Modulation of HIV and SIV neutralization sensitivity by DC-SIGN and mannose-binding lectin

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Abstract

The C-type lectin DC-SIGN binds to oligosaccharides on the human and simian immunodeficiency virus (HIV, SIV) envelope glycoproteins and promotes infection of susceptible cells. Here, we show that DC-SIGN recognizes glycans involved in SIV sensitivity to neutralizing antibodies and that binding to DC-SIGN confers neutralization resistance to an otherwise sensitive SIV variant. Moreover, we provide evidence that mannose-binding lectin (MBL) can interfere with HIV-1 neutralization by the carbohydrate-specific antibody 2G12.
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Introduction

DC-SIGN (CD209) is a calcium-dependent (C-type) lectin that was identified as a human immunodeficiency virus (HIV) envelope protein (Env) binding factor in placental tissue (Curtis et al., 1992). Subsequently, it was shown that DC-SIGN is expressed at high levels on dendritic cells (DCs) and proposed that DC-SIGN might be responsible for much of the well-documented capability of DCs to capture and transfer the virus to adjacent T-cells (trans-infection) (Geijtenbeek et al., 2000). Since the latter process might be important for dissemination of sexually transmitted HIV (Wu and KewalRamani, 2006), a role for DC-SIGN as an HIV transmission promoting factor has been suggested (Geijtenbeek et al., 2000, 2001b). While several

reports support important features of this model (Arrighi et al., 2004a, 2004b; Gurney et al., 2005; Hu et al., 2004; Liu et al., 2004, 2006; Martin et al., 2004), it has also been demonstrated that the majority of HIV taken up by DCs is degraded and processed for MHC presentation (Moris et al., 2004, 2006) and that DC-SIGN-dependent transmission is a short lived process (Burleigh et al., 2006; Nobile et al., 2005; Turville et al., 2004). Nevertheless, recent reports of DC-SIGN-mediated HIV interactions with platelets (Boukour et al., 2006; Chaipan et al., 2006) and activated B-cells (He et al., 2006; Rappocciolo et al., 2006) reinforce the notion that certain motile cells might capture HIV via DC-SIGN and might thereby promote viral spread in infected individuals.

Experimental infection of macaques with simian immunodeficiency virus (SIV) is one of the best models for HIV/AIDS in humans. Macaque and human DC-SIGN transmit HIV and SIV with similar efficiency (Baribaud et al., 2001; Geijtenbeek et al., 2001a; Ploquin et al., 2004; Wu et al., 2002a). However, the DC-SIGN-related proteins DC-SIGNR/L-SIGN/CD209L (humans)

(Bashirova et al., 2001; Pöhlmann et al., 2001b) and CD209L2 (macaques) (Bashirova et al., 2003) differ in their ability to interact with these viruses, with only DC-SIGNR being competent for HIV and SIV transmission (Bashirova et al., 2001, 2003; Pöhlmann et al., 2001b). DC-SIGN recognizes high-mannose carbohydrates on the HIV and SIV Env proteins (Appelmelk et al., 2003; Guo et al., 2004; Hong et al., 2002; Lin et al., 2003). Mutagenic analysis revealed that several glycans on HIV Env are involved in DC-SIGN binding (Hong et al., 2002) and that the DC-SIGN binding site partially overlaps with the epitope of the neutralizing antibody 2G12 (Binley et al., 2006; Hong et al., 2007). Glycans on the HIV and SIV Env proteins are required for protection from neutralizing antibodies (Pikora et al., 2006). Thus, the parental SIVmac239 virus is highly resistant to antibody-mediated neutralization, while SIVmac239 variants with mutations in certain N-linked glycosylation sites are neutralization sensitive (Reitter et al., 1998). However, it is currently unclear whether glycans involved in SIV neutralization sensitivity are also required for DC-SIGN binding. Here, we investigated if glycosylation sites known to impact SIV neutralization sensitivity also modulate DC-SIGN usage and we asked if DC-SIGN binding can confer neutralization resistance to an otherwise neutralization sensitive SIV variant with altered glycosylation signals. Moreover, we analyzed if mannose-binding lectin (MBL), a soluble lectin in human serum, modulates HIV-1 neutralization by the carbohydrate-specific monoclonal antibody 2G12 (Buchacher et al., 1994; Sanders et al., 2002).

