC-SIGN receptor comprises four CRDs, whereas Langerin exhibits three CRDs, which ought to enhance the intrinsically weak interactions with oligomeric or membrane-bound glycan target structures via avidity effects.

The CTLDs share a highly conserved three-dimensional structure (Zelensky and Gready, 2005). Since the N- and C-termini form a two-stranded β-sheet (β1 and β5) the entire fold can be considered as a large loop with two α-helices (α1 and α2) and a three-stranded β-sheet (β2 to β4) as predominant structural elements. The core of the domain is stabilized by conserved hydrophobic and polar residues, including the characteristic ‘WIGL’ motif, while up to two disulfide bridges at the base are important for structural integrity. The CRDs of both DC-SIGN and Langerin possess an additional β-strand designated β0 at the N-terminal side of strand β1 and therefore classify them as long-form CTLDs (Feinberg et al., 2001; Chatwell et al., 2008). Compared with most other CTLD structures the helix α2 of DC-SIGN is unusually long and packs more tightly against the rest of the CRD (Feinberg et al., 2001). In contrast, helix α2 of Langerin is shorter by one turn. In addition, the loop-connecting strands β2 and B3, the so-called LLR (long-loop region), comprise an additional β-sheet formed by prolongation of β2 and a new strand B2’ as well as a new 310-helix (α4) in Langerin (Chatwell et al., 2008). Despite the overall homology these structural differences should lead to distinct carbohydrate-binding patterns for DC-SIGN and Langerin, which was, however, not compared in detail till now.

Altogether four Ca2+-binding sites (Ca-1 to Ca-4) have been found within the C-type lectin family, with site
occupancies depending on the specific lectin (Drickamer, 1999; Zelensky and Gready, 2005). Glycan-binding usually occurs at the Ca-2 site via direct co-ordination of a monosaccharide. Amino acid residues around the metal co-ordination site make additional contacts to the monosaccharide and thereby determine the sugar specificity of each lectin. Typically, the ‘QPD’ motif located in the LLR is characteristic of Gal specificity, whereas C-type lectins such as DC-SIGN and Langerin, which preferentially bind Man and Fuc, carry the ‘EPN’ motif in the LLR (Drickamer, 1992). The DC-SIGN CRD crystal structure reveals three bound Ca\(^{2+}\) ions at the sites Ca-1, Ca-2 and Ca-3, with glycan complexation at the site Ca-2 (Feinberg et al., 2001). In contrast, only Ca-2 is occupied with Ca\(^{2+}\) in the Langerin CRD crystal structure and involved in glycan-binding. A second glycan bound at the site Ca-3 of Langerin in a calcium-independent manner was initially postulated (Chatwell et al., 2008); however, after careful reanalysis of the X-ray data the corresponding electron density was more reliably interpreted as a Trp residue from a neighboring molecule in the crystallographic unit cell (protein data bank (PDB): entries: 3P7G, 3P7F, 3P7H).

DC-SIGN and Langerin both occur on the cell surface of several dendritic cell (DC) subtypes. Langerin expression is mostly confined to a DC subset called Langerhans cells (LC) that populate the skin epidermis and all stratified epithelia. However, dermal DCs and resident DCs in the skin-draining lymph nodes were also shown to express Langerin (Merad et al., 2008). Langerin was demonstrated to be involved in the formation of the so-called Birbeck granules. These LC-specific cytoplasmic organelles were suggested to be part of a non-classical pathway for antigen-processing (Valladeau et al., 2000). Strikingly, Langerin gene transfection of murine fibroblasts and of a human melanoma cell line led to the formation of Birbeck granule-like structures (Valladeau et al., 2000; McDermott et al., 2004). In contrast, DC-SIGN is mainly expressed on subepithelial DCs and also on specialized macrophages in the placenta and lung as well as on peculiar DC populations in the peripheral blood as well as certain tissues (Soilleux et al., 2002).

DC-SIGN and Langerin both act as adhesion receptors in cell–cell interaction. DC-SIGN mediates rolling of DCs over the endothelial cell layer by binding to the glycoprotein ICAM-2, thus playing a role in DC migration (Geijtenbeek et al., 2000a). In addition, DC-SIGN is known to stabilize the interaction between DCs and T-cells by binding to the glycosylated receptor ICAM-3, thereby enabling T-cell engagement (Geijtenbeek et al., 2000c). Recent studies have also shown binding of DC-SIGN to the neutrophil cell surface glycoproteins CEACAM-1 and Mac-1 (van Gisbergen et al., 2005a,b), which might result in neutrophil-induced maturation of DCs. Langerin, which is less well studied, was found to immunoprecipitate with procollagen I and III as well as fibronectin (Tada et al., 2006), suggesting importance for LC localization, trafficking and maturation via binding to the extracellular matrix.

