Chapter 4.2

DC-SIGN enhances herpes simplex virus infection of dendritic cells *in cis.*

Lot de Witte[1*], Marein A.W.P. de Jong[1*], Anders Bolmstedt[2], Yvette van Kooyk[1] and Teunis B.H. Geijtenbeek[1*].

Dendritic cells (DCs) are essential to the induction of specific immune responses against invading pathogens. Herpes Simplex Virus (HSV) is a common human pathogen that causes painful, but mild infections of the skin and mucosa, which results in latency and recurrent infections. Of the two HSV subtypes described HSV-1 causes mainly oral-facial lesions while HSV-2 is associated with genital herpes. DCs are involved in HSV-induced immune suppression, but little is known about the molecular interactions between DCs and HSV.
Here we demonstrate that both HSV-1 and -2 interact with the DC-specific C-type lectin DC-SIGN. Further analyses demonstrate that DC-SIGN interacts with both gB and gC. Binding of HSV-1 to immature DCs depends on both DC-SIGN and heparan sulphate proteoglycans, since blocking antibodies against DC-SIGN and heparinase treatment abrogate HSV-1 interactions. Strikingly, HSV-1 infection of DCs is almost completely inhibited by blocking antibodies against DC-SIGN. Thus, DC-SIGN is an important attachment receptor for HSV-1 on immature DCs that enhances infection of DCs *in cis*. Our data suggest that DC-SIGN is a potential target to prevent HSV infection and virus dissemination. Further studies will show whether these interactions are involved in HSV-induced immune suppression.

---

[1]*Department of Molecular Cell Biology and Immunology, VU University Medical Center Amsterdam, van der Boechorststraat 7, 1081 BT Amsterdam, The Netherlands.* [2]*Department of Clinical Virology, University of Göteborg, Guldhedsgatan 10B, S-413 46 Göteborg, Sweden.*

[*]Authors contributed equally to this work

(Manuscript submitted for publication)

Introduction

Herpes simplex virus (HSV) is a double stranded DNA virus that belongs to the family of the *Alphaherpesviridae*. HSV is a common human pathogen that causes painful, but mild infections of the skin and mucosa, resulting in cold sores and blisters. Two subtypes of HSV have been described: HSV-1, which mostly causes oral-facial lesions and HSV-2, which is associated with genital herpes. HSV is transmitted by direct body contact with infected lesions or body fluids and enters the body at the mucosal tissues or lesions of the skin. The primary target cells for HSV are the epithelial cells and keratinocytes. During primary infection, HSV enters the cutaneous sensory neuron and travels to the dorsal root ganglia where it establishes the characteristic lifelong latent infection. At times when the immune system is suboptimal, such as during stress, trauma, UV-light, fatigue or a common cold, the virus escapes immune surveillance and migrates via the peripheral nerve towards the epithelium or skin, where it causes recurrent herpes simplex lesions (reviewed in Roizman *et al.*, 2007; Taylor *et al.*, 2002).

Aside from discomforting infectious cold sores and blisters, HSV can cause ocular herpes stromal keratitis, making it the major cause infectious cause of blindness in the western world (discussed in Pepose *et al.*, 2006). In addition, HSV can cause life-threatening infections in individuals with inadequate cellular immune responses, like newborns or immune compromised patients. This indicates that under normal conditions, dissemination of HSV throughout the body is prevented by the immune system. In addition, HSV has evolved strategies to escape from a clearing immune response and to cause latent infection.

As sentinels of the immune systems, dendritic cells (DCs) have an important regulatory function. Due to their specific location in the subepithelium and dermis, DCs are a target for invading pathogens such as HIV and measles virus. These viruses use DCs for infection, dissemination and immune evasion by targeting the DC-specific C-type lectin DC-SIGN (de Witte *et al.*, 2006; Burleigh *et al.*, 2006).

HSV-1 has developed several mechanisms to escape immune surveillance of DCs. DCs express the HSV entry receptors HVEM (Hve-A) and nectin-2 (Hve-B), and heparan sulfates that mediate attachment of HSV-1. DCs are productively infected with HSV (Mikloska *et al.*, 2001; Kruse *et al.*, 2000a), which results in apoptosis when high viral titres are used (PFU 5/ cell) (Bosnjak *et al.*, 2005). Moreover, HSV-1 infection strongly affects DC function by interfering with DC maturation including down-regulation of co-stimulatory molecules as well as CD83, CD1 molecules and MHC class I (Mikloska *et al.*, 2001;Raftery *et al.*, 2006; Kummer *et al.*, 2007). HSV-1 infection also decreases IL-12 production and lowers the allostimulatory capacity of DCs (Theodoridis *et al.*, 2007; Kruse *et al.*, 2000b). These data strongly suggest that DCs are involved in the suppression of the immune response against HSV-1.

The C- type lectin DC-SIGN, expressed exclusively by DCs, plays an important role in viral attachment and dissemination (de Witte *et al.*, 2006; Burleigh *et al.*, 2006). In addition, Halary *et al.* demonstrated that herpes family member CMV interacts with DC-SIGN, however, the authors concluded that HSV-1 glycoproteins do not bind to DC-SIGN (Halary *et al.*, 2002).

To better understand HSV pathology and to unravel targets to enhance the immune response to HSV, it is essential to understand the molecular interactions of HSV with DCs. Here we have investigated the receptors involved in the interaction between human monocyte-derived DCs and HSV. We demonstrate that HSV-1 and -2 interact with DC-SIGN via glycoproteins gB and gC. Binding of HSV to DCs depends on both heparan sulfate proteoglycans (HSPGs) and DC-SIGN. Although DC-SIGN is not an entry receptor, it does increase viral attachment and infection of target cells *in cis*, thereby contributing to the infectivity and transmission of HSV.

Results

Recombinant DC-SIGN interacts with both HSV-1 and -2.

DC-SIGN is a receptor for various viruses, including the herpes family member CMV (Halary *et al.*, 2002). HSV contains multiple envelope glycoproteins that are potential ligands for DC-SIGN. We therefore investigated whether this C-type lectin interacts with HSV-1 and -2. HSV virions were coated on the plates and binding of recombinant DC-SIGN was analyzed by ELISA.

