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Role of the C-type lectins DC-SIGN and L-SIGN in *Leishmania* interaction with host phagocytes

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Abstract

Leishmaniasis is a parasitic disease that courses with cutaneous or visceral clinical manifestations. The amastigote stage of the parasite infects phagocytes and modulates the effector function of the host cells. Our group has described that the interaction between *Leishmania* and immature monocyte-derived dendritic cells (DCs) takes place through dendritic cell-specific ICAM-3-grabbing nonintegrin (DC-SIGN), a C-type lectin that specifically recognizes fungal, viral and bacterial pathogens. The DC-SIGN-mediated recognition of *Leishmania* amastigotes does not induce DC maturation, and the DC-SIGN ligand/s on *Leishmania* parasites is/are still unknown. We have also found that the DC-SIGN-related molecule L-SIGN, specifically expressed in lymph node and liver sinusoidal endothelial cells, acts as a receptor for *L. infantum*, the parasite responsible for visceral leishmaniasis, but does not recognize *L. pifanoi*, which causes the cutaneous form of the disease. Therefore, DC-SIGN and L-SIGN differ in their ability to interact with *Leishmania* species responsible for either visceral or cutaneous leishmaniasis. A deeper knowledge of the parasite-C-type lectin interaction may be helpful for the design of new DC-based therapeutic vaccines against *Leishmania* infections.

Keywords: DC-SIGN; Dendritic cell; Infection; Leishmania; L-SIGN

Leishmaniasis

Leishmaniasis is a vector-borne parasitic disease whose clinical manifestations range from local cutaneous lesions (cutaneous leishmaniasis, CL) to life-threatening visceral disease (visceral leishmaniasis, VL), mainly as a result of differences among *Leishmania* species. *L. pifanoi* is responsible for CL, characterized by a localized infection at the site of inoculation by the

Abbreviations: CL, cutaneous leishmaniasis; DC, dendritic cell; DC-SIGN, dendritic cell-specific ICAM-3-grabbing nonintegrin (CD209); HSEC, hepatic sinusoidal endothelial cells; IMDDCs, immature monocyte-derived dendritic cells; LPG, lipophosphoglycan; CR3, complement receptor type 3; L-SIGN, liver-SIGN (DC-SIGNR, CD209L); VL, visceral leishmaniasis

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vector, while *L. infantum* causes VL due to parasite dissemination into internal organs. *Leishmania* parasites exist in two developmental stages. The flagellated promastigote is transmitted with the bite of the sand fly (insect vector) to the mammalian host, where it transforms into the amastigote stage. *Leishmania* amastigotes infect mononuclear phagocytes, where their intracellular location allows them to subvert the effector and regulatory functions of these cells (Duclos and Desjardins, 2000). Since epidermal Langerhans cells and dermal dendritic cells (DCs) contribute to immunosurveillance of the skin (Banchereau and Steinman, 1998; Mellman and Steinman, 2001) and are located in proximity to the site of parasite delivery, their role in the initiation of *Leishmania*-specific immune responses is an active area of research.

Leishmania host cells

To attain a successful infection, *Leishmania* needs to subvert the host immune response from the early steps after its inoculation. In the natural course of infection, these events occur in the dermis, where DCs may act as host cells for *Leishmania*, independently of the pathological outcome of the infection (McDowell et al., 2002). In this regard, Langerhans cells within cutaneous lesions are parasitized by *Leishmania* in vivo in both human and experimental murine CL (Blank et al., 1993; Moll, 1993). Studies on the interactions of *Leishmania* with murine or human DC have not yet clearly determined the range of parasite forms that these cells can internalize (Amprey et al., 2004; Bennett et al., 2001; Blank et al., 1993; Konecny et al., 1999; Marovich et al., 2000; Moll, 2000; Moll and Flohe, 1997; Qi et al., 2001; Sacks and Sher, 2002; Udey et al., 2001; von Stebut et al., 1998, 2000) or their influence on parasite survival.

Although *Leishmania* species might differentially subvert DC effector function (Antoine et al., 2004; Brandonisio et al., 2004; Chaussabel et al., 2003; Ghosh and Bandyopadhyay, 2004; Jebbari et al., 2002; Konecny, et al., 1999; Ponte-Sucre et al., 2001; Scott and Hunter, 2002; von Stebut et al., 1998), the receptors involved in the *Leishmania*–DC interaction remain largely undefined and could be critical for this process. In contrast, several macrophage receptors have been identified which mediate binding and subsequent uptake of *Leishmania* promastigotes (Blackwell, 1985; Russell and Talamas-Rohana, 1989). In this regard, lipophosphoglycan (LPG) and the metalloproteinase gp63 bind to complement receptor type 3 (CR3), mannose-fucose, and fibronectin receptors on macrophages (Blackwell, 1985; Da Silva et al., 1989; Guy and Belosevic, 1993; Mosser, 1994; Talamas-Rohana et al., 1990; Wilson and Pearson, 1988). However, the receptors implicated in amastigote uptake by DC and macrophages are poorly characterized, mainly due to the fact that isolation or in vitro culture of this intracellular form is difficult. The availability of axenic cultures has now opened the possibility of addressing the identification of *Leishmania* amastigotes receptors on macrophages and DCs (Armson et al., 1999; Bates et al., 1992; Debrabant et al., 2004; Doyle et al., 1991; Gupta et al., 2001; Hodgkinson et al., 1996).

Leishmania interaction with dendritic cells: exploiting DC-SIGN

Macrophages and DCs express a wide variety of pathogen-associated molecular pattern receptors, including numerous C-type lectin and lectin-like receptors (Engering et al., 2002; Figdor et al., 2002; McGreal et al., 2004). Since *Leishmania* spp. display an abundance of mannose-rich glycoconjugates on their surface that are important for parasite virulence (Ilgoutz and McConville, 2001; Garami and Ilg, 2001), a reasonable hypothesis is that lectin–oligosaccharide interactions are involved in parasite recognition by mononuclear phagocytes. DC-specific ICAM-3-grabbing nonintegrin (DC-SIGN, CD209) is a type II transmembrane C-type lectin expressed on DCs and macrophages, and was initially described as involved in cell–cell interactions through its capacity to bind ICAM-3 and ICAM-2 (Geijtenbeek et al., 2000a, b; van Kooyk and Geijtenbeek, 2002). The DC-SIGN extracellular domain comprises eight 23-residue tandem repeats and a C-terminal carbohydrate-recognition domain (Mitchell et al., 2001). DC-SIGN is now known to be a receptor for HIV (Geijtenbeek et al., 2000c; Pohlmann et al., 2001), *Ebola* virus (Alvarez et al., 2002), *Schistosoma mansoni* (van Die et al., 2003), *Sindbis* virus (Klimstra et al., 2003) *Candida albicans* (Cambi et al., 2003) *Mycobacterium tuberculosis* (Geijtenbeek et al., 2003), Hepatitis C (Wang et al., 2004), *Helicobacter pylori* (Bergman et al., 2004) and the fungal pathogen *Aspergillus fumigatus* (Serrano-Gomez et al., 2004).

