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Sialyl Lewis^x-dependent Binding of Human Monocyte-derived Dendritic Cells to Selectins

Zélia Silva[1,*], **ZiQiu Tong**[2,*], **M. Guadalupe Cabral**[1], **Catarina Martins**[1], **Rita Castro**[3], **Celso Reis**[4,5], **Hélder Trindade**[1], **Konstantinos Konstantopoulos**[2,6,7,¶], and **Paula A. Videira**[1]

[1] CEDOC, Departamento de Imunologia, Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Lisboa, Portugal

[2] Department of Chemical and Biomolecular Engineering, The Johns Hopkins University, Baltimore, Maryland, USA

[3] Metabolism & Genetics Group, Research Institute for Medicine and Pharmaceutical Sciences (iMed.UL), Faculdade de Farmácia da Universidade de Lisboa, Lisboa, Portugal

[4] Institute of Molecular Pathology and Immunology of University of Porto-IPATIMUP, Porto, Portugal

[5] Medical Faculty of the University of Porto, Porto, Portugal

[6] NCI PS-OC Johns Hopkins Physical Sciences in Oncology Center, Baltimore, Maryland, USA

[7] Institute for NanoBioTechnology, The Johns Hopkins University, Baltimore, Maryland, USA

Abstract

The limited efficacy of monocyte-derived dendritic cell (mo-DC)-based vaccines is primarily attributed to the reduced mo-DC migratory capacity. One undefined aspect is the initial binding of mo-DCs to endothelial cells and vascular selectins. In this study, we investigated the role and modulation of the selectin binding determinant sialyl Lewis^x (sLe^x) in selectin-dependent mo-DC binding. Our data reveal that sLe^x is required for maximal binding of mo-DCs to tumor necrosis factor (TNF)-α-activated endothelial cells under static conditions, as evidenced by the use of sialidase. Sialidase treatment also abrogated mo-DC cell tethering to immobilized, purified P-, L- or E-selectin under flow. The requirement of sLe^x-dependent binding of mo-DC to selectins was further substantiated by using sLe^x free sugar and anti-sLe^x antibody, which significantly suppressed mo-DC-selectin binding. P-selectin glycoprotein ligand-1 is required for mo-DC binding to both P- and L-selectin, but it is dispensable for E-selectin recognition. Interestingly, the extent of mo-DC tethering was maximal on P-selectin, followed by E- and L- selectin. Accordingly, L-selectin mediated faster mo-DC rolling than E- or P-selectin. Interferon (IFN)-γ induces a significant increase in mo-DC surface sLe^x expression, which is probably due to the enhanced synthesis of C2GnT-I. These findings may contribute to improving mo-DC-based vaccination protocols.

¶To whom correspondence should be addressed: Konstantinos Konstantopoulos, Ph.D., Department of Chemical and Biomolecular Engineering, The Johns Hopkins University, 3400 N. Charles Street, Baltimore, MD 21218, USA, Tel.: (410) 516-6290 Fax: (410) 516-5510, konstant@jhu.edu.
*Z. Silva and Z. Tong contributed equally to this work

Keywords

monocyte-derived dendritic cells; selectin; adhesion; shear flow; IFN-γ

INTRODUCTION

Dendritic cells (DCs) play a pivotal role in triggering and regulating immune responses due to their ability to uptake, process and present antigens to T lymphocytes [1]. DCs have been exploited in adoptive immune therapy to treat human malignancies and infectious diseases [2]. The most common approach involves the use of DCs derived *in vitro* from monocytes (mo-DCs), which after being loaded with tumour antigens, grant protective and therapeutic actions in cancer patients. Pro-inflammatory cytokines are often used to induce mo-DC maturation and promote efficient mo-DC interaction with T lymphocytes. However, the basic biology of mo-DCs remains uncovered, and vaccines based on mo-DCs still display a limited efficacy. One major bottleneck is the reduced migratory capacity of mo-DCs relative to the highly mobile natural DCs [3]. The clinical efficacy of these mo-DC-based vaccines lies in the accomplishment of two important steps: i) exit from the bloodstream to the tissue space and ii) subsequent homing to the draining lymph nodes, where antigen-specific T-cell stimulation takes place. Although the tethering of mo-DCs to the endothelial cell surface can regulate the extent of mo-DC homing, this step has not been carefully investigated so far.

It is well established that selectins mediate the initial tethering and rolling of leukocytes along the endothelial cell surface, followed by a cascade of molecular events which culminates in the leukocyte extravasation and migration into inflamed tissue [4]. Selectins (E-, P- and L-selectin) recognize the tetrasaccharide sialyl Lewis^x (sLe^x; NeuAc α2,3 Gal β1,4 [Fuc α1,3] GlcNAc-R; Fig. 1A), a terminal component of glycans attached to glycoproteins and glycolipids on most circulating immune cells and some endothelial cells. sLe^x biosynthesis requires the sequential action of different glycosyltransferases (Fig. 1A) [5]. In the case of the frequent sLe^x in core 2 O-glycans, the branch is initiated by core 2 β 1,6-N-acetylglucosaminyltransferase I (C2GnT-I), followed by the alternate action of β 1,4-galactosyltransferase I (β 4GalT-I) and β 1,3-N-acetylglucosaminyltransferase (β3GlcNAcT), forming polylactosamine chains. Elongation of core 2 branches is terminated by the addition of sialic acid by α 1,3-sialyltransferases (ST3Gal) followed by the addition of fucoses by α 1,3-fucosyltransferases (FucT) [6]. The role of sLe^x as selectin ligand, whilst extensively studied in leukocytes [5], has not been fully examined in mo-DCs. Regarding other subtypes, peripheral blood DCs express sLe^x and adhere to activated endothelial cells, under static conditions, via a selectin-dependent mechanism [7]. CD34$^+$-derived DCs bear an epitope similar to sLe^x, the cutaneous lymphocyte associated antigen (CLA), almost exclusively on P-selectin glycoprotein ligand-1 (PSGL-1) and bind efficiently to immobilized E- and P-selectin under both static and flow conditions [8]. More recently, Julien et al. [9] reported that immature mo-DCs express sLe^x on PSGL-1 but this determinant is lost upon mo-DCs maturation induced by tumor necrosis factor-alpha (TNF-α) in conjunction with prostaglandin (PG)E_2. Nevertheless, it remains to be determined whether sLe^x expression is functionally relevant to mo-DCs adhesion to selectins.