Like other C-type lectins and TLRs (Toll-like receptors) DC-SIGN and Langerin are involved in antigen-binding and uptake, resulting in migration of the DCs to the lymph nodes, followed by presentation of the antigen in the MHC (major histocompatibility complex) class I or II context. This maturation enables DCs to activate T-cells, thus initiating the adaptive immune response. Potential tolerogenic effects facilitated by DC-SIGN and Langerin are still under discussion (Zhou et al., 2006; Merad et al., 2008; van der Vlist and Geijtenbeek, 2010). Interestingly, downstream signaling upon DC-SIGN interaction seems to be dependent on the composition of the carbohydrate moiety bound, as capture of mannose- or fucose-expressing pathogens led to enhancement or suppression, respectively, of proinflammatory responses in DCs (Gringhuis et al., 2009).

Some pathogens, and even tumor cells, abuse C-type lectin receptors for evasion from the immune response. In particular, DC-SIGN was shown to bind to carbohydrate structures on the surface of viruses such as HIV-1 (Human immunodeficiency virus type 1) (Geijtenbeek et al., 2000b) or HCV (Hepatitis C virus) (Pohlmann et al., 2003) and also non-viral pathogens, such as Mycobacterium tuberculosis (Maeda et al., 2003), thus enabling their entry and infection. However, distinct effects seem to occur on HIV-1 binding to DC-SIGN and Langerin, respectively. Whereas the DC-SIGN/HIV-1 interaction plays an important role for HIV-1 survival, transmission and infection, its binding to Langerin was shown to result in internalization and viral degradation, hence conferring a protective effect (de Witte et al., 2007).

Consequently, the differential glycan-binding activities of DC-SIGN and Langerin deserve further attention. In the case of corresponding previous studies (see section Discussion) different recombinant versions of DC-SIGN and Langerin—including the CRD alone (Mitchell et al., 2001; Stambach and Taylor, 2003), the entire extracellular domain (ECD) of DC-SIGN (Mitchell et al., 2001; Guo et al., 2004), a truncated ECD of Langerin comprising the last seven heptad repeats of the neck structure (Feinberg et al., 2010) and immunoglobulin (Ig) Fc fusions of the Langerin as well as DC-SIGN CRDs (Galustian et al., 2004; Hsu et al., 2009; Tateno et al., 2010)—were used to analyze glycan specificity. Considering the mutually differing oligomeric states of the DC-SIGN and Langerin extracellular regions and also the dimeric quaternary structure of the recombinant Fc fusion proteins, both the spatial organization of the CRDs and any resulting avidity effects vary drastically in these constructs.

Here, we report for the first time a comprehensive side-by-side study with the recombinant monomeric CRDs of DC-SIGN and Langerin, prepared under essentially identical conditions, to analyze and compare their intrinsic glycan specificities.

Results

**Bacterial production of the CRDs of DC-SIGN and Langerin**

Both CRDs, which are naturally located at the C-termini of the full-length transmembrane receptors and—as typical extracellular domains—carry disulphide bonds, were produced in the periplasm of *Escherichia coli*. To this end, the sequences comprising residues 254–328 of mature DC-SIGN and residues 193–328 of mature Langerin were fused to the bacterial OmpA signal peptide at the N-terminus and to the Strep-tag II (Schmidt and Skerra, 2007) at the C-terminus and cloned on the vector pASK75 (Skerra, 1994). To allow efficient cleavage of the OmpA signal peptide and secretion into the periplasm, the N-terminal residue His254 of the
DC-SIGN CRD was substituted by Gln. Compared with the original published sequence, the cloned Langerin CRD carried the mutation Ala278Val (Chatwell et al., 2008), which occurs with an average allele frequency of 0.496. This mutation is known to have no effect on the stability or sugar-binding properties (Ward et al., 2006).

In order to improve the yield of soluble protein secretion, four periplasmic host chaperons and disulfide isomerases were simultaneously overexpressed using the helper plasmid pTUM4 (Schlapschy et al., 2006). When analyzing overexpression of the recombinant proteins by SDS-PAGE, in the case of Langerin a prominent band at about 18 kDa was seen in the whole cell extract, which most likely corresponded to the CRD with uncleaved OmpA signal peptide. The recombinant CRD purified from the periplasm, which was previously shown to represent the mature protein (Chatwell et al., 2008), appeared clearly smaller (Fig. 1), thus indicating oversaturation of the periplasmic export machinery. Nevertheless, a final yield of 6 mg isolated protein per liter bacterial culture for Langerin, compared with 1.1 mg for DC-SIGN, was satisfactory.