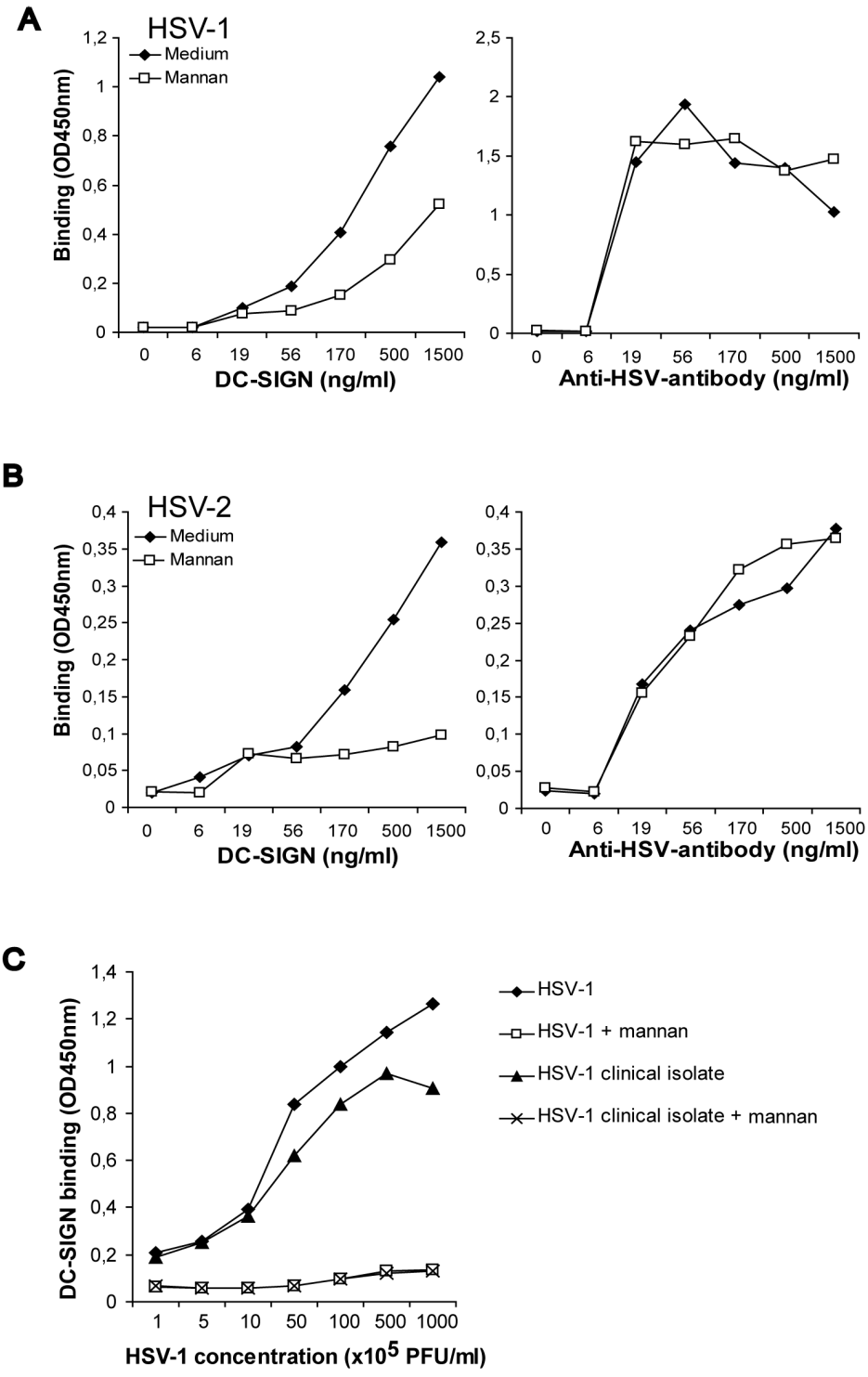


Figure 1 HSV-1 and HSV-2 interact with soluble DC-SIGN.
(a,b) HSV-1 (KOS321), a clinical isolate of HSV-1 and HSV-2 (333) were coated onto ELISA plates ($1x10^7$ PFU/ml, unless depicted otherwise) and the binding of DC-SIGN-Fc was determined by ELISA. Specificity of the DC-SIGN-Fc HSV interaction was determined by measuring binding in the presence of mannan. As a control for coating the viruses were detected by an antibody against HSV gB (B11D8). One representative experiment out of three is shown.

Both viruses were coated on the plates as determined by an antibody against HSV (Figure 1a,b; right panels). Recombinant DC-SIGN efficiently interacted with both HSV-1 and -2 in a concentration dependent manner (Figure 1a,b; left panels). The interaction was specific for the lectin domain of DC-SIGN, since pre-incubation of DC-SIGN with the polycarbohydrate mannan blocked the binding of DC-SIGN (Figure 1a,b).

To exclude that DC-SIGN ligands are acquired upon attenuation of the viruses, we investigated the binding of DC-SIGN to a clinical isolate. Both attenuated and clinical HSV strains strongly bound to DC-SIGN and this interaction was specific since mannan could completely block the binding (Figure 1c). These results demonstrate that soluble DC-SIGN interacts with both HSV-1 and HSV-2. To further investigate the role of DC-SIGN with HSV we have used the HSV-1 isolate Syn17^{+}.

HSV-1 gB and gC interact with DC-SIGN.

The envelope of HSV-1 contains 12 different glycoproteins. Glycoprotein gB and gC have been shown to mediate binding of HSV-1 to target cells, whereas the gB, gD and gH/L are important for entry of the virus into the target cells (Spear, 2004). We investigated the interaction of DC-SIGN with gB and gC glycoproteins using the soluble DC-SIGN binding assay. Soluble DC-SIGN interacted with both coated HSV-1 virions and the purified glycoproteins gB and gC (Figure 2a). The interaction was specific for the binding site of DC-SIGN, since the calcium chelator EGTA completely inhibited the interaction (Figure 2a). Next, cell lines expressing high levels of DC-SIGN and low levels of heparan sulfates (data not shown) were used to determine the interaction of gB and gC with cellular DC-SIGN. gB and gC were coated onto fluorescent beads and interaction with the mock-transfected K562 and K562-DC-SIGN cells was determined. K562-DC-SIGN efficiently interacted with gB and gC in contrast to the mock transfected K562 cells (Figure 2b).The interaction was specific for DC-SIGN, since antibodies against DC-SIGN, mannan and EGTA inhibited the interaction similar to the HIV-1 gp120-DC-SIGN interaction (Figure 2b).