Results

DC-SIGN and CD209L2 augment Ebola virus glycoprotein-driven cis-infection with similar efficiencies

In order to address the contribution of specific glycans in SIV Env to SIV interactions with DC-SIGN, we first attempted to confirm previous reports indicating that macaque DC-SIGN but not the related lectin CD209L2 can transmit SIV (Bashirova et al., 2003). Transmission of SIV by human and macaque DC-SIGN and macaque CD209L2 was assessed employing B-THP cell lines exogenously expressing these lectins as transmitters, as described previously (Baribaud et al., 2002; Bashirova et al., 2003). Lectin expression on these cell lines was comparable, as judged by FACS analysis (data not shown). In agreement with published studies (Baribaud et al., 2001; Bashirova et al., 2003; Geijtenbeek et al., 2001a; Ploquin et al., 2004; Wu et al., 2002a), we found that human and macaque DC-SIGN transmitted SIVmac239 and the macrophage tropic SIVmac239 variant 316 (Mori et al., 1993) with high efficiency, while transmission by CD209L2 was relatively inefficient (Figs. 1A and 2A). Similar
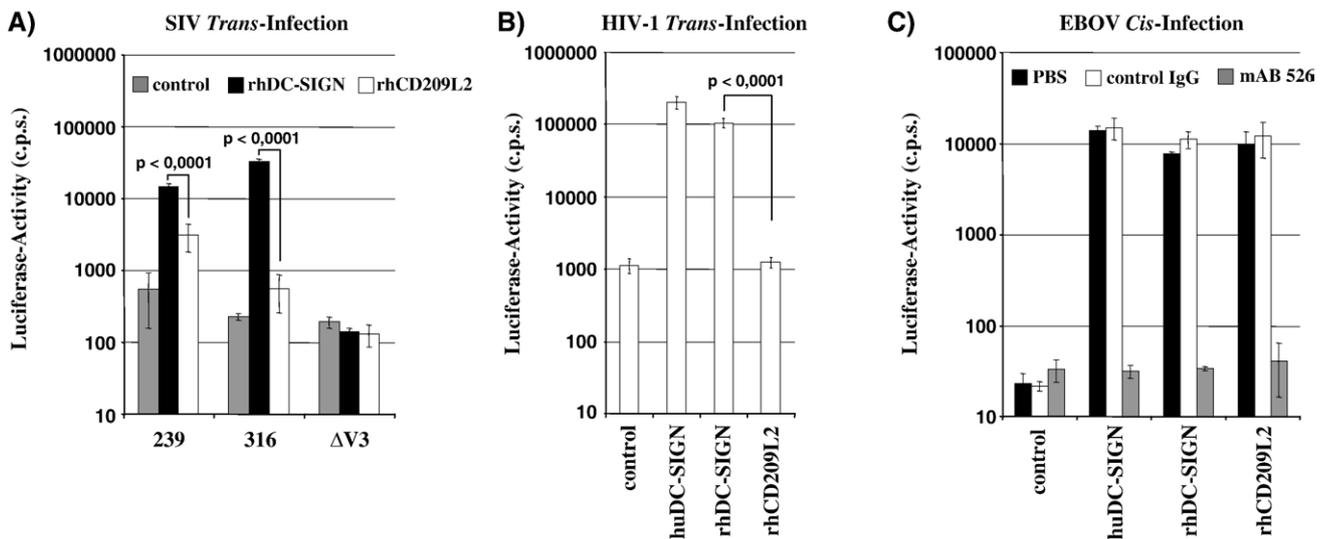