Both CRDs were purified from the periplasmic cell extract via StrepTactin affinity chromatography (Schmidt and Skerra, 2007). Subsequent gel filtration led to a homogenous monomeric protein preparation in each case (Figs. 1 and 2). SDS-PAGE revealed single bands at the expected molecular sizes of 15.9 kDa for DC-SIGN and 16.7 kDa for Langerin (Fig. 1). The increased electrophoretic mobility in the absence of a reducing agent indicated proper formation of the three intramolecular disulfide bonds of the DC-SIGN CRD and of the two disulfide bridges in the Langerin CRD, respectively.

Interestingly, during gel filtration both the recombinant DC-SIGN CRD and the Langerin CRD showed much enhanced retention on the Superdex 75 column when a calcium-containing running buffer was used (Fig. 2). In contrast, elution at the expected molecular size was observed in the presence of EDTA instead of Ca$^{2+}$. The Superdex material is a composite of agarose and dextran, polysaccharides comprising Gal and Glc units, respectively. Thus, the retention effect clearly demonstrates calcium-dependent binding of the DC-SIGN and Langerin CRDs to such oligosaccharide structures.

**Thermodynamic analysis of mono- and disaccharide-binding**

To test whether the monomeric CRDs of DC-SIGN and Langerin show similar binding behavior toward typical mono- or disaccharides, the interaction of Man and Man(α1-2)man was analyzed by isothermal titration calorimetry (ITC) (Fig. 3 and Table 1). As for other C-type lectins (Weis and Drickamer, 1996; Poget et al., 1999) only weak binding, albeit with characteristic saturation curves, was observed for these mono- and disaccharides. In all cases saccharide-binding was entropically disfavored, indicating an enthalpy-driven interaction, which is in agreement with the role of calcium complexation during sugar-binding. The measured dissociation constants ranged between 0.9 and 6.1 mM. The interaction with the disaccharide Man(α1-2)man was stronger than the one with Man by factors of 3.9 for DC-SIGN and 1.5 for Langerin.

This observation is consistent with additional contacts of the reducing sugar of Man(α1-2)man especially with DC-SIGN as apparent from the crystal structures of both
complexes (Feinberg et al., 2007; Chatwell et al., 2008). Although crystallographic analysis of Langerin initially indicated a secondary calcium-independent sugar-binding site (Chatwell et al., 2008), no interaction was detected on titration of Langerin with Man in an EDTA-containing buffer in the analyzed concentration range from 0.04 to 10 mM (Fig. 3). Nevertheless, this does not preclude potential supportive contacts at this site that may be involved in interactions with branched oligosaccharides which primarily bind to the neighboring calcium-dependent sugar-binding site.

Sugar recognition profile on a glycan array

For comprehensive analysis of the glycan specificities of the monomeric DC-SIGN and Langerin CRDs a glycan array provided by the Consortium for Functional Glycomics (Blixt et al., 2004) was probed with the fluorescently labeled recombinant proteins. The dyes were covalently attached to both recombinant CRDs in a similar manner via free amino groups (either Lys side chains or the polypeptide N-terminus) with a molar ratio of 0.97:1 for DC-SIGN labeled with fluorescein and of 0.49:1 for Langerin labeled with PromoFluor633 (see section Materials and methods). The glycan microarrays had been prepared by printing carbohydrate compounds onto N-hydroxysuccinimide-activated glass microscope slides to form covalent amide bonds via amino linkers (Blixt et al., 2004). In this way, binding of the DC-SIGN and Langerin CRDs to altogether 28 monosaccharides, 62 disaccharides and 185 larger oligosaccharides, including sulfatated compounds and sialic acid structures, was tested in parallel. Screening of the glycan array was conducted both in a calcium-containing and in a calcium-depleted (i.e. EDTA-containing) buffer. However, binding signals above background level were only detected in the presence of Ca²⁺ ions (Fig. 4).

As a result, screening of the glycan array revealed similarities as well as differences in glycan specificity between DC-SIGN and Langerin (Figs 4 and 5). Overall, DC-SIGN gave rise to a broader binding pattern while Langerin clearly showed fewer and more distinct signals. Both DC-SIGN and Langerin recognized Man-containing glycan structures, including several high-mannose-type and complex glycans (Fig. 5; sugar nos. 1–15). However, unlike DC-SIGN Langerin bound to compounds exhibiting terminal Man residues with a preference for small linear structures (Fig. 5; sugar nos. 4,5). The binding signal for the most highly branched high-mannose-type compound (Fig. 5; sugar no. 13) was negligible for Langerin. In contrast, DC-SIGN strongly bound to several highly branched high-mannose-type structures (Fig. 5; sugar nos. 10–13), whereas linear oligosaccharides were bound more weakly (Fig. 5; sugar nos. 4,5) and the monosaccharide Man was not recognized at all in the relevant affinity range (Fig. 5; sugar no. 1).