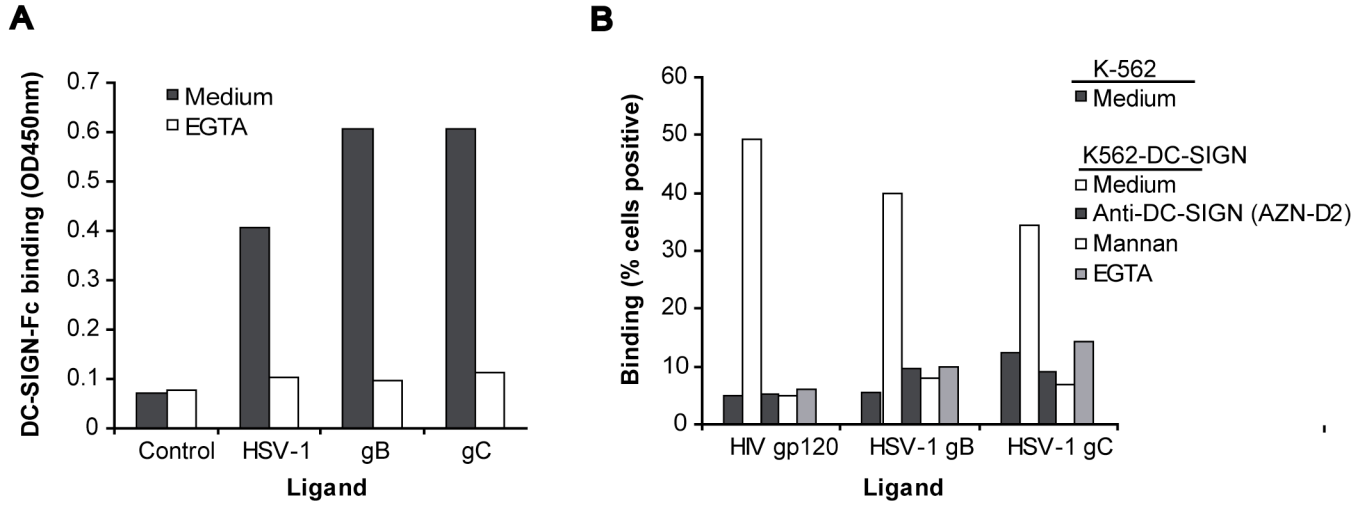


Figure 2. HSV-1 gB and gC interact with DC-SIGN. (a) DC-SIGN-Fc interaction with HSV-1, HSV-1 gB and gC was determined by a DC-SIGN-Fc-binding ELISA. Specificity of the DC-SIGN-Fc HSV-1 interaction was determined by measuring binding in the presence of EGTA. (b) The interaction of purified gB and gC or HIV-1 gp120 with either parental K562 cells or K562-DC-SIGN transfectants was measured by fluorescent bead binding assay. Specificity of the DC-SIGN interaction was determined by measuring binding in the presence of a blocking antibody against DC-SIGN (AZN-D2), mannan or EGTA. One representative experiment out of two is shown.

Both DC-SIGN and heparan sulfates are involved in HSV-1 binding to DCs.

Thus, HSV-1 gB and gC interact with cellular DC-SIGN. Since DCs are the first antigen presenting cells to interact with invading HSV, we investigated whether DC-SIGN plays a role in this interaction. In contrast to K562 cells, DCs express both DC-SIGN and HSV-1 attachment receptors heparan sulfates (Spear, 2004). We therefore investigated the role of DC-SIGN and heparan sulfates in HSV-1 binding to DCs. Both glycoproteins gB and gC interacted with DCs, and a blocking antibody against DC-SIGN completely blocked the interaction of gB with DCs (Figure 3a). Strikingly, the antibody against DC-SIGN inhibited the gC interaction to a lesser extent than the gB interaction, suggesting that gC also binds to

other receptors (Figure 3a). These data demonstrate that DC-SIGN interacts with HSV-1 through gB and to a lesser extent gC.Next, we investigated the interaction of HSV-1 with DCs and the involvement of heparan sulfates and DC-SIGN. DCs were treated with Heparinase III, which specifically digests cellular heparan sulfate chains on DCs (de Witte *et al.*, 2007). Indeed heparan sulfate expression was strongly decreased after treatment (Figure 3b). This was further confirmed by the increased expression of the 3G10 epitope, representing Heparinase-digested heparan sulfates on Heparinase-treated DCs (Figure 3b). The interaction of whole HSV-1 particles to DCs was further explored using HSV-1 particles coated onto fluorescent beads. DCs efficiently interacted with HSV-1 (Figure 3c), which was partially mediated through DC-SIGN, since antibodies against DC-SIGN inhibited the interaction (Figure 3c). Binding was not blocked to background levels, suggesting other receptors, such as heparan sulfates, also play a role. Indeed, heparinise treatment decreased binding of HSV-1 to DCs, and in combination with blocking antibodies against DC-SIGN, binding was further blocked. These data show that both heparan sulfates and DC-SIGN mediate binding of HSV-1 to DCs (Figure 3c).

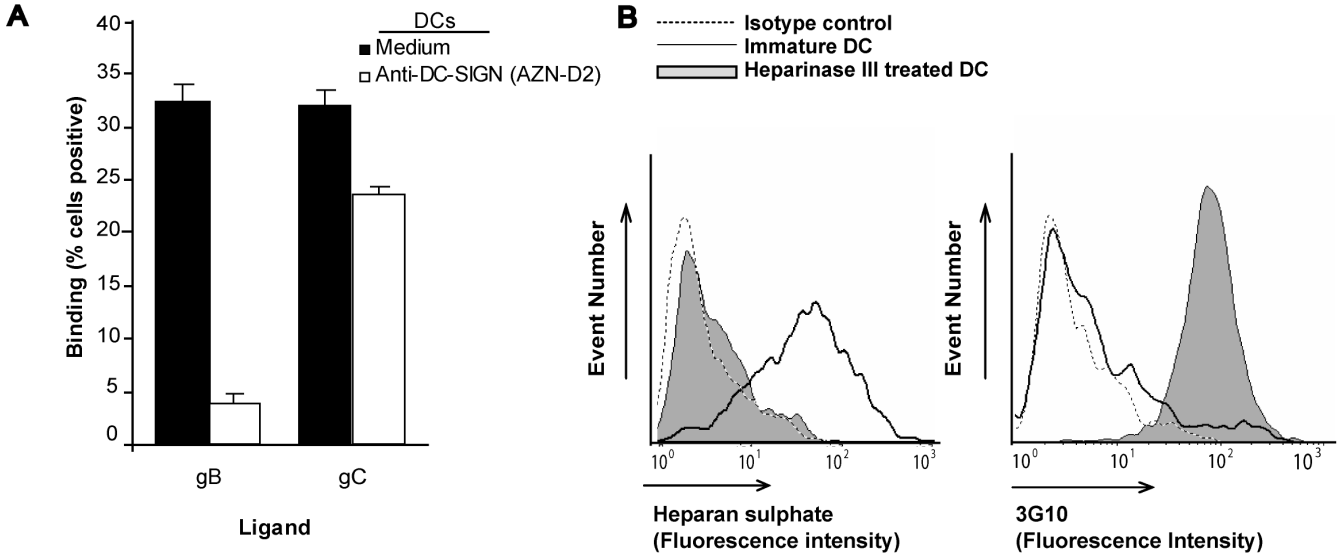


Figure 3. HSV-1 interacts with DCs through DC-SIGN and heparan sulfates. (a) HSV-1 gB or gC binding to DCs was measured using the fluorescent bead adhesion assay. Specificity of the binding for DC-SIGN was determined by measuring the adhesion in the presence of anti-DC-SIGN (AZN-D2). One representative experiment out of three is shown. Error bars represent standard errors of duplicates. (b,c) Immature DCs were treated with Heparinase III. (b) The treatment was analyzed by staining with an antibody against heparan sulfates (10E4) or an antibody against cleaved heparan sulfates (3G10) and subsequent analysis of expression by flow cytometry. (c) DCs were pre-incubated with mannan (1 mg/ml) and binding of HSV-1 coated beads was measured using the fluorescent bead adhesion assay. Error bars represent standard errors of duplicates. One representative experiment out of two is shown.