Fig. 1. CD209L2 does not transmit HIV and SIV but enhances *cis*-infection driven by Ebola virus glycoprotein. (A) DC-SIGN but not CD209L2 augments SIV transfer. B-THP cell lines expressing the indicated lectins were incubated with SIVmac239, the macrophage-tropic SIVmac239 variant 316 or a V3-deleted SIVmac239 variant as negative control (all produced in 293T cells). For this, 50,000 cells were mixed with 1,500 tissue culture infectious doses (as determined on CEMx174 R5 cells) of SIVmac239 and 316 or with a maximum volume of V3-deleted virus stock. Subsequently, the cells were washed and cocultivated with permissive CEMx174 R5 cells which express luciferase under control of the viral LTR. Three days after the start of the cocultures luciferase activities in cellular lysates were determined. The results of a representative experiment performed in quadruplicates are shown and were confirmed in three separate experiments. Error bars indicate standard deviation (SD), statistical significance was calculated using *t*-test for independent samples. Rh, rhesus macaque (B) CD209L2 does not transmit HIV-1. Lectin-dependent transmission of HIV-1 NL4-3 was assessed as described in panel A; however, input virus was normalized for p24-content. A representative experiment performed in quadruplicates is shown. Similar results were obtained in two separate experiments. Error bars indicate SD, statistical significance was calculated using *t*-test for independent samples. Hu, human; rh, rhesus macaque (C) CD209L2 augments infection driven by the Ebola virus glycoprotein (EBOV-GP). The indicated lectin expressing cells were seeded in 96-well plates at a concentration of 30,000 cells per well, incubated with mAb 526 or control IgG (10 μg/ml final concentration) and infected with SIV reporter virus bearing EBOV-GP. The luciferase activities in the cultures were determined 3 days post-infection. The results of a representative experiment performed in quadruplicates are presented and were confirmed in two separate experiments. Error bars indicate SD. Hu, human; rh, rhesus macaque.
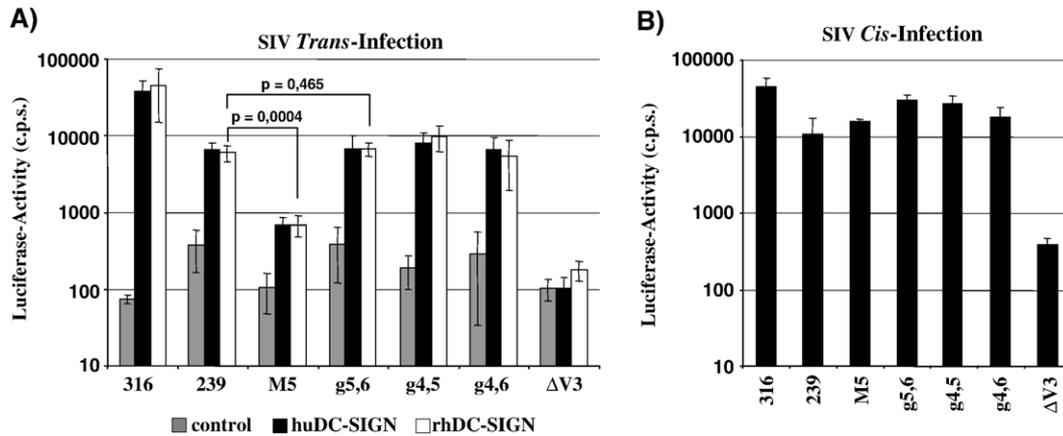
Fig. 2. DC-SIGN-mediated transmission of SIV variants with mutations in N-linked glycosylation signals. (A) Transmission of SIV glycosylation site variants by DC-SIGN. The transmission assay was carried out as described in the legend to Fig. 1A, employing infectivity normalized viruses. B-THP cell lines engineered to express human DC-SIGN (huDC-SIGN), rhesus macaque DC-SIGN (rhDC-SIGN) or parental B-THP cells were used as transmitters. In parallel, direct infection of CEMx174 R5 cells in the absence of transmitters was assessed and results are shown in panel B. Representative experiments performed in quadruplicates are presented in panels A and B. The results were confirmed in five separate experiments. Error bars indicate SD, statistical significance was calculated using *t*-test for independent samples.

results were obtained for HIV-1 (Fig. 1B). However, both macaque DC-SIGN and CD209L2 strongly augmented *cis*-infection (direct infection of the lectin expressing cells) driven by SIV-derived pseudotypes bearing the glycoprotein of Ebola virus (EBOV), a well-documented DC-SIGN ligand (Alvarez et al., 2002; Simmons et al., 2003), and antibody inhibition analysis confirmed that enhancement of infection was lectin-dependent (the monoclonal antibody (mAb) 526 recognizes DC-SIGN and CD209L2; Baribaud et al., 2002; Bashirova et al., 2003; Wu et al., 2002b) (Fig. 1C). Thus, CD209L2 might not impact SIV spread in macaques but might serve as a potent attachment factor in the context of EBOV infection.

Glycans involved in SIV sensitivity to neutralization also contribute to DC-SIGN binding

We next analyzed if well-characterized alterations of SIVmac239 Env glycosylation sites (Reitter and Desrosiers, 1998), which confer neutralization sensitivity (Reitter et al., 1998), also impact DC-SIGN-dependent SIV transfer to susceptible cells. Thus, variant M5 harbors changes in N-linked glycosylation signals 5, 6, 8, 12 and 13 while combinations of the glycosylation signals 4, 5 and 6 were inactivated in variants g4,5; g4,6 and g5,6; and all viral variants are known to be susceptible to neutralization (Johnson et al., 2003; Reitter et al., 1998). Analysis of DC-SIGN-mediated transmission of TCID$_{50}$-normalized viruses revealed that the variants g4,5; g4,6 and g5,6 interacted with DC-SIGN with similar efficiency as the wt virus, while transfer of M5 was severely reduced (Fig. 2A). Direct infection of target cells in the absence of lectin expressing cells confirmed comparable infectivity of all viruses analyzed except for a V3-loop deleted control virus (Fig. 2B), underlining that the diminished DC-SIGN-driven transfer of the M5 virus was not due to reduced infectivity for target cells. Therefore, N-linked glycosylation signals 5, 6, 8, 12 and 13 seem to be involved in

both, protection against neutralizing antibodies and engagement of DC-SIGN.