Most *Leishmania* amastigote-DC studies have been carried out with tissue-derived opsonized parasites, which might be bound via Fc and complement receptors, thus precluding the identification of opsonization-independent binding mechanisms. To analyze the participation of the receptor DC-SIGN in binding and internalization of *Leishmania*, we used axenic amastigotes (Armson et al., 1999; Pan and McMahon-Pratt, 1988), which are devoid of opsonizing antibodies. We first analyzed the interaction of *L. pifanoi* (that causes CL) axenic amastigote with K562-DC-SIGN transfectants and demonstrated that they are specifically recognized by DC-SIGN (Colmenares et al., 2002).

Subsequently, *L. pifanoi* amastigotes were found to bind DC-SIGN on the surface of immature monocyte-derived dendritic cells (IMDDCs), an interaction that was dramatically reduced in the presence of anti-DC-SIGN blocking antibodies (Colmenares et al., 2002). This set of results suggested an important role for DC-SIGN in the early stages of infection of DCs by *Leishmania*.

Since infection of immature dermal DCs is a common step shared by all *Leishmania* species, we next analyzed the capacity of IMDDCs to bind other *Leishmania* life cycle forms and species. Our results (Colmenares et al., 2004) underscored the relevance of the DC-SIGN–*Leishmania* interaction in both VL (*L. infantum*) and CL (*L. pifanoi*), as amastigotes and promastigotes from both species exhibited DC-SIGN-interaction capacity. Since the membrane composition of the parasite changes throughout its life cycle (Bahr et al., 1993; Wright and el Amin, 1989), we tested the ability of DC-SIGN to bind the three main life cycle forms of the parasite (amastigotes, procyclic promastigotes and metacyclic promastigotes). Amastigotes and metacyclic promastigotes showed the strongest DC-SIGN-dependent interaction with IMDDC (Fig. 1). Moreover, the avidity for DC-SIGN increased in the transition from procyclic (non-infective) to metacyclic (infective) promastigotes (i.e., procyclic and metacyclic promastigotes, respectively) (Fig. 1). Hence, the avidity of the different forms of the parasite for DC-SIGN appears to correlate with their virulence. On the other hand, a much lower ability for DC-SIGN recognition was observed in *Leishmania major* promastigotes (Fig. 1).

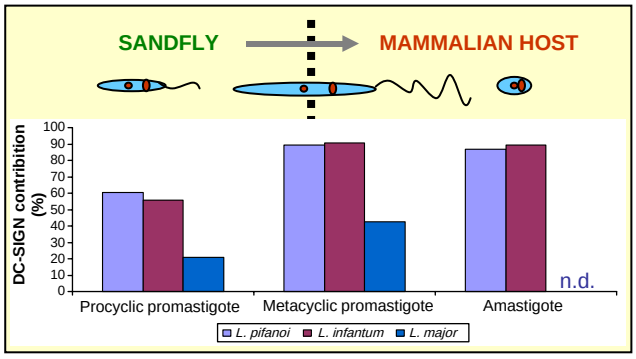

Fig. 1. Summary of experiments evaluating the contribution of DC-SIGN to immature monocyte-derived dendritic cell binding to different *Leishmania* life cycle forms and species. Immature monocyte-derived DCs were prepared as previously described, left untreated or pre-treated 10 min at 25 °C with the blocking anti-DC-SIGN monoclonal antibody (Relloso et al., 2002) and incubated with CFSE-labeled parasites at a 1:5 ratio for 1 h at 35 °C. Afterward, the percentage of cells with bound parasites was quantified by flow cytometry and the contribution of DC-SIGN (%) was calculated as: 100−((% MR-1-treated cells with bound parasites × 100)/% untreated cell with bound parasites).

DC-SIGN ligands on Leishmania: LPG is one of the most abundant glycoconjugates exposed on the cell surface of promastigotes, but scarcely expressed on amastigotes, and plays a pleiotropic role through the life cycle of *Leishmania* (Aebischer et al., 2005; Cunningham, 2002; Kamhawi et al., 2000; Naderer et al., 2004; Sacks et al., 2000; Turco and Descoteaux, 1992; Turco et al., 2001). This molecule is characterized by a high mannose content, and has been proposed to mediate promastigote interaction with DCs via DC-SIGN (Appelmelk et al., 2003). However, our results indicate that LPG is not an important *Leishmania* ligand for DC-SIGN because: (1) LPG is strongly down-regulated in amastigotes (Ilg et al., 1999; Ilgoutz and McConville, 2001; Turco and Descoteaux, 1992), which exhibit the highest DC-SIGN-binding ability; (2) LPG was unable to block *Leishmania* binding to DC-SIGN for all species and parasite developmental stages assayed; and (3) the LPG-defective *L. donovani* promastigotes (R2D2) bind to DC-SIGN-expressing cells. Furthermore, since LPG-defective promastigotes bound DC-SIGN with higher avidity than their wild-type counterparts, LPG might in fact mask other promastigote membrane ligands with affinity for DC-SIGN (Colmenares et al., 2004).