DC-SIGN is not an entry receptor for HSV-1 but enhances infection in cis.

Next, we investigated the role of DC-SIGN in HSV-1 infection. To determine whether DC-SIGN is an entry receptor for HSV-1, we used DC-SIGN transfected CHO cells, expressing high levels of DC-SIGN (Figure 4a). CHO cells were not susceptible to HSV-1 in contrast to the permissive GMK cells that were efficiently infected (Figure 4b). Even at high viral input, expression of DC-SIGN did not confer susceptibility to HSV-1, demonstrating that DC-SIGN is not an entry receptor for HSV-1(Figure 4b). Next, we investigated the function of DC-SIGN in HSV-1 infection of permissive cells. Parental Raji cells are infected with HSV-1 as determined by gB expression on the cell surface. Raji cells expressing DC-SIGN were more efficiently infected by HSV-1, demonstrating that DC-SIGN enhances HSV-1 infection *in cis* (Figure 4c). The increased levels of infection were inhibited by mannan, demonstrating that the enhancement is specific for DC-SIGN. Thus, although DC-SIGN is not an entry receptor for HSV, it does enhance HSV infection *in cis*.

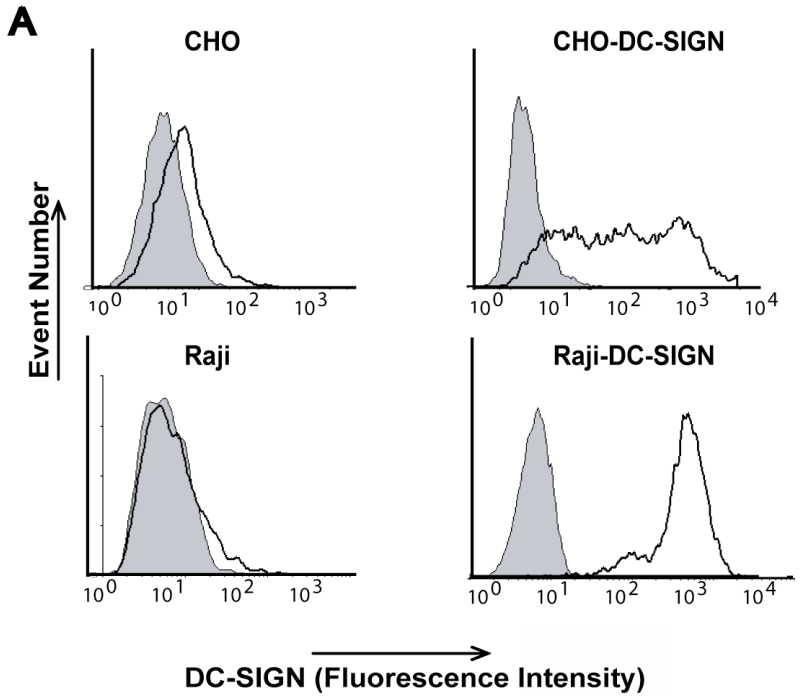
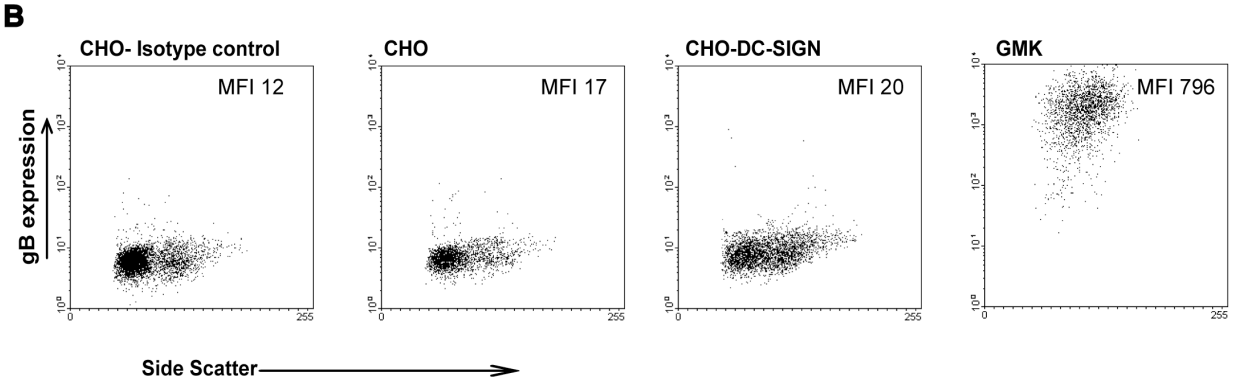
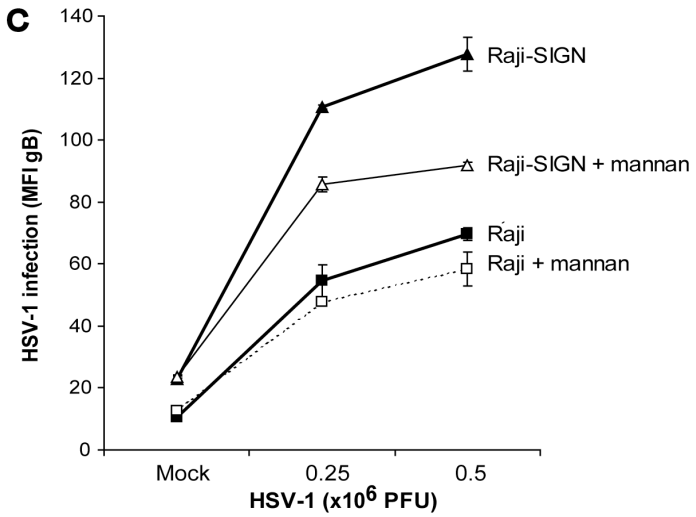


Figure 4. DC-SIGN mediates infection of immature DCs with HSV-1 *in cis*.

(a) CHO- and Raji-DC-SIGN transfectants were analyzed for DC-SIGN expression by flow cytometry. Open histograms represent specific staining; filled histograms the isotype controls. (b) GMK, CHO and CHO-DC-SIGN cells ($2x10^5$ cells) were infected with a high concentration of HSV-1 ($1x10^7$ PFU) (c) Raji and Raji-DC-SIGN cells ($5x10^4$ cells) were pre-treated with mannan and infected with the depicted concentrations of HSV-1. (b,c) gB expression was measured to determine the level of infection. Infection is depicted as mean fluorescent intensity (MFI). Error bars represent standard deviations of triplicates. One representative experiment out of three is shown.