![Fig. 3 Interaction of Langerin with Man in the presence of either Ca²⁺ or EDTA, measured by isothermal titration calorimetry (ITC). A 72.1 μM solution of the recombinant Langerin CRD was titrated with 60 mM Man either in 5 mM HEPES/NaOH pH 7.5, 115 mM NaCl, 2 mM CaCl₂ (left panel) or in 5 mM HEPES/NaOH pH 7.5, 115 mM NaCl, 1 mM EDTA (right panel). Temperature changes in the cell on injection of the ligand (upper panel) as well as the integrated areas of the corresponding peaks after subtraction of the dilution heat (lower panel) were plotted against the molar ratio of ligand to protein (concentration range: 0.04–10 mM).](https://academic.oup.com/peds/article-abstract/24/9/659/1554629/662)
Apart from Man-containing compounds DC-SIGN and Langerin appeared to bind terminal Fuc residues. Langerin recognized many structures containing Fuc linked through an α2-glycosidic bond to Gal (Fig. 5; sugar nos. 48–68), albeit in most cases the signals were weak. These compounds included the blood group antigens H, A and B (Fig. 5; sugar nos. 55, 64 and 62) as well as Leb, Lea and Leα (Fig. 5; sugar nos. 48–50, 53 and 54) and some of their derivatives. DC-SIGN bound to Fuc linked either to GlcNAc or Glc through an α3- or α4-glycosidic bond including the Lewis antigens Leb, Leα, Leb and Leα as well as their derivatives (Fig. 5; sugar nos. 19–52). Sulfatated derivatives of Leb and Lea (Fig. 5; sugar nos. 28, 42–46) as well as sialyl-Leα (Fig. 5; sugar no. 47) were also recognized by DC-SIGN. However, modification of Lea by sialic acid (see Supplementary material online, Table S1; array pos. 228–233, 259) abolished binding. Interestingly, DC-SIGN recognized the blood group antigens H, A and B, too (Fig. 5; sugar nos. 55, 64 and 62), even though these oligosaccharides do not exhibit Fuc linked to GlcNAc or Glc.

Langerin also showed binding signals for almost all compounds with terminal GlcNAc residues (Fig. 5; sugar nos. 15, 69–92) and for oligosaccharides containing 6SGal (Fig. 5; sugar nos. 93–101). This included sialyl-6SGal Leα (Fig. 5; sugar no. 101). Notably, chemically related sugars such as 3′- and 4′-sulfatated Gal or GalNAc, 6′-sulfatated Glc as well as GlcNAc, 6′-phosphorylated Man and double-sulfatated Gal were not or just poorly recognized by Langerin (see Supplementary material online, Table S1; array pos. 26–41, 139, 140, 216, 220, 227, 228, 244, 265–268, 280, 281).

Discussion

Using a two-dimensional glycan array provided by the Consortium for Functional Glycomics (http://www.functionalglycomics.org), in conjunction with our advanced E. coli expression system, we were able to perform a comparative side-by-side analysis of the isolated DC-SIGN and Langerin CRDs. Generally, the development of glycan array platforms has provided a deeper insight into carbohydrate−receptor interactions. In our study, a powerful glycan array prepared by robotic microarray printing technology to couple amine-functionalized synthetic carbohydrates to an amino-reactive glass slide (Blixt et al., 2004) was employed, offering a broad coverage of almost 300 different sugars. These synthetic glycan microarrays offer advantages over alternative systems, which often possess less diversity and/or require higher amounts of sample protein. For example, arrays of whole glycoproteins or synthetic lipid-linked oligosaccharide probes blotted onto nitrocellulose membranes or adsorbed to the wells of microtiter plates were used in other...
studies (Fukui et al., 2002; Galustian et al., 2004). Also, thiol-functionalized phosphatidylinositol mannosides printed on maleimide-activated glass slides (Boonyarattanakalin et al., 2008) have been described.