Leishmania ability to modulate the DC maturation state: Conflicting results have been obtained on the ability of various *Leishmania* species to induce DC maturation (Antoine et al., 2004). It is currently unknown whether this variability is due to variations in the experimental conditions or truly reflects species-specific or strain-specific interactions between *Leishmania* and DC. Since *Leishmania* spp. express highly polymorphic cell surface molecules, the DC response might vary according to the *Leishmania* species examined (Bennett et al., 2001; Flohe et al., 1998; Henri et al., 2002; Konecny et al., 1999; McDowell et al., 2002; von Stebut et al., 1998, 2000). We have evaluated the ability of *L. infantum* to alter the maturation state of IMDDC. Unlike LPS, *L. infantum* amastigotes, which bind to IMDDC via DC-SIGN, did not affect the cell surface expression of CD83, CD86 or MHC II, commonly considered as DC maturation parameters. Therefore, our results suggest that non-opsonized *L. infantum* amastigotes are unable to induce IMDDC maturation, at least during a 48-hour period (Fig. 2A). Moreover, LPS induced maturation of IMDDCs infected with *L. infantum* amastigotes (Fig. 2A), indicating that the parasites do not inhibit the capacity of DCs to be matured by other pathogen-derived products. The failure of IMDDC to mature in response to *Leishmania* capture and entry might represent a parasite strategy to avoid immunosurveillance and to allow their establishment and multiplication before the onset of immune responses. On the other hand, *L. infantum* amastigotes did not induce CCR7-directed IMDDC migration but

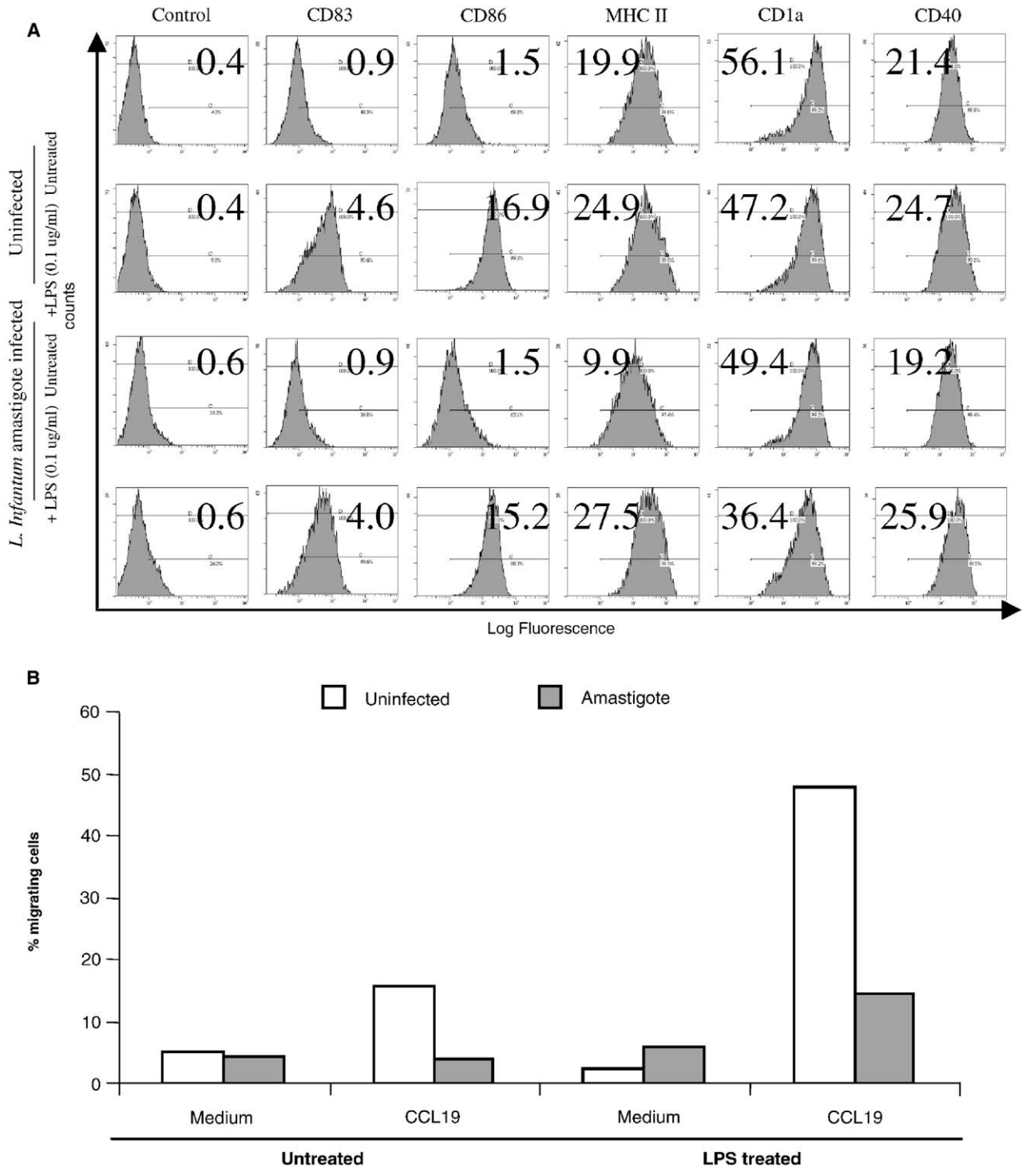

Fig. 2. Effect of *Leishmania* infection on dendritic cells maturation and migration. Immature monocyte-derived DCs were left untreated or pretreated for 1 h with LPS before adding or not *L. infantum* axenic amastigotes, and then incubated for 48 h. Afterward, cells were phenotypically characterized (A), and subjected to migration assays towards the CCR7 ligand CCL19 (B). Three independent experiments were performed with similar results, and a representative experiment is shown.

inhibited the CCR7-dependent migration induced upon LPS maturation (Fig. 2B). These data are in agreement with previous results demonstrating that cytokines abundantly produced during *Leishmania* infection (e.g., IL-10) down-regulate CCR7 expression (Antoine et al., 2004). Therefore, *Leishmania* might prevent the establishment of T cell-mediated immunity by interfering with the migratory properties of DCs.