DC-SIGN enhances infection of DCs in cis.
Next, we investigated the role of DC-SIGN in HSV-1 infection of DCs. Immature DCs were incubated with different concentrations of HSV-1 and infection was measured by staining for HSV-1 gB. As previously shown (Mikloska *et al.*, 2001), immature DCs were efficiently infected with HSV-1 at different viral inputs (Figure 5a,b,c), while the viability of the cells was not significantly decreased (data not shown). Non-infected DCs showed high DC-SIGN expression and low CD86 (Figure 5a; left panel). Infection of DCs with HSV-1 increased CD86 expression partially, but not as strong as observed with LPS-matured DCs.
The expression of DC-SIGN was reduced compared to non-infected cells, whereas the down-regulation of DC-SIGN by LPS-matured DC was stronger than HSV infected DCs (Figure 5a; middle and right panel). To specifically determine the role of DC-SIGN in HSV infection of DCs, we used mannan and blocking antibodies against DC-SIGN. Strikingly, HSV-1 infection was strongly decreased by blocking DC-SIGN with either mannan or anti-DC-SIGN antibodies (Figure 5b,c) while isotype antibodies did not have an effect (data not shown). These data show that DC-SIGN enhances DC infection of HSV *in cis*. Together, we demonstrate that HSV binds to DC-SIGN on DCs and this interaction is essential to enhance infection of DCs.

Discussion

DCs are the first antigen presenting cells to encounter HSV-1 and -2 at the site of infection, mucosal tissue in the oral and genital areas, respectively. HSV has been shown to escape from immune surveillance by DCs. Together with the incidence of HSV-1/ -2 co-infections with HIV-1 and the increased risk of acquiring HIV-1 in the present of HSV infection, it is important to understand the molecular interactions between HSV and DCs. The interaction of DCs and gB was primarily mediated by DC-SIGN, whereas both heparan sulfates and DC-SIGN are involved in binding of gC to DCs. In addition, we showed that although the interaction of HSV-1 with DC-SIGN does not mediate entry of the virus, it does enhance infection of DCs *in cis.*
DC-SIGN is a receptor for various viruses, including HIV-1, Ebola virus, measles virus, dengue virus and interestingly the herpes family member CMV (de Witte *et al.*, 2006; Tassaneetrithep *et al.*, 2003; Halary *et al.*, 2002). Here we demonstrate that HSV-1 also has to be included as ligand for DC-SIGN.
Both gB and gC bind to soluble and cellular DC-SIGN. This is in contrast to a previous report that demonstrated that HSV-1 gB did not bind to DC-SIGN. The recombinant HSV-1 gB used by Halary *et al.* (Halary *et al.*, 2002) was produced in insect cells by overexpression, whereas gB and gC used in our experiments were purified from eukaryotic cells. Thus, differences in cell-type and production methods might account for the differences observed. Indeed, infection by HSV-1 induces glycosylation changes (Olofsson *et al.*, 1980; Nystrom *et al.*, 2004), and insect cells lack glycosylation components necessary to generate glycosylations present in eukoryotic cells (Altmann *et al.*, 1999). Thus, the gB and gC produced in cells represent the glycosylations observed in HSV-1 particles and which are recognized by DC-SIGN.

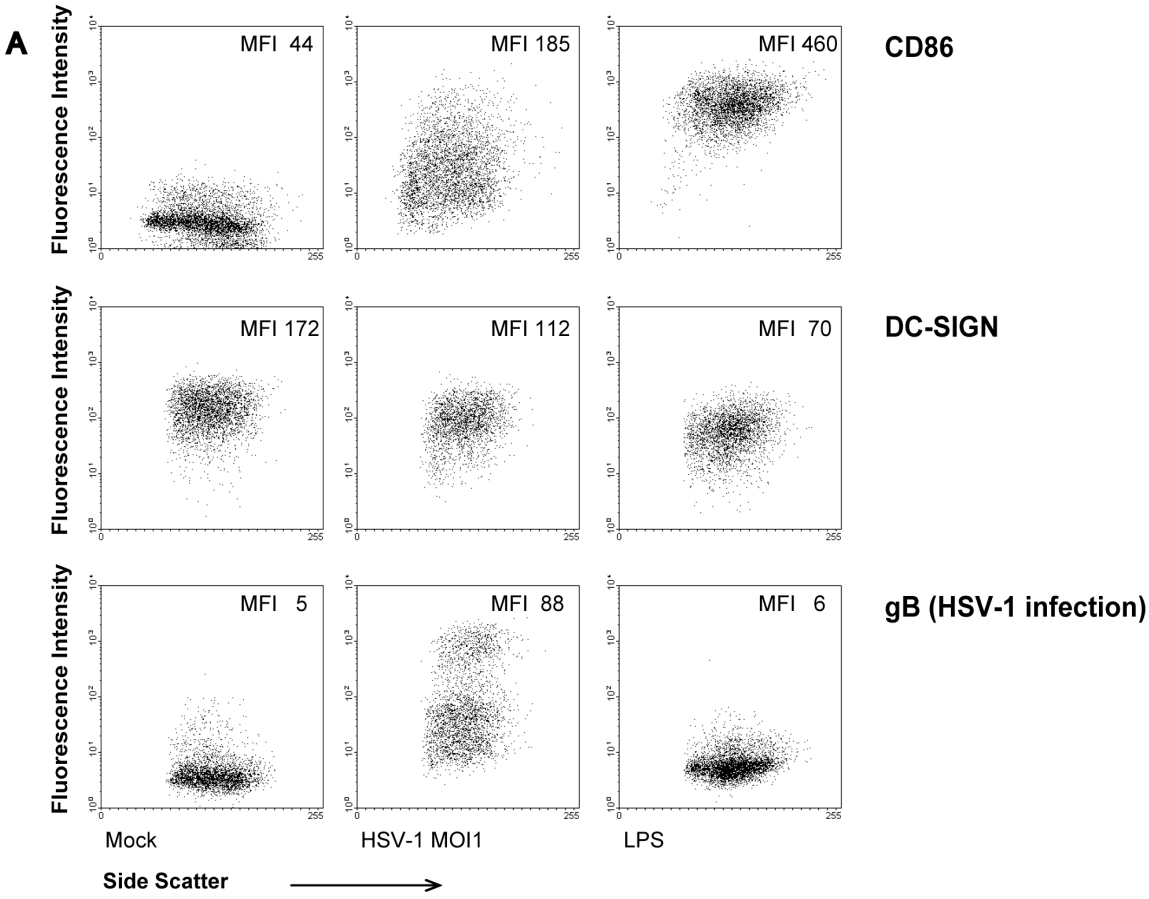


Figure 5. DC-SIGN enhances infection of immature DCs with HSV-1. (a,b,c) Immature DCs ($5x10^4$ cells) were infected with different concentrations of HSV-1 for 24 hours (a) CD86, DC-SIGN and HSV-1 gB staining on immature, LPS-matured and DCs ($1x10^6$ PFU) was measured by flow cytometry. (b,c) To determine the level of HSV-1 infection, HSV-1 gB expression was measured by flow cytometry. Cells were pre-treated with mannan and anti-DC-SIGN, to determine the contribution of DC-SIGN to DC infection. Error bars represent standard deviation of duplicates. One representative experiment out of two is shown. Significance was determined using a one-way analysis of variance (ANOVA) with the post hoc Bonferroni test using Graphpad prism software. Asterisks represent p- value compared to the medium condition, * $p< 0.05$, ** $p< 0.01$.