Our comparative analysis, which made use of the recombinant monomeric CRDs and thus excluded any effects caused by multimerization, complements several previous studies on the interaction of DC-SIGN and Langerin with carbohydrates (Mitchell et al., 2001; Stambach and Taylor, 2003; Guo et al., 2004; Takahara et al., 2004; Feinberg et al., 2010; Tateno et al., 2010). Generally, our data illustrate that DC-SIGN preferentially binds oligosaccharides containing Man or terminal Fuc (especially if linked to Gal) linked to either GlcNAc or Glc whereas Langerin predominantly recognizes terminal Man residues and, in particular, structures containing terminal Fuc (especially if linked to Gal) or GlcNAc and 6SGal. Indeed, however, some differences in the behavior of our monomeric Langerin CRD, for example, and of a previously applied Fe fusion protein probed with the same glycan array platform (Hsu et al., 2009) became apparent. While the Fe fusion showed strong binding signals for almost all high-mannose-type sugars, the monomeric CRD showed preference for the short, unbranched oligosaccharides, indicating higher selectivity in our study. Furthermore, among others, sugars with terminal β1–6- or α1–3-linked GlcNAc gave rise to signals with our Langerin CRD but not with the Fe fusion. Conversely, the structurally related sugars at array pos. 203, 204, 206 and 210, for example, were bound by the Langerin Fe fusion whereas no significant signal was seen for the isolated CRD. These findings underline the importance of our present comparative analysis between the Langerin and DC-SIGN CRDs that were prepared in the same (monomeric) oligomerization state.

A recent comparison between the crystal structures of DC-SIGN with bound GlcNAc3Man1 and Langerin with bound maltose revealed that in Langerin the sugar bound at the Ca-2 site is rotated by 180° with respect to the axis through the calcium ion and the C3–C4 bond (Chatwell et al., 2008). Hence, although DC-SIGN is known in principle to accept both orientations (Feinberg et al., 2007), the preferred binding mode differs between DC-SIGN and Langerin. The preference of Langerin for this orientation is caused by Lys299, which replaces Val372 in DC-SIGN (Chatwell et al., 2008). In this context it is noteworthy that Langerin appears to recognize terminal Man residues of predominantly linear and small glycans (cf. Fig. 5; sugar nos. 5, 6)—i.e. from the non-reducing end—while DC-SIGN

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**Fig. 5** Schematic overview of glycan structures recognized by the CRDs of DC-SIGN (black bars) or Langerin (grey bars). The sugar numbers assigned for this illustration are listed on the left while the array positions are given in brackets. Each glycan structure is depicted in the symbol nomenclature adopted by the Consortium for Functional Glycomics (http://www.functionalglycomics.org). The depicted glycan structures gave rise to average fluorescence signals of at least 5000 upon incubation with the fluorescein-labeled DC-SIGN and/or PromoFluor 633-labeled Langerin CRDs (cf. Fig. 4).
interacts with the α1–3 linked internal Man residue of the outer branch of high-mannose-type carbohydrates (Feinberg et al., 2001).

DC-SIGN and Langerin are known to bind terminal Fuc residues. However, our data suggest that DC-SIGN seeks glycan structures containing Fuc linked to GlcNAc or Glc whereas Langerin recognizes Fuc in a different structural context, especially when linked to Gal. In this regard, the preferred interaction of DC-SIGN with the Lewis antigens Le\(^a\), Le\(^b\), Le\(^x\) and Le\(^y\), which contain Fuc linked to GlcNAc, and of Langerin only with Le\(^b\) and Le\(^y\), which are difucosylated and additionally carry Fuc linked to Gal, can be explained. Hence, the orientation of Le\(^b\) and Le\(^y\) in the binding site of Langerin is most likely different from that of Lewis antigens in the binding site of DC-SIGN.

Irrespective of the general orientation of the oligosaccharide structure in the binding site, the complexation of the monosaccharide Fuc itself might be different. In contrast to DC-SIGN, which complexes Fuc mediated by the Ca-2 binding site via the hydroxyl groups of C3 and C4 (Guo et al., 2004), the rat liver mannose binding protein C (MBP-C) is known to interact with Fuc through the hydroxyl groups of C2 and C3 (Ng et al., 1996). The crystal structure of the Le\(^x\)-containing saccharide LNFP III (Guo et al., 2004) bound to DC-SIGN revealed that the GlcNAc that is linked to Fuc points away from the protein while the Gal residue that is linked to GlcNAc is complexed at a secondary binding site. Thus, it is possible that Fuc is not only recognized by DC-SIGN in context of GlcNAc but also if linked to other sugars. In this regard the strong binding signal of DC-SIGN for the blood group antigens H, A and B, which do not display Fuc linked to GlcNAc or Glc, can be understood.