Binding of SIV to DC-SIGN confers resistance to antibody-mediated neutralization

We then sought to determine if binding to DC-SIGN can protect SIV from attack by neutralizing antibodies. The g5,6 variant was chosen for these experiments since this virus is neutralization sensitive (Reitter et al., 1998) and engages DC-SIGN efficiently (Fig. 2A). Investigating a potential link between DC-SIGN binding and neutralization sensitivity required establishment of a cellular system in which binding to DC-SIGN readily occurs but does not augment viral infectivity; otherwise, it would be impossible to discriminate between DC-SIGN-dependent enhancement of infectious entry and DC-SIGN-mediated protection against neutralizing antibodies. In agreement with our previous unpublished observations, DC-SIGN-expressing 293 T-REx cells fulfilled this requirement, provided that no wash step was included in the transmission assay. Thus, virtually identical infection of CEMx174 R5 target cells by the g5,6 variant was observed independent of the presence of DC-SIGN T-REx cells, parental T-REx control cells or PBS in the cocultures (93.34 ± 8.16 and $84.94 \pm 8.76\%$ infectious entry in the presence of T-REx DC-SIGN and T-REx control cells, respectively, with entry in PBS-treated target cells being set as 100%. Results represent the average of six independent experiments ± standard error of the mean). This system was then employed for analysis of g5,6 neutralization. Coculture of T-REx control cells with CEMx174 R5 targets slightly increased neutralization resistance, particularly when low doses of antiserum were added, and this effect was further augmented by coculture of targets with DC-SIGN expressing T-REx cells (Figs. 3A and B). Inhibition analysis with mAb 526 confirmed that the enhanced neutralization
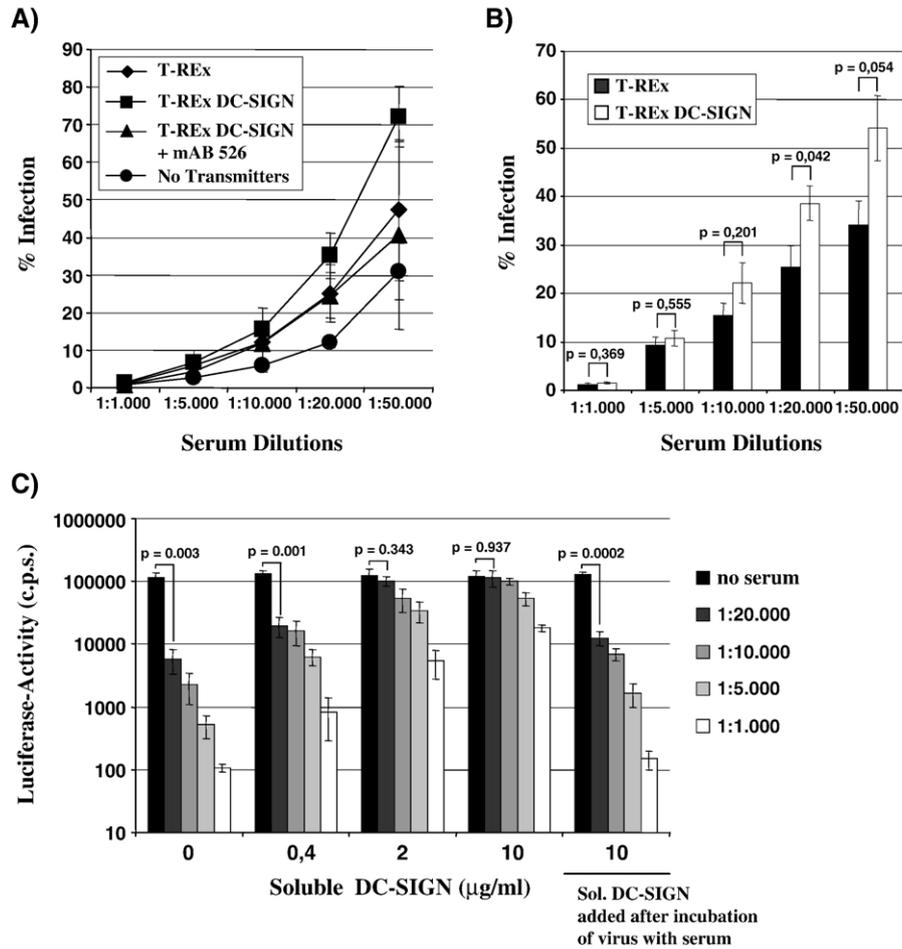
Fig. 3. Binding of SIV to DC-SIGN can protect from antibody-mediated neutralization. (A) Protection of SIV from neutralization by membrane-bound human DC-SIGN. Infectivity normalized SIVmac239 g5,6 virus was incubated with T-REx DC-SIGN cells (Pöhlmann et al., 2001a), in the presence or absence of mAb 526, or incubated with parental T-REx cells or PBS for 30 min in a total volume of 100 μl per well (the assay was carried out in 96-well plates). Subsequently, 50 μl of the indicated dilutions of neutralizing serum (obtained from a g5,6-infected macaque; Reitter et al., 1998) was added. After a 30-min incubation period, 50 μl of fresh medium containing CEMx174 R5 target cells was added to the cultures without removing free virus. Three days after the start of the cocultures, luciferase activities in cellular lysates were determined. The results of a representative experiment are shown and were confirmed in two to six separate experiments. Error bars indicate SD. (B) Same experimental setup as in panel A; however, the average ± SEM of six independent experiments is shown. Statistical significance was calculated using *t*-test for independent samples. (C) Protection of SIV from neutralization by soluble, recombinant human DC-SIGN. Infectivity normalized g5,6 virus was incubated for 30 min with the indicated final concentrations of soluble DC-SIGN, previously expressed in *Escherichia coli* and purified on mannose–sepharose (Mitchell et al., 2001), in a total volume of 100 μl. Subsequently, 50 μl of the indicated dilutions of neutralizing serum from a g5,6-infected macaque was added. As a control the g5,6 variant was first incubated with neutralizing antiserum and then the highest concentration of soluble DC-SIGN was added. After 30 min, 50 μl of fresh medium containing CEMx174 R5 target cells was added to the cultures. Luciferase activities in cellular lysates were measured 3 days after addition of target cells. The results of a representative experiment performed in quadruplicates are shown. Similar results were obtained in three separate experiments. Error bars indicate SD. Statistical significance was calculated using *t*-test for dependent samples.

