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DCIR interacts with ligands from both endogenous and pathogenic origin

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ABSTRACT

C-type lectins on dendritic cells function as antigen uptake and signaling receptors, thereby influencing cellular immune responses. Dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) is one of the best-studied C-type lectin receptors expressed on DCs and its glycan specificity and functional requirements for ligand binding have been intensively investigated. The carbohydrate specificity of dendritic cell immunoreceptor (DCIR), another DC-expressed lectin, was still debated, but we have recently confirmed DCIR as mannose/fucose-binding lectin. Since DC-SIGN and DCIR may potentially share ligands, we set out to elucidate the interaction of DCIR with established DC-SIGN-binding ligands, by comparing the carbohydrate specificity of DCIR and DC-SIGN in more detail. Our results clearly demonstrate that DC-SIGN has a broader glycan specificity compared to DCIR, which interacts only with mannotriose, sulfo-Lewis^a, Lewis^b and Lewis^a. While most of the tested DC-SIGN ligands bound DCIR as well, *Candida albicans* and some glycoproteins on some cancer cell lines were identified as DC-SIGN-specific ligands. Interestingly, DCIR strongly bound human immunodeficiency virus type 1 (HIV-1) gp140 glycoproteins, while its interaction with the well-studied DC-SIGN-binding HIV-1 ligand gp120 was much weaker. Furthermore, DCIR-specific ligands were detected on keratinocytes. Furthermore, the interaction of DCIR with its ligands was strongly influenced by the glycosylation of DCIR. In conclusion, we show that sulfo-Lewis^a is a high affinity ligand for DCIR and that DCIR interacts with ligands from both pathogenic and endogenous origin of which most are shared by DC-SIGN.

1. Introduction

Dendritic cells (DCs) play a critical role in shaping innate and adaptive immune responses. DCs are equipped with different receptor families, such as the Toll-like receptor (TLR) family that enables them to respond to pathogen associated molecular patterns (PAMPs) and danger associated molecular patterns (DAMPs) [1]. TLR stimulation will induce DC maturation, characterized by the upregulation of co-stimulatory molecules, the secretion of cytokines and the migration of DCs to the lymph nodes [2,3]. Other receptors, like the C-type lectin receptors (CLRs), are involved in antigen internalization and the routing to Major Histocompatibility complex (MHC) class I and II loading compartments, needed for MHC class I and II-dependent T cell activation [4,5]. In addition, some CLRs are capable of modulating TLR-induced responses by altering intracellular signaling pathways [6,7].

CLRs recognize glycan structures present on pathogens, commensals and self-glycoproteins [7]. The occurrence of a Glutamine

Abbreviations: CHO, Chinese Hamster Ovary; CLR, C-type lectin receptor; ConA, Concanavalin A; CRD, carbohydrate recognition domain; DAMP, danger associated molecular pattern; DC, dendritic cell; DC-SIGN, DC-specific intercellular adhesion molecule-3-grabbing non-integrin; DCIR, dendritic cell immunoreceptor; GalNAc, *N*-acetylgalactosamine; GlcNAc, *N*-acetylglucosamine; HCV, Hepatitis C Virus; HIV-1, human immunodeficiency virus type 1; ITIM, immunoreceptor tyrosine-based inhibitory motif; Methyl-Man/Glc, Methyl-α-D-glycopyranoside/Methyl-α-mannopyranoside; MHC, Major Histocompatibility complex; PAA, polyacrylamide; PAMP, pathogen associated molecular pattern; SP, soluble products; TLR, Toll-like receptor; TMB, 3,3′,5,5′-tetramethylbenzidine.