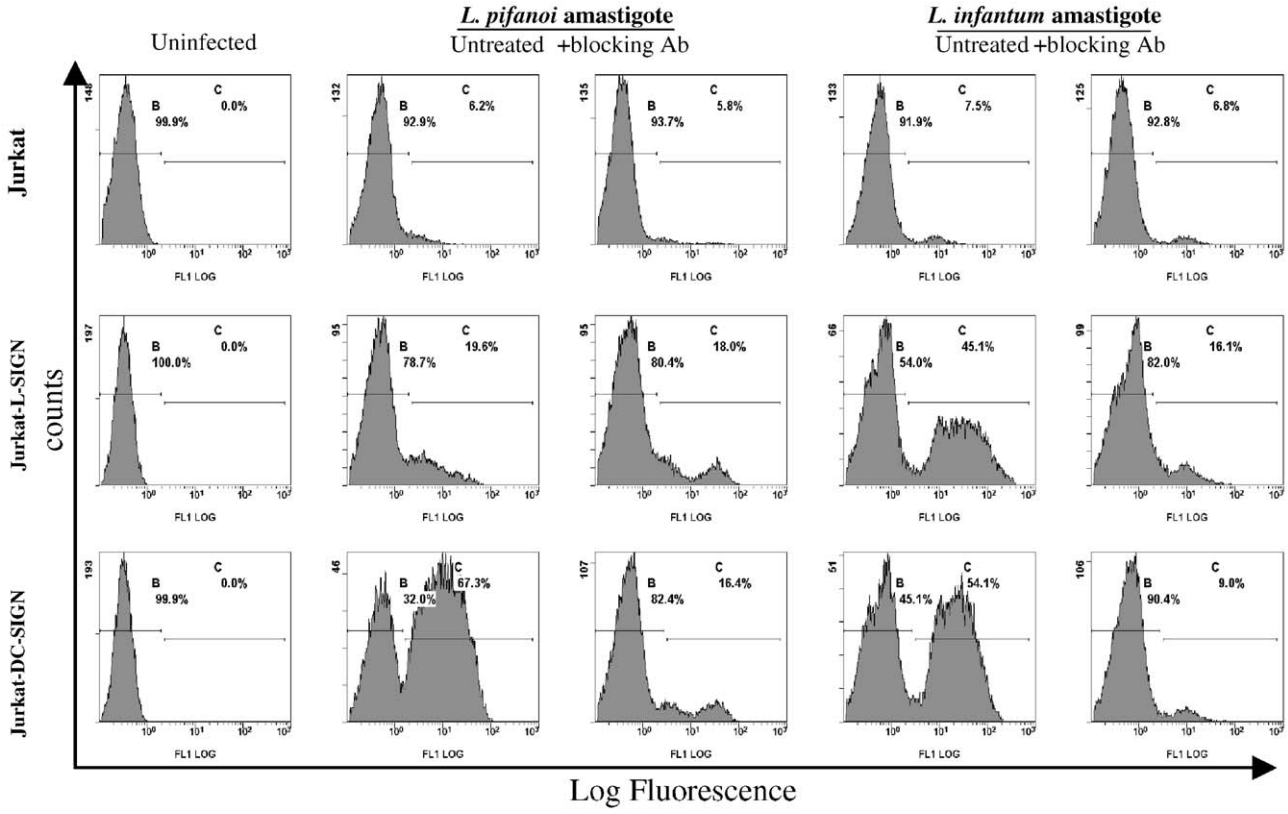

Fig. 3. *Leishmania infantum* binding to Jurkat cells expressing L-SIGN. L-SIGN-transfected Jurkat cells (Jurkat-L-SIGN), Jurkat-DC-SIGN and mock-transfected Jurkat cells (Alvarez et al., 2002) were left untreated or pretreated with receptor-specific blocking antibodies, and then incubated at 35 °C with the indicated CFSE-labeled parasites (1:5 cell:parasite ratio), or left uninfected (Colmenares et al., 2002). The percentage of cells with bound parasites was quantified by flow cytometry. Three independent experiments were performed with similar results, and a representative experiment is shown.

L-SIGN: a receptor implicated in VL: L-SIGN is a close homologue of DC-SIGN (77% amino acid sequence identity) that is expressed on human liver and lymph node sinusoidal endothelial cells (Soilleux et al., 2000). Like DC-SIGN, L-SIGN recognizes and binds high-mannose glycans, binds to ICAM-3 (Bashirova et al., 2001), and recognizes carbohydrate structures on pathogens such as ManLAM on *M. tuberculosis* (Koppel et al., 2004) and high-mannose moieties on HIV-1, HCV and Ebola. Besides, L-SIGN has been described as a receptor for severe acute respiratory syndrome coronavirus (Jeffers et al., 2004). Unlike DC-SIGN, L-SIGN does not bind to the fucose-containing Lewis^x antigens, suggesting that L-SIGN-expressing liver endothelial cells and lymph node are not involved in capture and internalization of Lewis^x-containing pathogens such as *H. pylori* and *S. mansoni* (Van Liempt et al., 2004). Besides, binding to L-SIGN is not reversible at low pH, suggesting that L-SIGN does not release internalized ligand in low-pH vesicles and that L-SIGN is degraded upon internalization (Guo et al., 2004). Because of their similar ligand specificity but differential tissue location, we have compared the capacity of DC-SIGN and L-SIGN to bind axenic amastigotes from *L. pifanoi* (responsible for CL), and *L. infantum* (responsible for VL). Binding experiments with Jurkat cells stably transfected with DC-SIGN or L-SIGN indicated that *L. infantum* amastigotes specifically bound to both DC-SIGN and L-SIGN, whereas *L. pifanoi* amastigotes were unable to bind to L-SIGN (Fig. 3). Therefore, only VL-causing parasites (*L. infantum*) appear to be recognized by L-SIGN. These results suggest that L-SIGN recognition of the distinct *Leishmania* species might play a role in the outcome of the parasite infection (CL vs. VL).

To further evaluate the relevance of L-SIGN in binding of *Leishmania* species causing VL, human hepatic sinusoidal endothelial cells (HSEC) were isolated from hepatic surgery donors, using a modification (Iñigo Martinez and Fernando Vidal-Vanaclocha, unpublished information) of previously described isolation procedures (Daneker et al., 1998; Heuff et al., 1994). Incubation of HSEC with axenic amastigotes showed that *L. infantum* amastigotes bound strongly to HSEC, whereas no binding was observed with *L. pifanoi* amastigotes (Fig. 4). In addition, the HSEC-*L. infantum*