resistance was indeed due to DC-SIGN (Figs. 3A and B). Thus, cell-bound SIV is somewhat less sensitive to neutralization than free virus and DC-SIGN expression increases protection.

The moderate protective effect against antibody-mediated neutralization of SIV observed with kidney-derived 293 cell lines (Figs. 3A and B) might not appropriately reflect the in vivo situation since in infected macaques DC-SIGN-expressing cells are arranged in tightly packed, three-dimensional arrays in lymphoid and mucosal tissues and might secrete soluble DC-SIGN in the supernatant (Furmonaviciene et al., 2007; Liu et al., 2005; Mummidi et al., 2001). Thus, a significantly higher number of Env trimers on the virion surface might be bound to DC-SIGN in vivo as compared to our two-dimensional cell

culture system. In order to investigate whether increasing DC-SIGN copy numbers and bypassing spatial constraints to DC-SIGN binding can confer neutralization resistance to the g5,6 variant, we pre-incubated the virus with rising concentrations of soluble recombinant tetrameric DC-SIGN (Mitchell et al., 2001) before adding neutralizing antiserum and target cells (Fig. 3C). In this experimental set-up DC-SIGN protected SIV g5,6 efficiently and in a dose-dependent manner from neutralization. Importantly, neutralization resistance was not observed when soluble DC-SIGN was added subsequent to the antiserum (Fig. 3C), suggesting that protection from neutralization was specific and most likely due to DC-SIGN tetramers shielding epitopes in SIV Env from recognition by neutralizing antibodies.
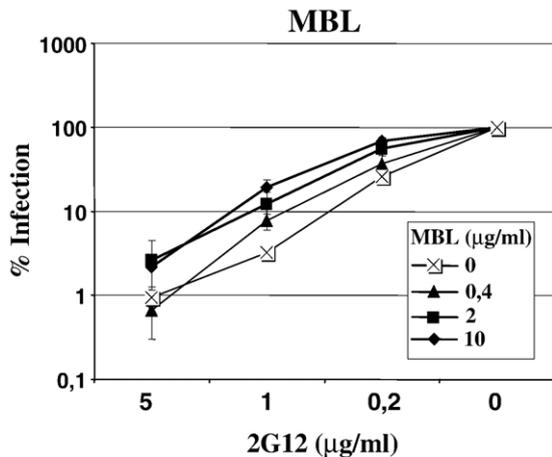
Fig. 4. MBL reduces 2G12-mediated neutralization of HIV-1. A HIV-1 NL4-3 reporter virus was incubated for 30 min with the indicated concentrations of recombinant MBL. Subsequently, the neutralizing antibody 2G12 was added at the indicated concentrations and the samples were incubated for 30 min before addition of CEMx174 R5 target cells. After cultivation of cells for 3 days, the luciferase activities in cellular lysates were determined. Enzyme activities measured in the absence of 2G12 were set as 100% and the average of three independent experiments performed in quadruplicates is shown. Error bars indicate SEM.