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acid-Proline-Asparagine (EPN) motif inside the carbohydrate recognition domain (CRD) predicts fucose and mannose binding, while the Glutamine-Proline-Aspartic acid (QPD) motif is involved in binding to galactose- or *N*-acetylgalactosamine (GalNAc)-containing glycans [8]. Dendritic cell immunoreceptor (DCIR) contains an unusual sequence in the putative CRD, whereby the asparagine residue is replaced by a serine residue, resulting in an EPS motif [9]. This receptor is the only classical CLR that contains an intracellular immunoreceptor tyrosine-based inhibitory motif (ITIM). The glycan specificity of this lectin has only recently been investigated, however with conflicting results, as both mannose/fucose specificity [10] as well as binding to sulfated LacNAc and Lac, and biantennary *N*-glycans [11] has been reported. We have elucidated the glycan specificity of DCIR as well and our results clearly demonstrate DCIR binding to mannotriose and Lewis^b [12], confirming DCIR as fucose/mannose-binding lectin.

DC-specific intercellular adhesion molecule-3 (ICAM-3)-grabbing non-integrin (DC-SIGN) is another fucose/mannose binding lectin expressed on DCs [13]. In contrast, DCIR is expressed on a variety of immune cells besides DCs, including monocytes, neutrophils, B-cells and activated T cells [9,14–16]. Compared to DCIR, the glycan specificity of DC-SIGN has been extensively studied, demonstrating DC-SIGN recognition of high mannose and unsialylated Lewis structures [17]. The interaction of DC-SIGN with fucose-containing glycans is of higher affinity than the DC-SIGN-mannose interaction [10].

Based on its glycan specificity, DC-SIGN binding to various endogenous and exogenous ligands has been suggested [17,18]. After the initial report on the binding of DC-SIGN to human immunodeficiency virus type 1 (HIV-1) gp120 [19], many other pathogens have been described as DC-SIGN ligands, including fungi, such as *Candida albicans* [20], viruses, like Hepatitis C Virus (HCV) [21,22], bacteria, such as *Helicobacter pylori* [17] and *Mycobacterium tuberculosis* [23] and helminths, like *Schistosoma mansoni* [24]. In addition, various endogenous proteins have been reported as DC-SIGN ligands, including the adhesion molecules ICAM-3, ICAM-2, CEACAM1 and Mac-1 [13,19,25–27]. Altered cellular glycosylation during oncogenesis results in the expression of DC-SIGN-binding glycans on tumor associated antigens, such as CEA [28,29].

Only recently the first DCIR-binding ligand, HCV, has been reported [30]. Furthermore, HIV-1 infectivity has been shown to be dependent on DCIR-induced signaling [31,32], however actual binding of HIV-1 to DCIR has not been demonstrated yet. The recognition of endogenous ligands by DCIR still needs to be established.

The potential overlapping glycan specificities of DC-SIGN and DCIR prompted us to investigate this in more detail, particularly as both are expressed simultaneously on DCs and the functional outcome might depend on the combined interaction of antigens with both lectins. Since DCIR and DC-SIGN most likely have distinct functional properties, we set out to investigate the glycan specificity of DCIR in more detail, thereby exploring whether DCIR was able to interact with known DC-SIGN-binding ligands. We here show that sulfo-Lewis^a is a high affinity ligand for DCIR and that DCIR interacts with ligands from both pathogenic and endogenous origin.