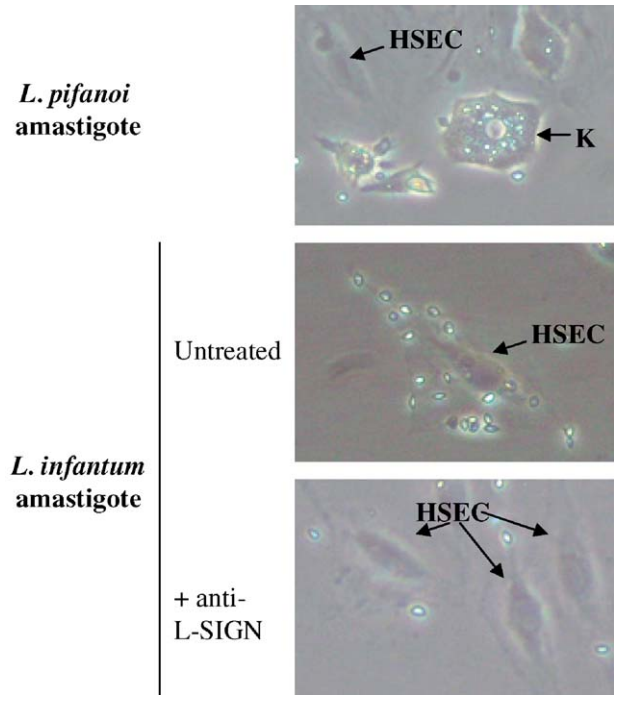

Fig. 4. *L. infantum* binding to hepatic sinusoidal endothelial cells (HSEC) is partially mediated by L-SIGN. Human HSEC were isolated from liver biopsies, and subjected to binding assays with *L. pifanoi* or *L. infantum* axenic amastigotes, as described (Colmenares et al., 2002). After incubation and washing, cells were photographed (HSEC, hepatic sinusoidal endothelial cells; K, Kupffer cells). Two independent experiments were performed with similar results, and a representative experiment is shown.

amastigote interaction was reduced in the presence of a blocking monoclonal antibody against L-SIGN (Fig. 4). These results confirmed the ability of VL-causing amastigotes to interact with L-SIGN. Given the mechanism described for the hepatitis C virus (Cormier et al., 2004), it is tempting to speculate that L-SIGN-mediated capture of *L. infantum* by HSEC could result in transinfection of Kupffer cells, which are the final targets of VL-causing *Leishmania* parasites (el Hag et al., 1994; Murray, 2001).

Concluding remarks

Taken together, the present work demonstrates that the C-type lectins DC-SIGN and L-SIGN are broad *Leishmania* receptors that differentially bind the distinct infective forms and species of the parasite. A deeper knowledge of the *Leishmania*–DC/L-SIGN interactions, and the subsequent immune consequences, may pave the way for the design of new therapeutic approaches against leishmaniasis. The recent description that macrophage treated with IL-4 (alternatively activated macrophages) also express DC-SIGN (Puig-Kroger et al., 2004) could be of major importance, due to the fact that the expression of this cytokine correlates with the pathology of the disease, and hence increases the potential relevance of these C-type lectins in leishmaniasis.

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References

Aebischer, T., Bennett, C.L., Pelizzola, M., Vizzardelli, C., Pavelka, N., Urbano, M., Capozzoli, M., Luchini, A., Ilg, T., Granucci, F., Blackburn, C.C., Ricciardi-Castagnoli, P., 2005. A critical role for lipophosphoglycan in proinflammatory responses of dendritic cells to *Leishmania mexicana*. Eur. J. Immunol. 35, 476–486.

Alvarez, C.P., Lasala, F., Carrillo, J., Muniz, O., Corbi, A.L., Delgado, R., 2002. C-type lectins DC-SIGN and L-SIGN mediate cellular entry by Ebola virus in cis and in trans. J. Virol. 76, 6841–6844.

Amprey, J.L., Spath, G.F., Porcelli, S.A., 2004. Inhibition of CD1 expression in human dendritic cells during intracellular infection with *Leishmania donovani*. Infect. Immun. 72, 589–592.

Antoine, J.C., Prina, E., Courret, N., Lang, T., 2004. *Leishmania* spp.: on the interactions they establish with antigen-presenting cells of their mammalian hosts. Adv. Parasitol. 58, 1–68.

Appelmelk, B.J., van Die, I., van Vliet, S.J., Vandenbroucke-Grauls, C.M., Geijtenbeek, T.B., van Kooyk, Y., 2003. Cutting edge: carbohydrate profiling identifies new pathogens that interact with dendritic cell-specific ICAM-3-grabbing nonintegrin on dendritic cells. J. Immunol. 170, 1635–1639.

Armson, A., Kamau, S.W., Grimm, F., Reynoldson, J.A., Best, W.M., MacDonald, L.M., Thompson, R.C., 1999. A comparison of the effects of a benzimidazole and the dinitroanilines against *Leishmania infantum*. Acta Trop. 73, 303–311.

Bahr, V., Stierhof, Y.D., Ilg, T., Demar, M., Quinten, M., Overath, P., 1993. Expression of lipophosphoglycan, high-molecular weight phosphoglycan and glycoprotein 63 in promastigotes and amastigotes of *Leishmania mexicana*. Mol. Biochem. Parasitol. 58, 107–121.

Banchereau, J., Steinman, R.M., 1998. Dendritic cells and the control of immunity. Nature 392, 245–252.

Bashirova, A.A., Geijtenbeek, T.B., van Duijnhoven, G.C., van Vliet, S.J., Eilering, J.B., Martin, M.P., Wu, L., Martin, T.D., Viebig, N., Knolle, P.A., KewalRamani, V.N., van Kooyk, Y., Carrington, M., 2001. A dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN)-related protein is highly expressed on human liver sinusoidal endothelial cells and promotes HIV-1 infection. J. Exp. Med. 193, 671–678.

Bates, P.A., Robertson, C.D., Tetley, L., Coombs, G.H., 1992. Axenic cultivation and characterization of *Leishmania mexicana* amastigote-like forms. Parasitology 105 (Pt 2), 193–202.

Bennett, C.L., Misslitz, A., Colledge, L., Aebischer, T., Blackburn, C.C., 2001. Silent infection of bone marrow-derived dendritic cells by *Leishmania mexicana* amastigotes. Eur. J. Immunol. 31, 876–883.

Bergman, M.P., Engering, A., Smits, H.H., van Vliet, S.J., van Bodegraven, A.A., Wirth, H.P., Kapsenberg, M.L., Vandenbroucke-Grauls, C.M., van Kooyk, Y., Appelmelk, B.J., 2004. Helicobacter pylori modulates the T helper cell 1/T helper cell 2 balance through phase-variable interaction between lipopolysaccharide and DC-SIGN. J. Exp. Med. 200, 979–990.