In the present study, we have demonstrated the functional role of sLe^x surface expression by mo-DCs in selectin-dependent binding under flow. We further investigated the ability of different maturation stimuli to modulate sLe^x expression, and showed that interferon-gamma (IFN-γ) induces a significant increase in the sLe^x surface expression, which is likely regulated by increased synthesis of C2GnT-I.

Materials and Methods

Reagents

Fluorescently-conjugated or unlabeled human anti-CD14 (M5E2), anti-CD83 (HB15e), anti-CD31 (L133.1), anti-CD45 (HI30), anti-sLe^x (CSLEX-1), anti-E-selectin (68-5H11), anti-P-selectin (AK4) and anti-PSGL-1 (KPL1) monoclonal antibodies (mAb), and rat anti-sLe^x IgM (HECA-452) mAb were purchased from BD Biosciences (San Jose, CA). The mouse anti-sLe^x IgM (KM93) mAb was from Calbiochem (La Jolla, CA). Blocking anti-E-selectin (CL2/6) mAb was purchased from AbDSerotec (Oxford, UK). Anti-HLA-DR (L243) was from Immunostep (Salamanca, Spain) and the anti-BDCA-1 (AD5-8E7) from Miltenyi Biotec (Bergisch Gladbach, Germany). The anti-CCR7 (150503) mAb, the P- and E-selectin-IgG Fc chimeras (consisting of the extracellular domain of the human P-selectin and E-selectin, respectively, linked to the human IgG-Fc) and the human cytokines TNF-α, IFN-γ, interleukin (IL)-6, IL-1β, IL-4 and Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) were purchased from R&D Systems (Minneapolis, MN). All other reagents were from Sigma (St. Louis, MO, USA) unless otherwise stated.

Cell isolation and culture

Monocytes were isolated from peripheral blood mononuclear cells by positive selection using anti-CD14 coated magnetic beads (Miltenyi Biotech) and cultured with IL-4 and GM-CSF [10]. After 5 days, mo-DC maturation was induced by lipopolysaccharide (LPS) (1 μg/mL), IL-1β (1000 U/mL), IFN-γ (1000 U/mL), TNF-α (1000 U/mL), IL-6 (10 ng/mL) or PGE_2 (10 μg/mL) or IL-1β, IL-6, PGE_2 and TNF-α mixture (IIPT) and on day 7 cells were collected. Differentiation and maturation of mo-DCs was confirmed by antibody staining and flow cytometry analysis. Human umbilical vein endothelial cells (HUVECs) were isolated by collagenase treatment as described in [11, 12].

Flow cytometry

Monocyte purity, mo-DC differentiation and maturation status were monitored by staining cells with fluorescently-conjugated anti-CD14, BDCA-1, HLA-DR, CCR7 and CD83 mAbs. sLe^x expression on mo-DCs was evaluated by indirect immunofluorescence using CSLEX-1, KM93 or HECA-452 as primary mAbs.

Confocal laser scanning microscopy

Mo-DCs were allowed to adhere to concanavalin-A coated cover glasses, fixed with 3.7% of paraformaldehyde and permeabilized with 0.1% TritonX. After blocking with 1% BSA, cells were stained using anti-sLe^x mAb CSLEX-1, followed by a biotinylated anti-mouse IgM antibody and PE-labelled streptavidin. The cell nuclei were stained with 1 μM TO-PRO-3 dye (Molecular Probes, Leiden, Netherlands). Images were acquired with a Leica TCS SP2 AOBS confocal microscope (Leica Microsystem, Mannheim, GmbH).

Isolation of RNA and Real-Time RT-PCR

Expression of glycosyltransferase genes was analyzed by real-time PCR [13–15] using TaqmanFast Universal PCR Master Mix, primers and Taqman probes provided by Applied Biosystems.

Adhesion of mo-DCs to HUVEC monolayers

HUVECs were cultured to confluency in 48-well flat bottom plates, and stimulated with TNF-α (10 ng/mL) for 4h at 37°C to obtain maximal levels of E-selectin expression [16]. Mo-DCs were overlaid, in duplicate wells, onto HUVEC monolayers at 5:1 (mo-DC:HUVEC) ratio and allowed to adhere for 30 min at 37°C. HUVEC and adherent mo-

DCs were detached from the plate with 0.05% trypsin-EDTA solution. Assessment to the expression of specific cell markers by flow cytometry analysis allowed the characterization of adherent mo-DC cells (HLA-DR$^+$) and HUVEC (CD31$^+$) in the mixture.