2. Materials and methods

2.1. Antibodies, Fc chimeric proteins, lectins, glycans and ligands

DCIR-Fc consists of the extracellular domains of DCIR (amino acid residues 208–689) fused at the C terminus to the Fc domain of human IgG1 [33] and was produced in Chinese Hamster Ovary (CHO) cells (DCIR-Fc) and in CHO Lec8 cells (DCIR-Fc Lec8) [34]. DC-SIGN-Fc was produced as described previously [35]. All Fc constructs were purified using Hi Trap Protein A HP columns (GE Healthcare Europe). Human IgG1 kappa (Serotec) was used as a control Fc protein. Antibodies used are α-Lewis^a (clone T174), α-Lewis^b (clone T218), α-Lewis^X (clone P12) and α-Lewis^Y (clone F3) (Calbiochem), goat-α-mouse-IgM-PO (Nordic Immunologic Laboratories), goat-α-mouse-IgM-FITC (Jackson Immunoresearch), goat-α-mouse-Ig-PO (Dako), goat α-mouse IgG Alexa Fluor 488 F(ab′)$_2$ (Invitrogen) and goat-α-human Fc-PO (Jackson Immunoresearch). Other reagents include streptavidin-Alexa Fluor 488 (Invitrogen), streptavidin-PO (Invitrogen), biotinylated lectin Concanavalin A (ConA, Vector Labs) and Methyl-α-D-glycopyranoside/Methyl-α-mannopyranoside (methyl-Man/Glc, Sigma Aldrich), α-D-mannose (Sigma Aldrich). Glycans used were biotin-labeled polyacrylamide (PAA) conjugates or unlabeled PAA conjugates (Lectinity and American Consortium of Functional Glycomics (www.functionalglycomics.org)), except for mannose-BSA (Sigma Aldrich), Mannotriose, 3′-sulfo-Lewis^a, 3′-sulfo-Lewis^X, Blood group A and B (Dextra Labs), which were BSA-conjugated and not biotinylated. *Trichinella spiralis* larvae were harvested from infected mice and kindly provided by Dr. J. van der Giessen (RIVM, Bilthoven, the Netherlands), *S. mansoni* cercariae were kindly provided by Dr. M. de Jong-Brink (FALW, VU University, Amsterdam, the Netherlands), and *Haemonchus contortus* larvae were a gift from Dr. J. Poot (Intervet Int., Boxmeer, the Netherlands). Soluble products (SP) of the helminth stages were prepared as described [36]. Larvae or cercariae were frozen in liquid nitrogen, and crushed with mortar and pestle into powder. The powder was dissolved in MilliQ and centrifuged to remove the insoluble fraction. After 30 min of centrifugation at 10,000 × *g* at 4 °C, supernatants were filtered (0.45 μm) and kept at −80 °C until further use. *C. albicans* yeast and hyphae were a kind gift from Prof. Dr. M. Netea (RUNMC, Nijmegen, the Netherlands). Different HIV proteins were provided by NIH AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: HIV-1 SF162 gp140 trimer, catalog number: 12026 from Dr. Leo Stamatatos; Recombinant HIV-1 IIIB gp120 (CHO), catalog number: 11784 from DIAIDS, NIAID (produced by Immunodiagnostics); HIV-1 gp120 CM, catalog number: 2968 from DAIDS, NIAID.

2.2. Cells

CHO, CHO Lec8 cells and Kato-III cells were cultured in RPMI1640 (Invitrogen) supplemented with 1000 U/ml penicillin/streptomycin (Lonza), 2 mM glutamine (Lonza) and 10% FBS (BioWhittaker). DCIR-Fc and DC-SIGN-Fc producing cell lines were cultured in RPMI supplemented with 1000 U/ml penicillin/streptomycin, 2 mM glutamine, 60 μg/ml glutamic acid, 60 μg/ml asparagine, 7 μg/ml adenosine, 7 μg/ml guanosine, 7 μg/ml cytidine, 7 μg/ml uridine and 2.4 μg/ml thymidine (all from Sigma Aldrich), 1 mM MEM non-essential amino acids, 1 mM sodium pyruvate MEM (Invitrogen) and 10% dialyzed FBS (Invitrogen). DCIR-Fc Lec8 cells were cultured in RPMI supplemented with 1000 U/ml penicillin/streptomycin, 2 mM glutamine, 10% FBS and 500 μg/ml hygromycin (Invitrogen) to select for DCIR-Fc producing cells. Prostate cancer cell lines VCaP, PC346C, LNCaP and LAPC-4, were a kind gift of Dr. W.M. van Weerden (Erasmus MC Rotterdam, the Netherlands). Colon cancer cell lines HCT116, HT-29 and SW480 were cultured in DMEM (Invitrogen) supplemented with 1000 Units/ml penicillin/streptomycin mixture (Lonza) and 10% FBS (BioWhittaker). Primary keratinocytes were obtained from healthy skin samples following plastic surgery, after obtaining informed consent from all donors. Epidermis and dermis were separated as described previously [37]. The CD1a negative keratinocytes were separated from the CD1a positive Langerhans cells using anti-CD1a-labeled immunomagnetic microbeads (Miltenyi Biotec).