Blackwell, J.M., 1985. Receptors and recognition mechanisms of *Leishmania* species. Trans. R. Soc. Trop. Med. Hyg. 79, 606–612.

Blank, C., Fuchs, H., Rappersberger, K., Rollinghoff, M., Moll, H., 1993. Parasitism of epidermal Langerhans cells in experimental cutaneous leishmaniasis with *Leishmania major*. J. Infect. Dis. 167, 418–425.

Brandonisio, O., Spinelli, R., Pepe, M., 2004. Dendritic cells in *Leishmania* infection. Microbes Infect. 6, 1402–1409.

Cambi, A., Gijzen, K., de Vries, J.M., Torensma, R., Joosten, B., Adema, G.J., Netea, M.G., Kullberg, B.J., Romani, L., Figdor, C.G., 2003. The C-type lectin DC-SIGN (CD209) is an antigen-uptake receptor for *Candida albicans* on dendritic cells. Eur. J. Immunol. 33, 532–538.

Chaussabel, D., Semnani, R.T., McDowell, M.A., Sacks, D., Sher, A., Nutman, T.B., 2003. Unique gene expression profiles of human macrophages and dendritic cells to phylogenetically distinct parasites. Blood 102, 672–681.

Colmenares, M., Puig-Kroger, A., Pello, O.M., Corbi, A.L., Rivas, L., 2002. Dendritic cell (DC)-specific intercellular adhesion molecule 3 (ICAM-3)-grabbing nonintegrin (DC-SIGN, CD209), a C-type surface lectin in human DCs, is a receptor for *Leishmania* amastigotes. J. Biol. Chem. 277, 36766–36769.

Colmenares, M., Corbi, A.L., Turco, S.J., Rivas, L., 2004. The dendritic cell receptor DC-SIGN discriminates among species and life cycle forms of *Leishmania*. J. Immunol. 172, 1186–1190.

Cormier, E.G., Durso, R.J., Tsamis, F., Boussemart, L., Manix, C., Olson, W.C., Gardner, J.P., Dragic, T., 2004. L-SIGN (CD209L) and DC-SIGN (CD209) mediate transinfection of liver cells by hepatitis C virus. Proc. Natl. Acad. Sci. USA 101, 14067–14072.

Cunningham, A.C., 2002. Parasitic adaptive mechanisms in infection by *leishmania*. Exp. Mol. Pathol. 72, 132–141.

Da Silva, R.P., Hall, B.F., Joiner, K.A., Sacks, D.L., 1989. CR1, the C3b receptor, mediates binding of infective *Leishmania major* metacyclic promastigotes to human macrophages. J. Immunol. 143, 617–622.

Daneker, G.W., Lund, S.A., Caughman, S.W., Swerlick, R.A., Fischer, A.H., Staley, C.A., Ades, E.W., 1998. Culture and characterization of sinusoidal endothelial cells isolated from human liver. In Vitro Cell Dev. Biol. Anim. 34, 370–377.

Debrabant, A., Joshi, M.B., Pimenta, P.F., Dwyer, D.M., 2004. Generation of *Leishmania donovani* axenic amastigotes: their growth and biological characteristics. Int. J. Parasitol. 34, 205–217.

Doyle, P.S., Engel, J.C., Pimenta, P.F., da Silva, P.P., Dwyer, D.M., 1991. *Leishmania donovani*: long-term culture of axenic amastigotes at 37 degrees C. Exp. Parasitol. 73, 326–334.

Duclos, S., Desjardins, M., 2000. Subversion of a young phagosome: the survival strategies of intracellular pathogens. Cell Microbiol. 2, 365–377.

el Hag, I.A., Hashim, F.A., el Toum, I.A., Homeida, M., el Kalifa, M., el Hassan, A.M., 1994. Liver morphology and function in visceral leishmaniasis (Kala-azar). J. Clin. Pathol. 47, 547–551.

Engering, A., Geijtenbeek, T.B., van Kooyk, Y., 2002. Immune escape through C-type lectins on dendritic cells. Trends Immunol 23, 480–485.

Figdor, C.G., van Kooyk, Y., Adema, G.J., 2002. C-type lectin receptors on dendritic cells and Langerhans cells. Nat. Rev. Immunol. 2, 77–84.

Flohe, S.B., Bauer, C., Flohe, S., Moll, H., 1998. Antigen-pulsed epidermal Langerhans cells protect susceptible mice from infection with the intracellular parasite *Leishmania major*. Eur. J. Immunol. 28, 3800–3811.

Garami, A., Ilg, T., 2001. Disruption of mannose activation in *Leishmania mexicana*: GDP-mannose pyrophosphorylase is required for virulence, but not for viability. EMBO J. 20, 3657–3666.

Geijtenbeek, T.B., Krooshoop, D.J., Bleijs, D.A., van Vliet, S.J., van Duijnhoven, G.C., Grabovsky, V., Alon, R., Figdor, C.G., van Kooyk, Y., 2000a. DC-SIGN-ICAM-2 interaction mediates dendritic cell trafficking. Nat. Immunol. 1, 353–357.

Geijtenbeek, T.B., Torensma, R., van Vliet, S.J., van Duijnhoven, G.C., Adema, G.J., van Kooyk, Y., Figdor, C.G., 2000b. Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. Cell 100, 575–585.

Geijtenbeek, T.B., Kwon, D.S., Torensma, R., van Vliet, S.J., van Duijnhoven, G.C., Middel, J., Cornelissen, I.L., Nottet, H.S., KewalRamani, V.N., Littman, D.R., Figdor, C.G., van Kooyk, Y., 2000c. DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells. Cell 100, 587–597.

Geijtenbeek, T.B., Van Vliet, S.J., Koppel, E.A., Sanchez-Hernandez, M., Vandenbroucke-Grauls, C.M., Appelmelk, B., Van Kooyk, Y., 2003. Mycobacteria target DC-SIGN to suppress dendritic cell function. J. Exp. Med. 197, 7–17.

Ghosh, M., Bandyopadhyay, S., 2004. Interaction of *Leishmania* parasites with dendritic cells and its functional consequences. Immunobiology 209, 173–177.