In addition to HSV-1 binding to DC-SIGN, we detected binding of recombinant DC-SIGN to HSV-2, suggesting a similar interaction between HSV-2 and DC-SIGN on DCs. Since glycoprotein B is highly conserved throughout the *Herpesviridae* family it is very likely that HSV-2 also interact with DCs via DC-SIGN, since HSV-1 gB and HSV-2 gB show 87% sequence homology (Cai *et al.*, 1988; Cai *et al.*, 1987; Cheshenko & Herold, 2002; Little *et al.*, 1981; Sarmiento *et al.*, 1979), and 83% homology in their protein coding region (Dolan *et al.*, 1998).

Heparan sulfates are glycosaminoglycan side-chains of cell-surface proteoglycans that have been shown to mediate attachment of HSV (Spear, 2004). On DCs, removal of heparan sulfates and blocking DC-SIGN decreased HSV-1 binding. These data demonstrate that both DC-SIGN and heparan sulfates are involved in binding of HSV-1 to DCs. This is similar to what we have observed for HIV-1, in which syndecan-3 on dendritic cells is together with DC-SIGN important for attachment of HIV-1 to DCs (de Witte *et al.*, 2007). Therefore, in analogy with HIV-1, it is tempting to speculate that the core protein containing the heparan sulfate side chains, involved in HSV-1 binding, is the heparan sulfate proteoglycan syndecan-3.

Both HSV-1 glycoprotein gB and gC are important for binding HSV-1 to target cells (Spear, 2004; Herold *et al.*, 1991). Although binding significantly enhances the efficiency of HSV-1 infection, it is not essential for infection, in contrast to the entry receptors. Using soluble DC-SIGN and DC-SIGN-transfected cell lines, we demonstrated that both gB and gC interact with DC-SIGN. The interaction of gB to DCs is completely dependent on DC-SIGN, whereas gC binding to DCs is partially mediated by DC-SIGN and heparan sulfates. These results suggest that gB has higher affinity for DC-SIGN and gC for heparan sulfates. Indeed, it was previously shown that gB has lower affinity for heparan sulfate than gC (Trybala *et al.*, 2000). We conclude that although gB and gC are not essential for viral entry, they do enhance viral attachment to target cells.

DC-SIGN plays an important role for DC infection by different viruses; it enhances the infection of DCs *in cis* for various viruses, including HIV-1 and measles virus (de Witte *et al.*, 2006; Burleigh *et al.*, 2006). DC-SIGN is highly expressed on DCs and efficiently captures the glycan structures on the viral envelope glycoproteins. This interaction enhances the contact of the virus with its entry receptors, resulting in infection. This process is thought to be important for entry receptors that have low affinity for the viral glycoproteins or are expressed at low levels on DCs. Here, we demonstrate that HSV-1 targets DC-SIGN for viral attachment and DC infection *in cis*, since the infection of DCs with HSV-1 can be inhibited with mannan and antibodies against DC-SIGN. Notably, mannan blocks HSV-1 infection more efficient than DC-SIGN antibodies. This could indicate the presence of an additional mannose-specific receptor on DCs or mannan is more efficient in inhibiting DC-SIGN function. Upon infection of DCs with HSV-1, we observed two populations of infected DCs that differed in their expression of gB, suggesting that not all DCs are productively infected.

Using a CHO cell line, which is naturally non-permissive to HSV entry, we demonstrate that DC-SIGN by itself is not an entry-receptor, since transfection of CHO cells with DC-SIGN does not result in HSV susceptibility of the cell line. Recently, Satoh *et al.* demonstrated that transfection of the CHO cell line with a novel identified entry receptor results in HSV infection, verifying that the CHO cell line is able to sustain viral infection (Satoh *et al.*, 2008).

Infection of DCs by HSV-1 down-regulates the immuno-stimulating phenotype of DCs (Novak & Peng, 2005; Mikloska *et al.*, 2001), and therefore our data strongly suggest that HSV-1 targets DC-SIGN to infect DCs and escape immunity. Moreover, DCs might facilitate dissemination throughout the body during primary infection or during complications such as herpes encephalitis. In conclusion, we have demonstrated that DC-SIGN captures HSV-1 and enhances infection of DCs *in cis*. Our data suggest

that DC-SIGN is a target to inhibit HSV-1 interactions with DCs and thereby prevent HSV induced immune escape and virus dissemination.

Materials and Methods

Antibodies, cells, proteins and viruses. The following antibodies were used: monoclonal mouse antibodies against DC-SIGN (AZN-D1 and AZN-D2) (Geijtenbeek *et al.*, 2003; Geijtenbeek *et al.*, 2000), monoclonal mouse antibodies against HSV-1 gB (B11D8) and gC (C12H12) (Bergstrom *et al.*, 1992), monoclonal mouse antibody against heparan sulfates (10E4) (a generous gift of Dr. G. Davids), monoclonal mouse antibody against digested heparan sulfates, (F-69-3G10; Seikagaku corporation), goat anti-human IgG conjugated with PO (Jackson Immunoresearch, West Grove, PA, USA), goat anti-mouse antibody conjugated with FITC (Zymed Laboratories Inc., South San Fransisco, Ca, USA).
Immature DCs were cultured as described before (Sallusto & Lanzavecchia, 1994). In short, human blood monocytes were isolated from buffy coats by use of a Ficoll gradient and a subsequent CD14 selection step using a MACS system (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). Purified monocytes were differentiated into immature DCs in the presence of interleukin-4 (IL-4) and granulocyte-macrophage colony-stimulating factor (GM-CSF) 500 and 800 U/ml, respectively (Schering-Plough, Brussels, Belgium). Experiments using immature DCs were performed at day 5 to 7 in RPMI containing 10% FCS, IL-4 and GM-CSF unless indicated otherwise. To generate mature DCs, immature DCs were incubated for 24 hours with 10ng/ml lipopolysaccharide (LPS) derived from *Salmonella Typhosa* (Sigma, St. Louis, MO, USA).
Parental and DC-SIGN transfected CHO (van Gisbergen *et al.*, 2005b), K562 (van Gisbergen *et al.*, 2005a) and Raji cells (Geijtenbeek *et al.*, 2003; Geijtenbeek *et al.*, 2000) were generated and cultured as described before. The viral strain HSV-1 ($Syn17^+$ and KOS321), HSV-1 clinical isolate and HSV-2 strain 333 were grown on green monkey kidney (GMK) cells. A plaque titration assay was performed to determine viral titers. Titers are depicted as plaque forming units (PFU). HSV-1 gB and gC proteins were purified as described before (Trybala *et al.*, 2000). In short, gB and gC were precipitated from a lysate of GMK cells using immunosorbent columns coated with antibodies against gB (B11D8) and gC (C12H12) and diluted at high concentrations in PBS.
Fluorescent bead adhesion assay. The streptavidin-coated TransFluorSpheres (488/645 nm, 1.0 μm; Molecular Probes, Eugene, OR, USA) beads were incubated with a biotinylated $F(ab')_2$ fragment goat anti-mouse (6 μg/ml; Jackson Immunoresearch), followed by an overnight incubation with antibodies against HSV gB or against HSV gC, at a concentration of 10 μg/ml at 4 °C. The beads were washed and incubated overnight with either HSV-1 or purified HSV gB or gC at 4 °C. HIV-1 gp120 beads were produced as described previously (Lekkerkerker *et al.*, 2004). The adhesion assay was performed as follows (Geijtenbeek *et al.*, 2003; Geijtenbeek *et al.*, 2000): $1x10^5$ cells were incubated with beads for 45 minutes at 37 °C. To determine specificity of adhesion, cells were pre-treated with mannan (1 mg/ml), EGTA (10 mM) or blocking antibodies against DC-SIGN (20 μg/ml) for 15 minutes at 37 °C. Binding was measured by flow cytometry.