Flow-based adhesion assay

Octadecyltrichlorosilane treated glass slides were first incubated with anti-human IgG Fc and then with the appropriate P-, L- or E-selectin IgG Fc chimera, and blocked with 1% BSA, as described in [19]. Mo-DCs (1×10^6 cells/mL) suspended in D-PBS buffer containing Ca^{2+}/Mg^{2+} and 0.1% BSA were perfused over immobilized P-, L- or E-selectin-coated slides [20] at 1 dyn/cm^2 using a microfluidic channel affixed to the selectin-coated slides. In select experiments, the selectin-coated slides were incubated with 20 μg/mL of a function-blocking anti-P- or E-selectin mAb or with 1 mM of sLe^x sugar. In other experiments, mo-DCs were incubated with 40 μg/mL of an anti-sLe^x antibody (CSLEX-1) or treated with sialidase [17, 18], before perfusion over selectin-coated slides. The extent of adhesion was quantified by enumerating the total number of tethering events in a single ×10 field of view during a 3 min period. Average rolling velocities were computed as the distance traveled by the centroid of the translating cell divided by the time interval at the given wall shear stress.

Results and Discussion

sLe^x is expressed by human mo-DCs

In view of the critical involvement of sLe^x-decorated glycoproteins and glycolipids [21] in the initial tethering and rolling of immune cells along the endothelial cell surface, we investigated the expression of sLe^x on the surface of mo-DCs by flow cytometry. Fig. 1B reveals that mo-DCs from all donors display moderate sLe^x expression, as assessed by the use of three different anti-sLe^x mAbs: CSLEX-1, KM93 and HECA-452. Despite the minor donor variability, the staining intensity detected using the CSLEX-1 mAb was consistently higher than that of the KM93 and HECA-452 antibodies. The different intensities of surface immunostaining observed with the three distinct mAbs is attributed to intrinsic differences in the antibody specificities with respect to the extent of polylactosamine chain as reported by others [9]. The flow cytometry results were confirmed by confocal-laser scanning microscopy visualization of CSLEX-1 staining, which showed that sLe^x is distributed uniformly throughout each mo-DC surface and cytoplasm (Fig. 1C).

The adhesion of mo-DCs to activated endothelium under static conditions requires sialylated structures

The observed sLe^x surface expression by mo-DCs prompted us to assess their ability to adhere to cytokine-activated endothelial cells under static conditions, and the potential contribution of sLe^x:selectin interaction to this process. Because treatment of HUVECs with TNF-α induces maximal E-selectin expression within 4h of stimulation, static adhesion assays were performed by incubating mo-DCs with TNF-α-activated (4h) HUVECs. After 30 min of co-incubation, 43.8±5.9% of mo-DCs remained adherent to the TNF-α-treated endothelial cells (Table 1). In contrast, only 25.5±5.8% of mo-DCs adhered to untreated (No TNF-α) HUVECs. To evaluate the potential involvement of sialylated structures in mo-DC binding to TNF-α-activated endothelium, mo-DCs were treated with sialidase (0.2 U/mL), which cleaves terminal sialic acid residues from the cell surface, prior to their incubation with HUVECs. This intervention resulted in a significant decrease in the extent of mo-DCs (33.3±8.4%) adherent to activated endothelium (Table 1). However, addition of the anti-sLe^x blocking mAb, CSLEX-1, or soluble sLe^x sugar to the medium did not alter the extent of mo-DC adhesion to HUVECs relative to appropriate controls (Table 1). Interestingly, similar observations were previously reported using blood DCs [7]. Use of an anti-E-selectin mAb failed to inhibit the extent of adhesion under static conditions (Table 1). The lack of an

inhibitory effect by the anti-E-selectin mAb may be attributed to the engagement of other adhesion molecules in this process such as β_2-integrin binding to endothelial ICAM-1.