Our data clearly demonstrate specificity of Langerin for GlcNAc-terminated carbohydrates. Strikingly, irrespective of the glycosidic linkage to the sugar residue preceding in the oligosaccharide chain, of 29 GlcNAc-terminated structures present on the glycan array a high fraction of 25 were recognized by Langerin, which is remarkable in the light of the overall much smaller number of binding signals compared with DC-SIGN. The crystal structures of MBP-C in complex with Man or GlcNAc show that both sugars are bound via the hydroxyl groups of C3 and C4. In addition, GlcNAc forms a van der Waals contact between the acetamidomethyl carbon and a Pro residue (Ng et al., 1996). A superposition of GlcNAc on the coordinated sugar in the Ca-2 site of the crystal structure of Langerin with bound maltose (PDB code: 3P7H) revealed that the acetamido group of bound GlcNAc indeed can be accommodated in a hydrophobic pocket formed by Pro310, Lys313 and Phe315.
DC-SIGN and Langerin are known to recognize Gal with very weak affinity (Stambach and Taylor, 2003; Guo et al., 2004). Nevertheless, the binding activity of Fc fusions of the murine and human Langerin CRDs and also of a truncated version of the extracellular region of human Langerin toward structures containing 6SGal have been demonstrated (Galustian et al., 2004; Feinberg et al., 2010; Tateno et al., 2010). Similarly, our data indicate that human Langerin recognizes 6SGal. Gal is bound by C-type lectins via the so-called ‘QPD’ motif through the equatorial and axial hydroxyl groups of C3 and C4, respectively (Kolatkar and Weis, 1996; Poget et al., 1999). However, Langerin contains the ‘EPN’ motif that is associated with specificity for Man with the hydroxyl groups of C3 and C4 both in an equatorial configuration. Interestingly, in the case of the so-called ‘QPD’ motif through the equatorial and axial hydroxyl groups of C3 and C4, respectively (Kolatkar and Weis, 1996; Poget et al., 1999). However, Langerin contains the ‘EPN’ motif that is associated with specificity for Man with the hydroxyl groups of C3 and C4 both in an equatorial configuration. Interestingly, in the case of the 6SGal-containing compounds 100 and 101 (cf. Fig. 5) the OH-groups at C1’, C3’ and C6’ are occupied by substituents, leaving only the OH-groups at positions 2’ and 4’ for direct complexation of the calcium in the binding site. Recently, it was shown by mutational analysis that two positively charged residues (Lys299 and Lys313) that are located in close proximity to the Ca-2 site in Langerin and are not present in DC-SIGN are involved in binding 6’S-lactosamine, which contains 6SGal (Tateno et al., 2010).

When interpreting these data obtained for the monomeric CRDs it should be kept in mind that the spatial organization of the carbohydrate-binding sites of DC-SIGN and Langerin in the oligomeric receptor proteins obviously differs, since DC-SIGN is a tetramer and Langerin is a trimer. This clustering is believed to be important for glycan recognition and specificity in vivo and has to be investigated in the future. First analyses have shown that the interaction between carbohydrates and DC-SIGN coincide with a reorientation of the CRDs, thus indicating flexibility in their mutual arrangement (Menon et al., 2009; Satomaa et al., 2009).

The differences in glycen specificity of DC-SIGN and Langerin that have become apparent from the present analysis should lead to biological implications. GlcNAc-terminated glycans were recognized by Langerin, but not by DC-SIGN (Tateno et al., 2010). Indeed, Langerin could bind to certain pathogens through exposed GlcNAc-terminated surface glycans, thus facilitating pathogen degradation and eliciting an immune response. For example, the interaction of the West Nile virus with the Langerin homolog DC-SIGNR and of the Ebola virus glycoprotein with the C-type lectin LSECtin was shown to be mediated by GlcNAc-terminated glycans (Davis et al., 2006; Powlesland et al., 2008). In addition, the lipopolysaccharide (LPS) of the bacterial cell wall commonly contains terminal GlcNAc. Accordingly, MBP and the horse shoe crab lectin tachylectin-2 bind to GlcNAc-containing LPS structures of several bacteria (van Emmerik et al., 1994; Kawabata and Iwanaga, 1999). In humans GlcNAc-terminated glycans are rare since GlcNAc is usually further modified by several glycosyltransferases. Recently, however, it was shown that GlcNAc-terminated protein-linked glycans as well as glycosphingolipids accumulate on the surface of tumor cells in the lung, kidney, breast and ovary (Satomaa et al., 2009). Also, on tissue damage proteins and lipids with immature or truncated glycans containing terminal GlcNAc might be released from intracellular compartments. Consequently, Langerin could be involved in the detection of cancer cells as well as tissue damage and facilitate corresponding immune responses.