To investigate the role of heparan sulfates, immature DCs ($1x10^5$ cells/well) were seeded in a total volume of 30 µl phosphate buffer saline (PBS) and Heparinase III (Prozyme, Glyko, San Leandro, CA, USA) was added at a concentration of 0.1 IU/ml. The plate was incubated for 1 hour at room temperature. After one hour the cells were washed with TSA and directly used for subsequent experiments. Efficiency of the digestion was valuated by staining the cells by 10E4 (antibody against heparan sulfates) and 3G10 (antibody against Δheparan sulfate, which recognizes digested heparan sulfate chains). The cells were analyzed by flow cytometry. A strong reduction of the mean fluorescence intensity of the heparan sulfate staining and a positive staining of the population for 3G10 confirmed an efficient treatment.

DC-SIGN-Fc binding. Recombinant DC-SIGN consists of the extracellular portion of DC-SIGN (aa residues 64-404) fused at the C terminus to the human IgG1-Fc domain. DC-SIGN-Fc was produced in Chinese hamster ovary K1 cells after transfection with the DC-SIGN-Sig-pIgG1-Fc vector (5 µg/ $1x10^6$ cells). The soluble DC-SIGN-Fc binding ELISA was performed as previously described (Geijtenbeek *et al.*, 2002). In short, different concentrations of HSV-1, HSV-2 or purified HSV-1 glycoproteins (gB and gC) were coated onto ELISA plates overnight at room temperature. Unspecific binding was blocked by incubating the plate with 1% BSA for 1 hour at 37 °C. Soluble DC-SIGN-Fc supernatant was added for 1 hour at 37 °C. Unbound DC-SIGN-Fc was washed away and binding was determined using a peroxidase-conjugated goat anti-human Fc antibody. Specificity was determined (unless indicated otherwise) in the presence of mannan (1 mg/ml) or EGTA (10 mM). The amount of HSV coated onto the plate was detected by using anti-gB and peroxidase-conjugated goat anti-mouse Fc antibodies.

HSV-1 infection. Immature DCs, Raji or Raji-DC-SIGN cells ($5x10^4$ cells) were seeded in a round-bottom 96-wells plate in complete RPMI. The cells were pre-incubated with medium, a blocking antibody against DC-SIGN (AZN-D1; 20µg/ml) or mannan (1mg/ml) for 1 hour at 37 °C in 5% CO_2 before infecting them with different concentrations of HSV-1 syn 17^+. The virus-DC mixture was incubated at 37°C for 24 hours. Next, the cells were stained with antibodies against HSV gB for 30 minutes at 4 °C, followed with a 1:50 dilution of goat anti mouse FITC IgG for 30 minutes at 4 °C. At last the cells were incubated with 2% PFA and gB expression was measured by flow cytometry.

CHO, CHO-DC-SIGN and GMK ($2x10^5$ cells) were seeded in a flat-bottom 96-wells plate 24 hours before infection with HSV-1 syn 17^+ ($10x10^6$ PFU). Cells were harvested after 48 hours of infection and analysed for gB expression as described above.

Statistical Analysis. To determine the differences for HSV-1 infection between treated and non-treated DCs, the mean values of the triplicates were used in a one-way analysis of variance (ANOVA). When the overall F-test was significant, differences were further investigated with the post hoc Bonferroni test using Graphpad Prism software. A probability of $p < 0.05$ was considered statistically significant.

Acknowledgements

We would like to thank Dr. G. Davids for providing us with the 10E4 antibody and Dr. G. Verjans for supplying the clinical strain of HSV-1. This work was supported by the Dutch Scientific Organization (NWO) grants 91746367 (LdW) and 91204025 (MdJ).

References

Altmann, F., Staudacher, E., Wilson, I. B. & Marz, L. (1999). Insect cells as hosts for the expression of recombinant glycoproteins. *Glycoconj J* **16**, 109-123.

Bergstrom, T., Sjogren-Jansson, E., Jeansson, S. & Lycke, E. (1992). Mapping neuroinvasiveness of the herpes simplex virus type 1 encephalitis-inducing strain 2762 by the use of monoclonal antibodies. *Mol Cell Probes* **6**, 41-49.

Bosnjak, L., Miranda-Saksena, M., Koelle, D. M., Boadle, R. A., Jones, C. A. & Cunningham, A. L. (2005). Herpes simplex virus infection of human dendritic cells induces apoptosis and allows cross-presentation via uninfected dendritic cells. *J Immunol* **174**, 2220-2227.

Burleigh, L., Lozach, P. Y., Schiffer, C. & other authors (2006). Infection of dendritic cells (DCs), not DC-SIGN-mediated internalization of human immunodeficiency virus, is required for long-term transfer of virus to T cells. *J Virol* **80**, 2949-2957.

Cai, W. H., Gu, B. & Person, S. (1988). Role of glycoprotein B of herpes simplex virus type 1 in viral entry and cell fusion. *J Virol* **62**, 2596-2604.

Cai, W. Z., Person, S., Warner, S. C., Zhou, J. H. & DeLuca, N. A. (1987). Linker-insertion nonsense and restriction-site deletion mutations of the gB glycoprotein gene of herpes simplex virus type 1. *J Virol* **61**, 714-721.

Cheshenko, N. & Herold, B. C. (2002). Glycoprotein B plays a predominant role in mediating herpes simplex virus type 2 attachment and is required for entry and cell-to-cell spread. *J Gen Virol* **83**, 2247-2255.

de Witte, L., Abt, M., Schneider-Schaulies, S., van Kooyk Y. & Geijtenbeek, T. B. (2006). Measles Virus Targets DC-SIGN To Enhance Dendritic Cell Infection. *J Virol* **80**, 3477-3486.

de Witte, L., Bobardt, M., Chatterji, U., de Geest, G., David, G., Geijtenbeek, T. B. & Gallay, P. (2007). Syndecan-3 is a dendritic cell-specific attachment receptor for HIV-1. *Proc Natl Acad Sci U S A* **104**, 19464-19469.