Guo, Y., Feinberg, H., Conroy, E., Mitchell, D.A., Alvarez, R., Blixt, O., Taylor, M.E., Weis, W.I., Drickamer, K., 2004. Structural basis for distinct ligand-binding and targeting properties of the receptors DC-SIGN and DC-SIGNR. Nat. Struct. Mol. Biol. 11, 591–598.

Gupta, N., Goyal, N., Rastogi, A.K., 2001. In vitro cultivation and characterization of axenic amastigotes of *Leishmania*. Trends Parasitol. 17, 150–153.

Guy, R.A., Belosevic, M., 1993. Comparison of receptors required for entry of *Leishmania major* amastigotes into macrophages. Infect. Immun. 61, 1553–1558.

Henri, S., Curtis, J., Hochrein, H., Vremec, D., Shortman, K., Handman, E., 2002. Hierarchy of susceptibility of dendritic cell subsets to infection by *Leishmania major*: inverse relationship to interleukin-12 production. Infect. Immun. 70, 3874–3880.

Heuff, G., Meyer, S., Beelen, R.H., 1994. Isolation of rat and human Kupffer cells by a modified enzymatic assay. J. Immunol. Methods 174, 61–65.

Hodgkinson, V.H., Soong, L., Duboise, S.M., McMahon-Pratt, D., 1996. *Leishmania amazonensis*: cultivation and characterization of axenic amastigote-like organisms. Exp. Parasitol. 83, 94–105.

Ilg, T., Handman, E., Stierhof, Y.D., 1999. Proteophosphoglycans from *Leishmania* promastigotes and amastigotes. Biochem. Soc. Trans. 27, 518–525.

Ilgoutz, S.C., McConville, M.J., 2001. Function and assembly of the *Leishmania* surface coat. Int. J. Parasitol. 31, 899–908.

Jebbari, H., Stagg, A.J., Davidson, R.N., Knight, S.C., 2002. *Leishmania major* promastigotes inhibit dendritic cell motility in vitro. Infect. Immun. 70, 1023–1026.

Jeffers, S.A., Tusell, S.M., Gillim-Ross, L., Hemmila, E.M., Achenbach, J.E., Babcock, G.J., Thomas Jr., W.D., Thackray, L.B., Young, M.D., Mason, R.J., Ambrosino, D.M., Wentworth, D.E., Demartini, J.C., Holmes, K.V., 2004. CD209L (L-SIGN) is a receptor for severe acute respiratory syndrome coronavirus. Proc. Natl. Acad. Sci. USA 101, 15748–15753.

Kamhawi, S., Modi, G.B., Pimenta, P.F., Rowton, E., Sacks, D.L., 2000. The vectorial competence of *Phlebotomus sergenti* is specific for *Leishmania tropica* and is controlled by species-specific, lipophosphoglycan-mediated midgut attachment. Parasitology 121 (Pt 1), 25–33.

Klimstra, W.B., Nangle, E.M., Smith, M.S., Yurochko, A.D., Ryman, K.D., 2003. DC-SIGN and L-SIGN can act as attachment receptors for alphaviruses and distinguish between mosquito cell- and mammalian cell-derived viruses. J. Virol. 77, 12022–12032.

Konecny, P., Stagg, A.J., Jebbari, H., English, N., Davidson, R.N., Knight, S.C., 1999. Murine dendritic cells internalize *Leishmania major* promastigotes, produce IL-12 p40 and stimulate primary T cell proliferation in vitro. Eur. J. Immunol. 29, 1803–1811.

Koppel, E.A., Ludwig, I.S., Hernandez, M.S., Lowary, T.L., Gadikota, R.R., Tuzikov, A.B., Vandenbroucke-Grauls, C.M., van Kooyk, Y., Appelmelk, B.J., Geijtenbeek, T.B., 2004. Identification of the mycobacterial carbohydrate structure that binds the C-type lectins DC-SIGN, L-SIGN and SIGNR1. Immunobiology 209, 117–127.

Marovich, M.A., McDowell, M.A., Thomas, E.K., Nutman, T.B., 2000. IL-12p70 production by *Leishmania major*-harboring human dendritic cells is a CD40/CD40 ligand-dependent process. J. Immunol. 164, 5858–5865.

McDowell, M.A., Marovich, M., Lira, R., Braun, M., Sacks, D., 2002. *Leishmania* priming of human dendritic cells for CD40 ligand-induced interleukin-12p70 secretion is strain and species dependent. Infect. Immun. 70, 3994–4001.

McGreal, E.P., Martinez-Pomares, L., Gordon, S., 2004. Divergent roles for C-type lectins expressed by cells of the innate immune system. Mol. Immunol. 41, 1109–1121.

Mellman, I., Steinman, R.M., 2001. Dendritic cells: specialized and regulated antigen processing machines. Cell 106, 255–258.

Mitchell, D.A., Fadden, A.J., Drickamer, K., 2001. A novel mechanism of carbohydrate recognition by the C-type lectins DC-SIGN and DC-SIGNR. Subunit organization and binding to multivalent ligands. J. Biol. Chem. 276, 28939–28945.

Moll, H., 1993. Experimental cutaneous leishmaniasis: Langerhans cells internalize *Leishmania major* and induce an antigen-specific T-cell response. Adv. Exp. Med. Biol. 329, 587–592.

Moll, H., 2000. The role of dendritic cells at the early stages of *Leishmania* infection. Adv. Exp. Med. Biol. 479, 163–173.

Moll, H., Flohe, S., 1997. Dendritic cells induce immunity to cutaneous leishmaniasis in mice. Adv. Exp. Med. Biol. 417, 541–545.

Mosser, D.M., 1994. Receptors on phagocytic cells involved in microbial recognition. Immunol. Ser. 60, 99–114.

Murray, H.W., 2001. Tissue granuloma structure–function in experimental visceral leishmaniasis. Int. J. Exp. Pathol. 82, 249–267.

Naderer, T., Vince, J.E., McConville, M.J., 2004. Surface determinants of *Leishmania* parasites and their role in infectivity in the mammalian host. Curr. Mol. Med. 4, 649–665.

Pan, A.A., McMahon-Pratt, D., 1988. Monoclonal antibodies specific for the amastigote stage of *Leishmania pifanoi*. I. Characterization of antigens associated with stage- and species-specific determinants. J. Immunol. 140, 2406–2414.