Mo-DCs tether and roll on purified P- E- and L-selectin under flow conditions

To eliminate the potential contribution of other adhesion molecules, such as ICAM-1, flow-based adhesion experiments were performed by perfusing mo-DCs over purified selectins under controlled kinematic conditions. As shown in Fig. 2, mo-DCs tethered and rolled on P, E- or L-selectin-coated surfaces at a wall shear stress of 1 dyn/cm^2. The extent of mo-DC tethering was maximal on purified P-selectin-, intermediate on E-selectin-, and low on L-selectin-coated surfaces (Fig. 2A). In agreement with previous work using neutrophils [22], L-selectin mediated faster mo-DC rolling than E- or P-selectin (Fig. 2B). Treatment of mo-DCs with sialidase nearly abrogated their binding to P-selectin (Fig. 3A), E-selectin (Fig. 3B) and L-selectin (data not shown) under flow. We next evaluated the potential effects the anti-sLe^x blocking antibody, CSLEX-1 (40 μg/mL), and the sugar sLe^x (1 mM) itself on mo-DC binding to purified selectins. Interestingly, addition of either the anti-sLe^x antibody or the sugar to the perfusion medium was equally effective in decreasing the extent of mo-DC tethering to P-selectin by ~30% relative to untreated control cells or cells perfused in the presence of an isotype control antibody (Fig. 3A). The inhibitory effects of these agents were more pronounced on E-selectin-dependent binding under flow, as evidenced by a >50% reduction in the extent of mo-DC binding relative to controls (Fig. 3B). Use of an anti-P-selectin or anti-E-selectin mAb reduced mo-DC binding to the respective selectins by •85% and 65%, respectively (Fig. 3). The specificity of mo-DC tethering to selectins was demonstrated by the lack of any cell binding on bare slides (Fig. 3). Taken together, these data illustrate the role of sLe^x-decorated glycoconjugates in mo-DC binding to selectins. Although P-, E- and L-selectins share the ability to recognize the tetrasaccharide sLe^x determinant, the binding affinity of selectins for isolated monovalent sLe^x is very low [23]. PSGL-1 has fucose- and sialic acid-containing polylactosamine side chains, many of which terminate in sLe^x [24]. Although PSGL-1 is recognized by all three selectins [20], it represents the major counter-receptor only for P- and L-selectin in human leukocytes [21, 25]. In agreement with these previous observations, a function-blocking anti-PSGL-1 mAb completely abolished the binding of mo-DC to P-selectin (Fig. 3A) under flow, and reduced binding to L-selectin by ~50% (data not shown). In agreement with previous work using neutrophils [21], PSGL-1 appears to be dispensable in the tethering of mo-DCs to E-selectin under flow (Fig. 3B).

sLe^x expression on mo-DCs can be modulated by maturation stimuli

The induction of maturation is a standard protocol used in the development of mo-DC vaccines to improve their effectiveness in cancer therapy [26]. We previously reported that maturation induces significant glycosylation changes in mo-DCs [10, 27]. We therefore reasoned that sLe^x expression may be altered upon mo-DC maturation induced by stimuli used in the establishment of mo-DC-based vaccines [26]. To this end, we analyzed the sLe^x surface expression in both immature and mature mo-DCs by flow cytometry using the CSLEX-1 antibody. As shown in Fig. 4, IFN-γ significantly increased the sLe^x surface expression in 11 of 15 donors examined in this work. These data are consistent with previous observations showing that intradermal injection of IFN-γ leads to increased expression of sLe^x in a specialized type of DCs, the Langerhans cells [28]. Maturation stimuli such as LPS, IL-6, IL-1β, PGE_2 and TNF-α, or a combination of IL-6, IL-1β, PGE_2 and TNF-α, used in some vaccine protocols [26], failed to increase sLe^x expression. We confirmed the induction of maturation for all the aforementioned stimuli through the upregulation of the maturation markers HLA-DR, CD83 and CCR7 (data not shown).

We next aimed to determine whether the increase of sLe^x expression by IFN-γ was due to transcriptional changes of critical enzymes involved in sLe^x biosynthesis. In view of the fact that selectin ligands, such as PSGL-1, terminate in sLe^x on core 2 O-linked glycan chains, we investigated the following enzymes: C2GnT-I and β 4GalT-I, involved in core 2 elongation; and α 1,3-sialyltransferases, ST3Gal-IV, -VI or -III, as well as the α 1,3-fucosyltransferases, FucT-III, -IV, -VI or -VII (Fig. 1) [6, 29]. Table 2 reveals that IFN-γ stimulation induces a marked upregulation of C2GnT-I, whereas modest and non-significant increases are noted in the expression levels of and β 4GalT-I and FucT-VII. While FucT-III and -VI are not expressed (data not shown), treatment of mo-DCs with IFN-γ reduced ST3Gal-III expression. Taken together, these data suggest that IFN-γ acts at the transcriptional level, upregulating the expression of C2GnT-I glycosyltransferase, which leads to the synthesis of core 2 decorated O-glycans carrying sLe^x. Interestingly, others have reported that LPS-induced maturation causes a great decrease of sLe^x in mo-DCs, which was associated with a decrease in the C2GnT-I expression [9].

While sLe^x:selectin interactions have been comprehensively described as essential for the initial tethering and rolling steps of different leukocyte subpopulations to the endothelial lining, this has not been demonstrated for mo-DCs. Some aspects of the relevance of sLe^x:selectin interactions have been previously reported for other DC subsets [8]. While all DCs share certain features, their subtypes actually represent a variety of cell types with different phenotypic traits and effector functions [1]. To the best of our knowledge, this is the first thorough *in vitro* study demonstrating that human mo-DCs effectively bind to P-, E- and L-selectins via a sLe^x-dependent pathway.

Our finding that sLe^x expression can be modulated upon IFN-γ-mediated maturation may have important implications in the clinical setting. IFN-γ has been more recently introduced in mo-DC vaccines protocols to treat cancer due to its potent T helper 1 (Th1) polarization and pro-inflammatory activity [30]. IFN-γ stimulates multiple mo-DC effector functions, acting as an inducer and a regulator of inflammation that can be helpful in mo-DC-based vaccination [31]. Our data suggest a new role for IFN-γ as a regulator of sLe^x biosynthesis with an expected impact on selectin-dependent binding and eventual extravasation of mo-DCs into tissues.