6SGal is a structural element of the proteoglycan keratan sulfate and the Lewis antigen sialyl-6SGal Leα. KS6ST (keratan sulfate 6-O-sulfotransferase), an enzyme transferring sulfate to position 6 of Gal in keratan, was found to generate Langerin ligands (Tateno et al., 2010). Keratan sulfate was shown to be associated with several epithelial cells like uterine endometrial cells, keratinocytes, the corneal endothelial cells and also epithelia-derived carcinoma cells (Funderburgh, 2000). The transmembrane glycoproteins MUC1 and CD44 are decorated with keratan sulfate, for example, (Takahashi et al., 1996; Aplin et al., 1998). MUC1, an apical epithelial protein, has been demonstrated to exhibit both adhesive and anti-adhesive properties depending on the glycosylation state of the protein (Aplin et al., 1998; Jeschke et al., 2009). CD44, which occurs on keratinocytes, DCs, and activated lymphocytes in the adult, mediates cell–cell and cell–matrix interactions (Ponta et al., 1998). CD44 exists in several cell-type specific and differentiation-dependent alternative splice forms, some of which carry proteoglycans such as keratan sulfate and other polysaccharide structures (Zhou et al., 1999). Hence, an interaction between Langerin and keratan sulfate could mediate cell–cell communication as well as localization and trafficking of LCs in epithelial tissues. Recently, it was demonstrated that LN299 glioblastoma cells producing sulfatated keratan are targeted by Langerin, suggesting that Langerin is involved in the detection of tumor-specific glycosylation and anti-tumor immunity (Tateno et al., 2010).

Sialyl-6SGal Leα structures were detected on the glycoprotein GlyCAM-1 (Yoshino et al., 1997). The C-type lectin L-selectin is known to bind to GlyCAM-1 as well as to CD34 and MadCAM-1, thereby facilitating the rolling of leukocytes over the vascular endothelium. Like L-selectin, Langerin might interact with sialyl-6SGal Leα-decorated glycoproteins to promote LC migration. Lewis antigens are produced by mammas as well as viral and bacterial pathogens. Their function lies in cell localization and trafficking and in immune modulation. Cancer cells carry altered Lewis antigens, thus promoting tumor progression and metastasis (Brookshen, 2006; Ma et al., 2006). In this regard interactions between DC-SIGN and Lewis antigens have been described. For example, a Leα structure on ICAM-2 is known to mediate the DC-SIGN-dependent rolling of DCs over endothelial cells (Garcia-Vallejo et al., 2008). Further, interaction between DC-SIGN and Leα/Leβ glycans on CEA or CEACAM-1 produced by SW1116 colorectal carcinoma cells was suggested to result in failure of the host to induce an effective anti-tumor response (Nonaka et al., 2008). Pathogens are known to produce Lewis antigens as a form of molecular mimicry to evade the host immune response (Moran, 2008), e.g. Helicobacter pylori was shown to bind DC-SIGN through Lewis antigens (Bergman et al., 2004).

We have observed that DC-SIGN and Langerin both recognize sialoglycans, which is commonly overproduced by cancer cells, indicating that Langerin, like DC-SIGN, may be involved in immune response activation or, likewise, suppression triggered by certain tumors. However, in contrast to DC-SIGN, which recognizes Leα, Leβ Leα, Leβ, sialyl-Leα and sulfatated derivatives of Leα and Leβ, Langerin just weakly binds to Leβ and Leα and sialyl-Leα. This finding

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the soluble recombinant protein was recovered. The protein extract was dialyzed against 150 mM NaCl, 1 mM EDTA, 100 mM Tris-HCl pH 8.0 and applied to a column with immobilized StreptTactin (Schmidt and Skerra, 2007) equilibrated with the same buffer. The recombinant CRDs were eluted by application of 2.5 mM D-desthiobiocin in the chromatography buffer. Elution fractions were concentrated using Amicon Ultra 15 centrifugal filter units with a molecular weight cut off of 10 kDa (Millipore, Eschborn, Germany), applied to a Superdex 75 HR 16/60 gel filtration column (Amersham Biosciences, Uppsala, Sweden) equilibrated with PBS (4 mM KH₂PO₄, 16 mM Na₂HPO₄, 115 mM NaCl) containing 1 mM EDTA and eluted in a homogeneous peak corresponding to the monomeric protein.

Protein concentrations were determined according to the absorption at 280 nm using calculated extinction coefficients (Gill and von Hippel, 1989) of 59 640 M⁻¹ cm⁻¹ for the DC-SIGN CRD and of 54 960 M⁻¹ cm⁻¹ for the Langerin CRD.

Analytical gel filtration

Analytical gel filtration was performed by loading 0.5 mg of the affinity-purified DC-SIGN or Langerin CRD (ca. 1 mg/ml) onto a Superdex 75 HR 10/30 column (Amersham Biosciences) equilibrated either with 20 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA or with 20 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM CaCl₂, 0.5 ml elution fractions were collected and subsequently analyzed by SDS–PAGE (15% w/v), followed by staining with Coomassie Brilliant Blue R-250.