Pohlmann, S., Soilleux, E.J., Baribaud, F., Leslie, G.J., Morris, L.S., Trowsdale, J., Lee, B., Coleman, N., Doms, R.W., 2001. DC-SIGNR, a DC-SIGN homologue expressed in endothelial cells, binds to human and simian immunodeficiency viruses and activates infection in trans. Proc. Natl. Acad. Sci. USA 98, 2670–2675.

Ponte-Sucre, A., Heise, D., Moll, H., 2001. *Leishmania major* lipophosphoglycan modulates the phenotype and inhibits migration of murine Langerhans cells. Immunology 104, 462–467.

Puig-Kroger, A., Serrano-Gomez, D., Caparros, E., Dominguez-Soto, A., Relloso, M., Colmenares, M., Martinez-Munoz, L., Longo, N., Sanchez-Sanchez, N., Rincon, M., Rivas, L., Sanchez-Mateos, P., Fernandez-Ruiz, E., Corbi, A.L., 2004. Regulated expression of the pathogen receptor dendritic cell-specific intercellular adhesion molecule 3

(ICAM-3)-grabbing nonintegrin in THP-1 human leukemic cells, monocytes, and macrophages. J. Biol. Chem. 279, 25680–25688.

Qi, H., Popov, V., Soong, L., 2001. *Leishmania amazonensis*-dendritic cell interactions in vitro and the priming of parasite-specific CD4(+) T cells in vivo. J. Immunol. 167, 4534–4542.

Relloso, M., Puig-Kroger, A., Pello, O.M., Rodriguez-Fernandez, J.L., de la Rosa, G., Longo, N., Navarro, J., Munoz-Fernandez, M.A., Sanchez-Mateos, P., Corbi, A.L., 2002. DC-SIGN (CD209) expression is IL-4 dependent and is negatively regulated by IFN, TGF-beta, and anti-inflammatory agents. J. Immunol. 168, 2634–2643.

Russell, D.G., Talamas-Rohana, P., 1989. *Leishmania* and the macrophage: a marriage of inconvenience. Immunol. Today 10, 328–333.

Sacks, D., Sher, A., 2002. Evasion of innate immunity by parasitic protozoa. Nat. Immunol. 3, 1041–1047.

Sacks, D.L., Modi, G., Rowton, E., Spath, G., Epstein, L., Turco, S.J., Beverley, S.M., 2000. The role of phosphoglycans in *Leishmania*-sand fly interactions. Proc. Natl. Acad. Sci. USA 97, 406–411.

Scott, P., Hunter, C.A., 2002. Dendritic cells and immunity to leishmaniasis and toxoplasmosis. Curr. Opin. Immunol. 14, 466–470.

Serrano-Gomez, D., Dominguez-Soto, A., Ancochea, J., Jimenez-Heffernan, J.A., Leal, J.A., Corbi, A.L., 2004. Dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin mediates binding and internalization of *Aspergillus fumigatus* conidia by dendritic cells and macrophages. J. Immunol. 173, 5635–5643.

Soilleux, E.J., Barten, R., Trowsdale, J., 2000. DC-SIGN; a related gene, DC-SIGNR; and CD23 form a cluster on 19p13. J. Immunol. 165, 2937–2942.

Talamas-Rohana, P., Wright, S.D., Lennartz, M.R., Russell, D.G., 1990. Lipophosphoglycan from *Leishmania mexicana* promastigotes binds to members of the CR3, p150,95 and LFA-1 family of leukocyte integrins. J. Immunol. 144, 4817–4824.

Turco, S.J., Descoteaux, A., 1992. The lipophosphoglycan of *Leishmania* parasites. Annu. Rev. Microbiol. 46, 65–94.

Turco, S.J., Spath, G.F., Beverley, S.M., 2001. Is lipophosphoglycan a virulence factor? A surprising diversity between *Leishmania* species. Trends Parasitol. 17, 223–226.

Udey, M.C., von Stebut, E., Mendez, S., Sacks, D.L., Belkaid, Y., 2001. Skin dendritic cells in murine cutaneous leishmaniasis. Immunobiology 204, 590–594.

van Die, I., van Vliet, S.J., Nyame, A.K., Cummings, R.D., Bank, C.M., Appelmelk, B., Geijtenbeek, T.B., van Kooyk, Y., 2003. The dendritic cell-specific C-type lectin DC-SIGN is a receptor for *Schistosoma mansoni* egg antigens and recognizes the glycan antigen Lewis x. Glycobiology 13, 471–478.

van Kooyk, Y., Geijtenbeek, T.B., 2002. A novel adhesion pathway that regulates dendritic cell trafficking and T cell interactions. Immunol. Rev. 186, 47–56.

Van Liempt, E., Imberty, A., Bank, C.M., Van Vliet, S.J., Van Kooyk, Y., Geijtenbeek, T.B., Van Die, I., 2004. Molecular basis of the differences in binding properties of the highly related C-type lectins DC-SIGN and L-SIGN to Lewis X trisaccharide and *Schistosoma mansoni* egg antigens. J. Biol. Chem. 279, 33161–33167.

von Stebut, E., Belkaid, Y., Jakob, T., Sacks, D.L., Udey, M.C., 1998. Uptake of *Leishmania major* amastigotes results in activation and interleukin 12 release from murine skin-derived dendritic cells: implications for the initiation of anti-*Leishmania* immunity. J. Exp. Med. 188, 1547–1552.

von Stebut, E., Belkaid, Y., Nguyen, B.V., Cushing, M., Sacks, D.L., Udey, M.C., 2000. *Leishmania major*-infected murine langerhans cell-like dendritic cells from susceptible mice release IL-12 after infection and vaccinate against experimental cutaneous Leishmaniasis. Eur. J. Immunol. 30, 3498–3506.

Wang, Q.C., Feng, Z.H., Nie, Q.H., Zhou, Y.X., 2004. DC-SIGN: binding receptors for hepatitis C virus. Chin. Med. J. (Engl.) 117, 1395–1400.

Wilson, M.E., Pearson, R.D., 1988. Roles of CR3 and mannose receptors in the attachment and ingestion of *Leishmania donovani* by human mononuclear phagocytes. Infect. Immun. 56, 363–369.

Wright, E.P., el Amin, E.R., 1989. *Leishmania* infection: surfaces and immunity. Biochem. Cell Biol. 67, 525–536.