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References

1. Banchereau J, Palucka AK. Dendritic cells as therapeutic vaccines against cancer. Nat Rev Immunol. 2005; 5:296–306. [PubMed: 15803149]
2. Figdor CG, de Vries IJ, Lesterhuis WJ, Melief CJ. Dendritic cell immunotherapy: Mapping the way. Nat Med. 2004; 10:475–480. [PubMed: 15122249]
3. Ridolfi R, Riccobon A, Galassi R, Giorgetti G, Petrini M, Fiammenghi L, Stefanelli M, Ridolfi L, Moretti A, Migliori G, Fiorentini G. Evaluation of in vivo labelled dendritic cell migration in cancer patients. J Transl Med. 2004; 2:27. [PubMed: 15285807]
4. McEver RP. Selectins: Lectins that initiate cell adhesion under flow. Curr Opin Cell Biol. 2002; 14:581–586. [PubMed: 12231353]
5. Sperandio M, Gleissner CA, Ley K. Glycosylation in immune cell trafficking. Immunol Rev. 2009; 230:97–113. [PubMed: 19594631]

6. Carvalho AS, Harduin-Lepers A, Magalhaes A, Machado E, Mendes N, Costa LT, Matthiesen R, Almeida R, Costa J, Reis CA. Differential expression of alpha-2,3-sialyltransferases and alpha-1,3/4-fucosyltransferases regulates the levels of sialyl lewis a and sialyl lewis x in gastrointestinal carcinoma cells. Int J Biochem Cell Biol. 2010; 42:80–89. [PubMed: 19781661]
7. Srinivas U, Larsson M, Lundblad A, Forsum U. E-selectin involvement in in vitro adhesion of blood dendritic cells to human umbilical cord endothelial cells. Scand J Immunol. 1993; 38:273–278. [PubMed: 7689249]
8. Kieffer JD, Fuhlbrigge RC, Armerding D, Robert C, Ferenczi K, Camphausen RT, Kupper TS. Neutrophils, monocytes, and dendritic cells express the same specialized form of PSGL-1 as do skin-homing memory T cells: Cutaneous lymphocyte antigen. Biochem Biophys Res Commun. 2001; 285:577–587. [PubMed: 11453631]
9. Julien S, Grimshaw MJ, Sutton-Smith M, Coleman J, Morris HR, Dell A, Taylor-Papadimitriou J, Burchell JM. Sialyl-lewis(x) on P-selectin glycoprotein ligand-1 is regulated during differentiation and maturation of dendritic cells: A mechanism involving the glycosyltransferases C2GnT1 and ST3Gal I. J Immunol. 2007; 179:5701–5710. [PubMed: 17947642]
10. Videira PA, Amado IF, Crespo HJ, Alguero MC, Dall'Olio F, Cabral MG, Trindade H. Surface alpha 2–3- and alpha 2–6-sialylation of human monocytes and derived dendritic cells and its influence on endocytosis. Glycoconj J. 2008; 25:259–268. [PubMed: 18080182]
11. Burdick MM, McCaffery JM, Kim YS, Bochner BS, Konstantopoulos K. Colon carcinoma cell glycolipids, integrins, and other glycoproteins mediate adhesion to HUVECs under flow. Am J Physiol Cell Physiol. 2003; 284:C977–87. [PubMed: 12477667]
12. Burdick M, Konstantopoulos K. Platelet-induced enhancement of LS174T colon carcinoma and THP-1 monocytoid cell adhesion to vascular endothelium under flow. Am J Physiol Cell Physiol. 2004; 287:C539–47. [PubMed: 15084476]
13. Zhu F, Wang P, Kontrogianni-Konstantopoulos A, Konstantopoulos K. Prostaglandin (PG)D(2) and 15-deoxy-delta(12,14)-PGJ(2), but not PGE(2), mediate shear-induced chondrocyte apoptosis via protein kinase A-dependent regulation of polo-like kinases. Cell Death Differ. 2010; 17:1325–1334. [PubMed: 20150912]
14. Zhu F, Wang P, Lee NH, Goldring MB, Konstantopoulos K. Prolonged application of high fluid shear to chondrocytes recapitulates gene expression profiles associated with osteoarthritis. PLoS One. 2010; 5:e15174. [PubMed: 21209926]
15. Videira PA, Correia M, Malagolini N, Crespo HJ, Ligeiro D, Calais FM, Trindade H, Dall'Olio F. ST3Gal.I sialyltransferase relevance in bladder cancer tissues and cell lines. BMC Cancer. 2009; 9:357. [PubMed: 19811634]
16. Bevilacqua MP, Stengelin S, Gimbrone MA Jr, Seed B. Endothelial leukocyte adhesion molecule 1: An inducible receptor for neutrophils related to complement regulatory proteins and lectins. Science. 1989; 243:1160–1165. [PubMed: 2466335]
17. Thomas SN, Schnaar RL, Konstantopoulos K. Podocalyxin-like protein is an E-/L-selectin ligand on colon carcinoma cells: Comparative biochemical properties of selectin ligands in host and tumor cells. Am J Physiol Cell Physiol. 2009; 296:C505–13. [PubMed: 19118161]
18. Thomas SN, Zhu F, Schnaar RL, Alves CS, Konstantopoulos K. Carcinoembryonic antigen and CD44 variant isoforms cooperate to mediate colon carcinoma cell adhesion to E- and L-selectin in shear flow. J Biol Chem. 2008; 283:15647–15655. [PubMed: 18375392]
19. Ghosh M, Alves C, Tong Z, Tettey K, Konstantopoulos K, Stebe KJ. Multifunctional surfaces with discrete functionalized regions for biological applications. Langmuir. 2008; 24:8134–8142. [PubMed: 18582132]
20. Hanley WD, Wirtz D, Konstantopoulos K. Distinct kinetic and mechanical properties govern selectin-leukocyte interactions. J Cell Sci. 2004; 117:2503–2511. [PubMed: 15159451]
21. Nimrichter L, Burdick MM, Aoki K, Laroy W, Fierro MA, Hudson SA, Von Seggern CE, Cotter RJ, Bochner BS, Tiemeyer M, Konstantopoulos K, Schnaar RL. E-selectin receptors on human leukocytes. Blood. 2008; 112:3744–3752. [PubMed: 18579791]
22. Puri KD, Finger EB, Springer TA. The faster kinetics of L-selectin than of E-selectin and P-selectin rolling at comparable binding strength. J Immunol. 1997; 158:405–413. [PubMed: 8977216]