MBL modulates HIV-1 neutralization by the carbohydrate-specific antibody 2G12

In order to further explore the relevance of lectin engagement for lentiviral spread in the face of neutralizing antibodies, we asked if lectins in human serum can affect neutralization sensitivity of HIV-1. To this end, we investigated if soluble MBL, a lectin present in concentrations of up to 10 µg/ml in human serum, modulates recognition of HIV-1 by the carbohydrate-specific, broadly neutralizing antibody 2G12. Treatment of HIV-1 with MBL alone caused a moderate increase in viral infectivity (6.30 ± 1.29-fold at the maximal MBL concentration, average $\pm$ standard error of the mean (SEM) of three independent experiments and data not shown). Exposure of virions to recombinant MBL prior to antibody addition provided appreciable protection against 2G12-mediated neutralization (Fig. 4), with at least the difference in HIV-1 infectivity in the presence of 1 µg/ml 2G12 and either 0 or 10 µg/ml MBL being statistically significant ($p = 0.03$). Thus, our results are generally compatible with the idea that MBL and maybe other lectins present in human serum might interfere with HIV-1 recognition by carbohydrate-specific agents.

Discussion

DC-SIGN and DC-SIGNR are calcium-dependent (C-type) lectins that can augment HIV and SIV infectivity when expressed exogenously on cell lines. Endogenous DC-SIGN expression on DCs (Geijtenbeek et al., 2000), platelets (Boukour et al., 2006; Chaipan et al., 2006) and B-cells (He et al., 2006; Rappocciolo et al., 2006) has also been shown to enhance infection by primate lentiviruses and it has been proposed that DC-SIGN on these cell types might promote viral

dissemination in and between hosts. However, the role of DC-SIGN in DC-mediated transfer of HIV and SIV is under discussion and an important contribution of other factors has been established (Baribaud et al., 2002; Boggiano et al., 2006; Gummuluru et al., 2003; Turville et al., 2002; Wu et al., 2002a). Moreover, it should be noted that DC-SIGN can augment *cis*- and *trans*-infection (Burleigh et al., 2006; Lee et al., 2001; Nobile et al., 2005; Turville et al., 2004) and it can not be excluded that a minor part of the transmission activity of the B-THP cell lines examined here was due to progeny virus released from infected transmitters. The expression of DC-SIGN and DC-SIGNR differs among humans and non-human primates. Humans express DC-SIGN and DC-SIGNR, while the latter is absent in monkeys which instead encode a related protein termed CD209L2 (Bashirova et al., 2003). CD209L2, like DC-SIGNR, has been detected in liver and lymph nodes (Bashirova et al., 2003). However, in contrast to DC-SIGNR, CD209L2 was found to be unable to efficiently augment *trans*-infection of HIV-1 and SIV (Bashirova et al., 2003). We confirmed this observation but also noted that CD209L2 was able to robustly augment EBOV-driven *cis*-infection and might therefore modulate EBOV spread in infected macaques.

It has long been proposed that oligosaccharides on the surface of HIV and SIV Env protect these proteins from recognition by neutralizing antibodies. Strong experimental evidence for such an effect was reported by Reitter and colleagues (1998) who demonstrated that inactivation of highly conserved glycosylation signals in SIVmac239 Env by side-specific mutagenesis yields viral variants which, in contrast to the wt virus, are highly neutralization sensitive. Sera from macaques experimentally infected with some of these variants exhibited augmented reactivity against peptides spanning the altered glycosylation signals and were capable of neutralizing the wt virus (Reitter et al., 1998), indicating that certain glycans in SIV Env prevent the generation of an effective neutralizing antibody response. Our results show that some of these oligosaccharides are also involved in binding to DC-SIGN. Thus, removal of glycosylation signals 5, 6, 8, 12 and 13 in variant M5, which rendered the mutant virus neutralization sensitive (Reitter et al., 1998), strongly reduced DC-SIGN engagement. In contrast, mutation of glycosylation signals 5 and 6 together or mutation of a single site in combination with alteration of glycosylation site 4 did not appreciably diminish interactions with DC-SIGN. These results suggest that glycosylation signals 4, 5 and 6, which are used in the wt virus (Reitter and Desrosiers, 1998), might not be involved in DC-SIGN binding. Alternatively, the glycans attached to signals 5 and 6 might be recognized by DC-SIGN and defects in these glycosylation sites might be compensated by carbohydrates attached at positions 8, 12 and 13. In fact, mutation of glycosylation site 5 and 6 in the M5 variant might affect processing of glycans 8, 12 and 13, which, under these conditions, might be exclusively of the high-mannose type (the type of carbohydrate bound by DC-SIGN). In any event, the diminished but not abrogated DC-SIGN interactions with the M5 virus clearly suggest that several glycans in SIV Env might be required for DC-SIGN binding and most or all of these

glycans are also important for protection of SIV against neutralizing antibodies (Reitter et al., 1998).