Isothermal titration calorimetry

The DC-SIGN and Langerin CRDs were dialyzed against 5 mM HEPES/NaOH pH 7.5, 115 mM NaCl, 2 mM CaCl₂ or 5 mM HEPES/NaOH pH 7.5, 115 mM NaCl, 1 mM EDTA. Man and Man(α1–2)man were dissolved in the corresponding buffers. All solutions were sterile-filtered (0.22 μm; Millipore) prior to use. The sample cell of a MicroCal VP-ITC calorimeter (GE Healthcare, Freiburg, Germany) was loaded with the DC-SIGN or Langerin CRD at a concentration of 30 to 72.1 μM and then titrated with a solution of 60 mM Man or 27.3 mM Man(α1–2)man. The experiments were carried out using the following settings: one initial 1 μl injection, followed by 14 automatic injections of 20 μl ligand solution each; delay between injections: 1200 s; stirring: 260 rpm; temperature: 25°C. For all measurements a control experiment with buffer in the absence of protein was conducted to determine the dilution heat of the ligand, and the integrated heats were then subtracted from the integrated heats for the ligand-binding measurement. The data were fitted with the instrument software using the single binding site model (setting n = 1), resulting in the binding constant K, the molar enthalpy ΔH and the molar entropy ΔS for complex formation. The dissociation constant K_d and the Gibbs free energy ΔG were calculated using K_d = 1/K and ΔG = ΔH − TΔS.

Protein labeling

For fluorescent labeling the purified DC-SIGN CRD, dissolved at 22.8 μM in 100 mM NaHCO₃ pH 9, 2 mM EDTA, was rapidly mixed with a 8.5 mM solution of 6-[fluorescein-5(6)-carboxamido]hexanoic acid N-hydroxysuccinimide ester

suggests that the interaction of Langerin with Lewis antigens should play a lesser role in vivo.

Taken together, the present biochemical analysis indicates that Langerin may function in LC localization and migration and in the recognition of pathogens as well as cancer cells although intracellular downstream signaling as a result of sugar antigen-binding remains to be shown. With the knowledge of the distinct glycan specificities of DC-SIGN and Langerin the possibility emerges that these C-type lectins may be independently targeted by certain glycans or glycan-like compounds. This is of importance because it is known that the interaction of HIV with DC-SIGN promotes virus infection while binding to Langerin causes degradation of the virus. Hence, based on the described specificity profiles one might devise a microbicide that inhibits the former interaction but does not interfere with the latter.

Materials and methods

Vector construction and protein purification

For periplasmic production of the recombinant Langerin (SWISS-PROT entry: Q9UJ71) and DC-SIGN (SWISS-PROT entry: Q9NX6) CRDs in E. coli the plasmids pLA1 (Chatwell et al., 2008) and pDC1, respectively, were used.

For construction of pDC1 the coding sequence of the DC-SIGN CRD was amplified via polymerase chain reaction (PCR) from a mammalian expression vector carrying the full-length cDNA for human DC-SIGN with the following primers, which were phosphorylated using T4 polynucleotide kinase: 5’-CCC AGC CCT GTC CTT GGG AAT GG-3’ and 5’-GCT TTT GTA CAA GAA TTT GGC-3’. The amplification product was purified by agarose gel electrophoresis and ligated with the expression vector pASK75 strepII (Skerra, 1994; Voss and Skerra, 1997) that had been cut with Eco147I and Eco47III and dephosphorylated using shrimp alkaline phosphatase. After transformation of E. coli XL1-Blue (Bullock et al., 1987), the resulting plasmid pDC1 was isolated and its composition was confirmed by restriction digest as well as double-stranded dyeosequencing (ABI PRISM 310 Genetic Analyzer, Applied Biosystems, Darmstadt, Germany). pDC1 encodes the DC-SIGN CRD comprising residues 254–381 of the mature membrane protein with a His254Gln substitution, an N-terminal OmpA signal peptide and the Strep-tag II (Schmidt and Skerra, 2007) at its C-terminus.

Recombinant DC-SIGN and Langerin CRDs were produced via periplasmic secretion in the E. coli K12 strain JM83 (Yanisch-Perron et al., 1985) harboring pDC1 or pLA1, respectively, together with the helper plasmid pTUM4 (Schlapschy et al., 2006). Cultures were grown in 2 L LB medium (Sambrook et al., 1989) supplemented with 100 mg/l ampicillin and 30 mg/l chloramphenicol at 22°C. Gene expression was induced (Skerra, 1994) at a cell density of 0.8 ml l⁻¹ and incubation on ice for 30 min. The resulting spheroplasts were sedimented by centrifugation and the supernatant containing...
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References


Supplementary data

Supplementary data are available at PEDS online.

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