23. Varki A. Selectin ligands: Will the real ones please stand up? J Clin Invest. 1997; 99:158–162. [PubMed: 9005982]
24. McEver RP, Cummings RD. Role of PSGL-1 binding to selectins in leukocyte recruitment. J Clin Invest. 1997; 100:S97–103. [PubMed: 9413410]
25. Walcheck B, Moore KL, McEver RP, Kishimoto TK. Neutrophil-neutrophil interactions under hydrodynamic shear stress involve L-selectin and PSGL-1. A mechanism that amplifies initial leukocyte accumulation of P-selectin in vitro. J Clin Invest. 1996; 98:1081–1087. [PubMed: 8787668]
26. Lee AW, Truong T, Bickham K, Fonteneau JF, Larsson M, Da Silva I, Somersan S, Thomas EK, Bhardwaj N. A clinical grade cocktail of cytokines and PGE2 results in uniform maturation of human monocyte-derived dendritic cells: Implications for immunotherapy. Vaccine. 2002; 20(Suppl 4):A8–A22. [PubMed: 12477423]
27. Crespo HJ, Cabral MG, Teixeira AV, Lau JT, Trindade H, Videira PA. Effect of sialic acid loss on dendritic cell maturation. Immunology. 2009; 128:e621–31. [PubMed: 19740323]
28. Ross EL, Barker JN, Allen MH, Chu AC, Groves RW, MacDonald DM. Langerhans' cell expression of the selectin ligand, sialyl lewis x. Immunology. 1994; 81:303–308. [PubMed: 7512530]
29. Ellies LG, Sperandio M, Underhill GH, Yousif J, Smith M, Priatel JJ, Kansas GS, Ley K, Marth JD. Sialyltransferase specificity in selectin ligand formation. Blood. 2002; 100:3618–3625. [PubMed: 12393657]
30. Schroder K, Sweet MJ, Hume DA. Signal integration between IFNgamma and TLR signalling pathways in macrophages. Immunobiology. 2006; 211:511–524. [PubMed: 16920490]
31. Frasca L, Nasso M, Spensieri F, Fedele G, Palazzo R, Malavasi F, Ausiello CM. IFN-gamma arms human dendritic cells to perform multiple effector functions. J Immunol. 2008; 180:1471–1481. [PubMed: 18209042]

Highlights

- The role of the selectin binding determinant sLe^x was studied in mo-DCs.
- sLe^x is required for maximal mo-DC binding to endothelial cells.
- Mo-DCs bind to selectins via a sLe^x-dependent pathway.
- PSGL-1 is indispensable for P- and L-, but not E-selectin recognition.
- IFN-γ increases sLe^x expression in mo-DCs, probably by enhancing C2GnT-I synthesis.

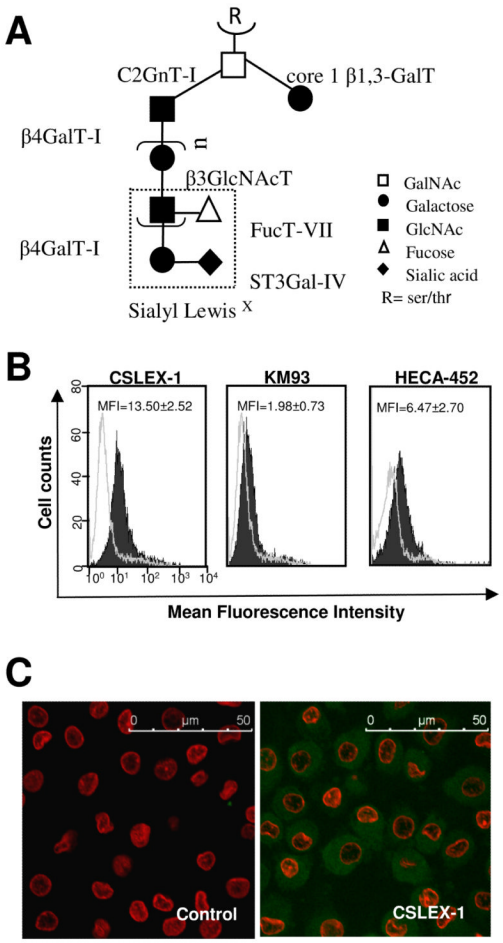

Fig. 1.

sLe^x expression in mo-DCs. A: simplified biosynthetic pathway of sLe^x core 2 decorated O-glycans, with the involved glycosyltransferases represented. B: Flow cytometry histograms of sLe^x expression in mo-DCs from a representative donor. Mo-DCs were stained with CSLEX-1, KM93 or HECA-452 (filled histograms) or with secondary antibody only (open histograms). The mean fluorescence intensity (MFI) ± SE of sLe^x labeling with CSLEX-1 (n=9 independent experiments), KM93 (n=3) or HECA-452 (n=3) antibodies is shown. C: Confocal laser-scanning microscopy of mo-DCs stained with secondary antibody only (control) or CSLEX-1 primary antibody.