Since an overlapping set of glycans was involved in DC-SIGN engagement and in SIV sensitivity to neutralization, we analyzed whether DC-SIGN binding protects SIV from antibody-mediated neutralization. Attachment of virus to DC-SIGN expressing cells indeed exerted a modest but readily detectable protective effect. More impressively, pre-incubation of virions with soluble, tetrameric DC-SIGN resulted in highly efficient, dose-dependent protection from neutralizing antibodies. Why was soluble DC-SIGN more efficient in protecting SIV from antibody recognition than membrane-bound protein? First, roughly 1,000 to 10,000 more DC-SIGN tetramers were available for SIV binding in the assays carried out with soluble relative to membrane-bound DC-SIGN. Second, the spatial constraints to ligand recognition imposed by membrane insertion of DC-SIGN did not afflict SIV binding to soluble DC-SIGN. Thus, usage of soluble relative to membrane-associated DC-SIGN most likely allowed DC-SIGN binding to a higher percentage of virion incorporated Env trimers and thereby provided more efficient protection against antibodies. Since soluble DC-SIGN, in agreement with previous data (Kwon et al., 2002), did not alter SIV infectivity and had no protective effect when added subsequently to the neutralizing antiserum, we believe that protection was most likely due to steric hindrance, with DC-SIGN covering epitopes recognized by neutralizing antibodies.

Whether DC-SIGN can indeed shield neutralization sensitive HIV and SIV variants from antibody recognition in the infected host needs to be elucidated. However, three-dimensional arrays of DC-SIGN-positive cells in lymphoid tissues or in the anogenital mucosa may simultaneously bind to more Env trimers on the virion surface than cell lines commonly used to study DC-SIGN function. Moreover, several studies reported that alternative splicing of endogenous DC-SIGN and DC-SIGNR readily occurs in primary cells and can result in production of secreted lectins lacking the transmembrane domain (Liu et al., 2005; Mummidi et al., 2001). Finally, recent data indicate the presence of a protease-sensitive region within DC-SIGN, at an N-terminal sequence close to the outer bilayer of the plasma membrane, that is highly susceptible to cleavage by a number of eukaryotic enzymes (Feinberg et al., 2005; Furmonaviciene et al., 2007). Thus, certain tissues and compartments of the infected host might contain sufficient soluble and membrane-bound DC-SIGN to shield a fraction of the local viruses against neutralizing antibodies. In addition, a very recent study by van Montfort and colleagues (2007) suggests that DC-SIGN might protect HIV-1 from neutralization by antibodies even after antibody binding. This group demonstrates that DC-SIGN on cell lines and monocyte-derived dendritic cells mediates efficient capture of HIV-1 bound by broadly neutralizing antibodies and that captured virions are highly infectious for co-cultured T-cells. It is proposed that antibody-bound virus is internalized into endosomal compartments where low pH dissociates the virus–antibody complexes, followed by recycling of infectious virus to the cell surface and transfer to target cells (van Montfort et al., 2007). Therefore,

DC-SIGN might provide HIV and SIV with an efficient two-step protection against neutralizing antibodies, shielding of epitopes from antibody recognition and release of bound antibodies upon intracellular uptake of virions, which can subsequently be transmitted to T-cells.

A potential impact of lectins on the neutralization sensitivity of lentiviruses is further highlighted by our observation that MBL can interfere with HIV-1 recognition by the carbohydrate-specific antibody 2G12. MBL is present in human serum in concentrations ranging from 20 to 10,000 ng/ml and readily opsonizes HIV-1 (Dommett et al., 2006). However, MBL binding does not induce substantial HIV-1 neutralization (Ying et al., 2004). In contrast to previous studies (Dommett et al., 2006), we even observed slight augmentation of infectivity upon HIV-1 exposure to MBL. Differences in the cell types used for virus production (kidney-derived 293T cells versus T-cell lines) might account for this discrepancy. Pre-incubation with MBL moderately protected the CXCR4-tropic HIV-1 NL4-3 against neutralization by 2G12, a broadly neutralizing antibody, which, like MBL and DC-SIGN, recognizes mannose-rich N-glycans (Sanders et al., 2002). It is therefore conceivable that the level of MBL in patients might impact the efficiency of 2G12 treatment, which can reduce virus replication in infected patients (Trkola et al., 2005).

In summary, we provide evidence that engagement of DC-SIGN by otherwise neutralization sensitive SIV variants can confer efficient protection against neutralization. Moreover, our data suggest that MBL can modulate HIV-1 recognition by the broadly neutralizing antibody 2G12. These results and a recent report (van Montfort et al., 2007) demonstrate that attachment to DC-SIGN, MBL and possibly other lectins might allow some degree of viral spread despite the presence of neutralizing antibodies, which has implications for the design of vaccines and antibodies for therapy and prevention.