Dolan, A., Jamieson, F. E., Cunningham, C., Barnett, B. C. & McGeoch, D. J. (1998). The genome sequence of herpes simplex virus type 2. *J Virol* **72**, 2010-2021.

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

Geijtenbeek, T. B., van Duijnhoven, G. C., van Vliet, S. J., Krieger, E., Vriend, G., Figdor, C. G. & van Kooyk Y. (2002). Identification of different binding sites in the dendritic cell-specific receptor DC-SIGN for intercellular adhesion molecule 3 and HIV-1. *J Biol Chem* **277**, 11314-11320.

Geijtenbeek, T. B. & van Kooyk Y. (2003).DC-SIGN: a novel HIV receptor on DCs that mediates HIV-1 transmission. *Curr Top Microbiol Immunol* **276**, 31-54.

Halary, F., Amara, A., Lortat-Jacob, H. & other authors (2002). Human cytomegalovirus binding to DC-SIGN is required for dendritic cell infection and target cell trans-infection. *Immunity* **17**, 653-64.

Herold, B. C., WuDunn, D., Soltys, N. & Spear, P. G. (1991). Glycoprotein C of herpes simplex virus type 1 plays a principal role in the adsorption of virus to cells and in infectivity. *J Virol* **65**, 1090-1098.

Kruse, M., Rosorius, O., Kratzer, F., Bevec, D., Kuhnt, C., Steinkasserer, A., Schuler, G. & Hauber, J. (2000a). Inhibition of CD83 cell surface expression during dendritic cell maturation by interference with nuclear export of CD83 mRNA. *J Exp Med* **191**, 1581-1590.

Kruse, M., Rosorius, O., Kratzer, F., Stelz, G., Kuhnt, C., Schuler, G., Hauber, J. & Steinkasserer, A. (2000b). Mature dendritic cells infected with herpes simplex virus type 1 exhibit inhibited T-cell stimulatory capacity. *J Virol* **74**, 7127-7136.

Kummer, M., Turza, N. M., Muhl-Zurbes, P., Lechmann, M., Boutell, C., Coffin, R. S., Everett, R. D., Steinkasserer, A. & Prechtel, A. T. (2007). Herpes simplex virus type 1 induces CD83 degradation in mature dendritic cells with immediate-early kinetics via the cellular proteasome. *J Virol* **81**, 6326-6338.

Lekkerkerker, A. N., Ludwig, I. S., van Vliet, S. J., van Kooyk, Y. & Geijtenbeek, T. B. (2004). Potency of HIV-1 envelope glycoprotein gp120 antibodies to inhibit the interaction of DC-SIGN with HIV-1 gp120. *Virology* **329**, 465-476.

Little, S. P., Jofre, J. T., Courtney, R. J. & Schaffer, P. A. (1981).A virion-associated glycoprotein essential for infectivity of herpes simplex virus type 1. *Virology* **115**, 149-160.

Mikloska, Z., Bosnjak, L. & Cunningham, A. L. (2001). Immature monocyte-derived dendritic cells are productively infected with herpes simplex virus type 1. *J Virol* **75**, 5958-5964.

Novak, N. & Peng, W. M. (2005). Dancing with the enemy: the interplay of herpes simplex virus with dendritic cells. *Clin Exp Immunol* **142**, 405-410.

Nystrom, K., Biller, M., Grahn, A., Lindh, M., Larson, G. & Olofsson, S. (2004). Real time PCR for monitoring regulation of host gene expression in herpes simplex virus type 1-infected human diploid cells. *J Virol Methods* **118**, 83-94.

Olofsson, S., Khanna, B. & Lycke, E. (1980). Altered kinetic properties of sialyl and galactosyl transferases associated with herpes simplex virus infection of GMK and BHK cells. *J Gen Virol* **47**, 1-9.

Pepose, J. S., Keadle, T. L. & Morrison, L. A. (2006). Ocular herpes simplex: changing epidemiology, emerging disease patterns, and the potential of vaccine prevention and therapy. *Am J Ophthalmol* **141**, 547-557.

Raftery, M. J., Winau, F., Kaufmann, S. H., Schaible, U. E. & Schonrich, G. (2006).CD1 antigen presentation by human dendritic cells as a target for herpes simplex virus immune evasion. *J Immunol* **177**, 6207-6214.

Roizman D, Knipe D & Whitley, R. (2007). Herpes simplex viruses. In *Fields Virology*, 5th edn, pp. 2501-2601. Edited by Knipe DM, Howley D, Griffin DE, Lamb R & Martin B: Lippincott, Williams and Wilkins, Philadelphia, PA.

Sallusto, F. & Lanzavecchia, A. (1994). Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. *J Exp Med* **179**, 1109-18.

Sarmiento, M., Haffey, M. & Spear, P. G. (1979). Membrane proteins specified by herpes simplex viruses. III. Role of glycoprotein VP7(B2) in virion infectivity. *J Virol* **29**, 1149-1158.

Satoh, T., Arii, J., Suenaga, T. & other authors (2008).PILRalpha is a herpes simplex virus-1 entry coreceptor that associates with glycoprotein B. *Cell* **132**, 935-944.

Spear, P. G. (2004). Herpes simplex virus: receptors and ligands for cell entry. *Cell Microbiol* **6**, 401-410.

Tassaneetrithep, B., Burgess, T. H., Granelli-Piperno, A. & other authors (2003).DC-SIGN (CD209) Mediates Dengue Virus Infection of Human Dendritic Cells. *J Exp Med* **197**, 823-9.

Taylor, T. J., Brockman, M. A., McNamee, E. E. & Knipe, D. M. (2002). Herpes simplex virus. *Front Biosci* **7**, d752-d764.

Theodoridis, A. A., Prechtel, A. T., Turza, N. M., Zenke, M. & Steinkasserer, A. (2007). Infection of human dendritic cells with herpes simplex virus type 1 dramatically diminishes the mRNA levels of the prostaglandin E(2) receptors EP2 and EP4. *Immunobiology* **212**, 827-838.

Trybala, E., Liljeqvist, J. A., Svennerholm, B. & Bergstrom, T. (2000). Herpes simplex virus types 1 and 2 differ in their interaction with heparan sulfate. *J Virol* **74**, 9106-9114.

van Gisbergen, K. P., Aarnoudse, C. A., Meijer, G. A., Geijtenbeek, T. B. & van Kooyk, Y. (2005a). Dendritic cells recognize tumor-specific glycosylation of carcinoembryonic antigen on colorectal cancer cells through dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin. *Cancer Res* **65**, 5935-5944.

van Gisbergen, K. P., Ludwig, I. S., Geijtenbeek, T. B. & van Kooyk, Y. (2005b). Interactions of DC-SIGN with Mac-1 and CEACAM1 regulate contact between dendritic cells and neutrophils. *FEBS Lett* **579**, 6159-6168.