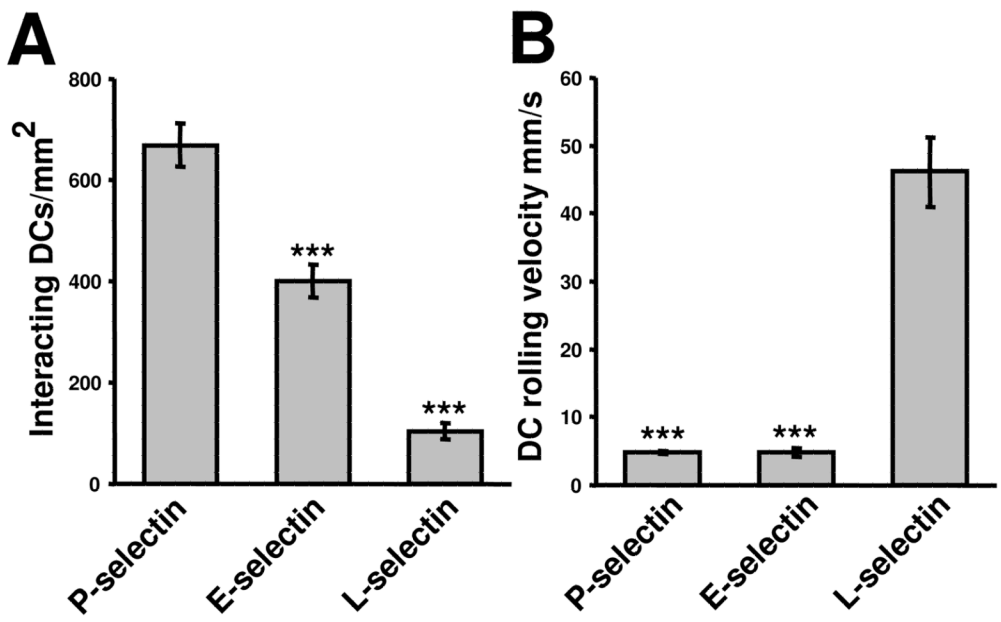

Fig. 2.

Adhesion of mo-DCs to immobilized selectins under flow. Mo-DCs were perfused over P-, E- or L- selectin immobilized surface at 1 dyn/cm^2 in a microfluidic device. A: The number of interacting mo-DCs per mm^2 was quantified using videomicroscopy after 3 min of cell perfusion. The data represent the mean ± SE of at least three independent experiments. B: The average mo-DC rolling velocity was calculated by videomicroscopy/digital image processing using at least 20 cells from 3 independent experiments. (***P<0.001).

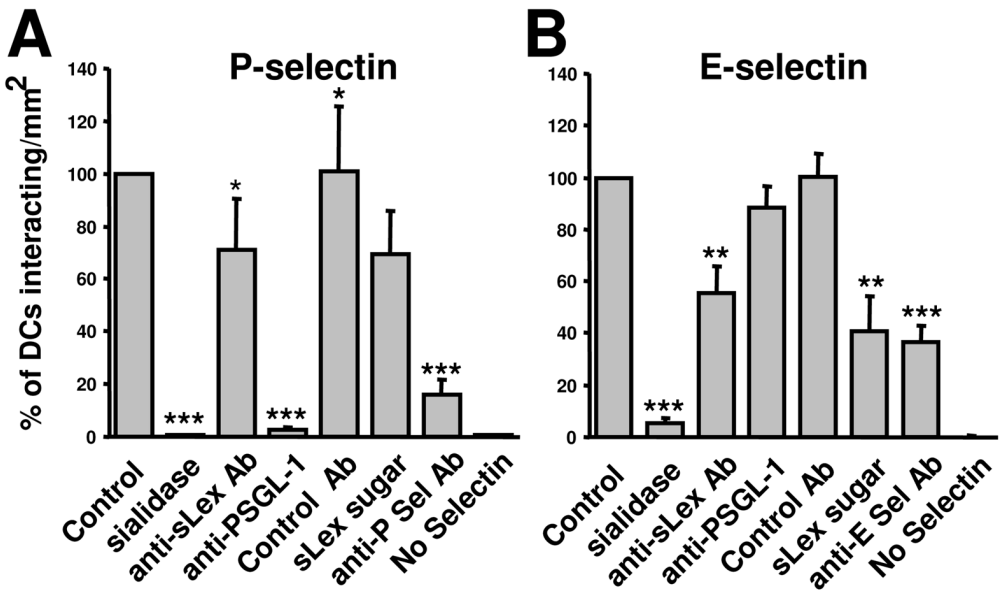

Fig. 3.