Materials and methods

Plasmid construction and cell culture

The envelope sequences of SIVmac239 variants 316, M5, g4,5; g4,6 and g5,6 (Reitter and Desrosiers, 1998; Reitter et al., 1998) were SphI and NheI inserted into the SIVmac239 genome contained in a pBR322-derived plasmid. The SIVmac239 ΔV3 variant has been described previously (Pöhlmann et al., 1999). Generation of 293 T-REx and B-THP cell lines expressing DC-SIGN has been described previously (Geijtenbeek et al., 2000; Pöhlmann et al., 2001a; Wu et al., 2004). The CEMx174 R5 cells express exogenous CCR5 and contain expression cassettes for EGFP and luciferase under control of the viral LTR (Hsu et al., 2003). T-REx cells and 293T cells were maintained in Dulbecco's modified Eagle's medium (DMEM; PAA) supplemented with 10% FCS, penicillin and streptomycin. In addition, the culture medium for parental T-REx cells was supplemented with 2.5 μg/ml blasticidin (Invitrogen), while medium for T-REx DC-SIGN cells contained 2.5 μg/ml blasticidin and 50 μg/ml zeocin (Invitrogen). Culture medium for parental and lectin expressing B-THP cell lines and CEMx174 M7 R5 cells was

RPMI (PAA), supplemented with 10% FCS, penicillin and streptomycin.

Virus production and normalization

SIVmac239 viruses were produced by transfection of 293T cells with plasmids encoding proviral DNA. Two days after transfection the culture supernatants were harvested, passed through 0.4 μm pore-size filters, aliquoted and stored at −80 °C. For subsequent infection experiments, the $TCID_{50}$ of each SIV stock was assessed using serial virus dilutions for infection of CEMx174 M7 R5 cells. For each dilution 6 parallel wells of a 96-well plate were infected. After 3 days the infected wells were counted and the results were used to determine the $TCID_{50}$ employing a previously described method. Lentiviral pseudotypes bearing ZEBOV-GP and encoding luciferase were generated as described (Marzi et al., 2006). Pseudotypes were normalized for luciferase production upon infection of 293T cells using a commercially available kit as recommended by the manufacturer (Promega, WI, USA). Replication-competent HIV-1 NL4-3 luciferase encoding reporter virus (NL4-3luc) was produced as described (Pöhlmann et al., 2001a) and stocks were normalized for p24-antigen content employing a commercially available antigen capture ELISA (Murex; Abbott Diagnostics).

Infection, transmission and neutralization experiments

Transmission of SIV and HIV-1 was analyzed as described previously (Baribaud et al., 2002; Pöhlmann et al., 2001a). In brief, 5×10^4 B-THP cells were incubated with equal volumes of viral supernatants containing 1,500 $TCID_{50}$ of each SIVmac239 variant or 5 ng p24-antigen of HIV-1 NL4-3 luc. Thereafter, cells were washed and cocultured with 3×10^4 CEMx174 M7 R5 target cells. Three days after the start of the cocultivation cells were lysed and luciferase activities in cellular lysates determined. Lectin-mediated enhancement of ZEBOV-GP-driven infection was analyzed as follows. The indicated B-THP cell lines were seeded into 96-well plates at a density of 3×10^4 per well, pre-incubated with either PBS or the DC-SIGN/R-specific mAb 526 (R&D Systems) at a final concentration of 10 μg/ml for 30 min at 37 °C and then infected with infectivity-normalized ZEBOV-GP pseudotypes. Luciferase activities in cell lysates were determined 3 days after infection. For neutralization experiments with T-REx cell lines, the cells were seeded into 96-well plates at a density of 3×10^4 per well and lectin expression induced overnight with doxycycline (dox) at a final concentration of 100 ng/ml. The cells were then pre-incubated with PBS or mAb 526 (10 μg/ml final concentration) for 30 min followed by incubation with 1,500 $TCID_{50}$ per well of the SIV variants. After incubation for 30 min at 37 °C, the serum of a SIVmac239 g5,6-infected macaque (Reitter et al., 1998) was added to the mixture at the indicated dilutions and the cells again incubated for 30 min. Finally, 3×10^4 CEMx174 M7 R5 target cells were added to each well and 72 h later luciferase activities in cellular lysates were determined.

The setup for inhibition experiments with soluble tetrameric DC-SIGN (sol DC-SIGN) and recombinant human MBL (R&D Systems) was similar: 1,500 $TCID_{50}$ of the SIVmac239 variant g5,6 or 7 ng HIV-1 NL4-3luc were pre-incubated with the indicated final concentrations of soluble DC-SIGN or MBL for 30 min at 37 °C in a 96-well plate (the generation of soluble DC-SIGN has been previously described in detail; Mitchell et al., 2001). Afterwards the serum of a SIVmac239 g5,6-infected macaque or the HIV-1 neutralizing monoclonal antibody 2G12 (NIH AIDS Research and Reference Program) was added to the mixture at the indicated dilutions/concentrations. After a second incubation period, 3×10^4 CEMx174 M7 R5 cells were added to each well and 72 h later luciferase activities in cellular lysates were determined.

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