Contribution of sLe^x to mo-DC:selectin interactions under flow. Mo-DCs were perfused over immobilized P-selectin (A) or E- selectin (B) for 3 min at 1 dyn/cm^2. In select experiments, mo-DCs were treated with sialidase, or incubated with an anti-sLe^x or an anti-PSGL-1 or an isotype control antibody (Control) before perfusion over immobilized selectins. In other experiments, the P- or E-selectin-coated slides were incubated with an anti-P-selectin or an anti-E-selectin antibody or with the sLe^x sugar. Control experiments without immobilized selectins were carried out to test any non-specific binding. The number of interacting mo-DCs per mm^2 with P- or E-selectin-coated surfaces was quantified for each experimental condition. Data are reported as percentage of interacting cells relative to untreated mo-DCs (control). Data represents the mean ± SE of at least three independent experiments (** $P<0.01$, *** $P<0.001$).

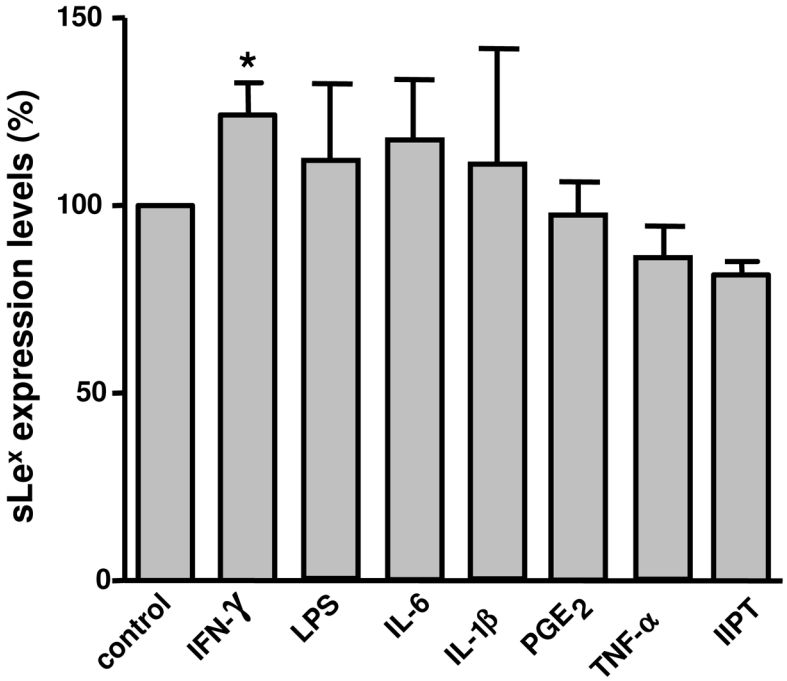

Fig. 4.

Effect of maturation stimuli on sLe^x expression. The maturation of mo-DCs was achieved by treating mo-DCs with either IFN-γ, LPS, IL-6, IL-1β, PGE_2, TNF-α alone or a cocktail mixture of IL-6, IL-1β, PGE_2 plus TNF-α (IIPT). The expression of sLe^x was analyzed by flow cytometry using an anti-sLe^x antibody (CSLEX-1). The sLe^x expression levels are reported as percentage of CSLEX-1 staining relative to that in immature untreated mo-DCs (control). Data represent the mean ± SE of at least three independent experiments (*P<0.05).

Table 1

Contribution of sLe^x to mo-DC interaction with HUVECs under static conditions

Assay condition	% of Interacting Cells	*P* Value
control	43.8±5.9	—
sialidase treatment	33.3±8.4	0.020
anti-sLe^x	42.0±3.0	0.250
sugar sLe^x	37.7±9.9	0.395
anti-E-selectin	37.0±9.1	0.228
No Ca^{2+}/Mg^{2+}	11.5±0.5	0.005
No TNF-α	25.5±5.8	0.020

Mo-DCs were overlaid on TNF-α-stimulated HUVECs and incubated for 30 min at 37°C. Values represent the percentage of interacting mo-DCs with HUVECs determined for the following conditions: no treatment (control), sialidase treatment of mo-DCs [17, 18], pre-incubation of mo-DCs with an anti-sLe^x antibody or in the presence of sLe^x sugar. In select experiments, HUVECs were pre-incubated with an anti-E-selectin antibody prior to the addition of mo-DCs. Other control experiments were performed using PBS lacking Ca^{2+}/Mg^{2+} or non-TNF-α-stimulated HUVECs. Data represent the mean ± SE of n=2–5 experiments.

Table 2

Effect of IFN-γ on the gene expression of glycosyltransferases involved in the sLe^x biosynthesis in mo-DCs

	mRNA expression level		
Glycosyltransferases	**Non-stimulated**	**IFN-γ stimulated**	***P Value***
ST3Gal-III	0.38±0.04	0.15±0.03	0.005
ST3Gal-IV	0.14±0.04	0.098±0.02	0.351
ST3Gal-VI	3.17±0.40	2.89±0.39	0.647
FucT-IV	1.60±0.42	1.68±0.31	0.875
FucT-VII	1.92±0.68	2.24±0.45	0.699
34GalT-I	34.38±14.99	37.62±8.24	0.859
C2GnT-I	5.55±2.49	10.45±2.76	0.035

The expression of relevant glycosyltransferases involved in the biosynthetic pathway of sLe^x core 2 decorated O-glycans (Fig. 1A) was evaluated by quantitative real-time PCR in total RNA extracted from 1×10^6 cells. The mRNA expression levels of the glycosyltransferases in non-stimulated and IFN-γ stimulated mo-DCs are expressed as the permillage (‰) of the expression of the endogenous controls (β-actin/GAPDH mean). Data represent the mean ± SE of at least three independent assays.