

Lectin Switching During Dengue Virus Infection

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Dengue virus receptors are relatively poorly characterized, but there has been recent interest in 2 C-type lectin molecules, dendritic cell-specific intercellular adhesion molecule 3 (ICAM-3)-grabbing nonintegrin (DC-SIGN) and its close homologue liver/lymph node-specific ICAM-3-grabbing integrin (L-SIGN), which can both bind dengue and promote infection. In this report we have studied the interaction of dengue viruses produced in insect cells, tumor cell lines, and primary human dendritic cells (DCs) with DC-SIGN and L-SIGN. Virus produced in primary DCs is unable to interact with DC-SIGN but remains infectious for L-SIGN-expressing cells. Skin-resident DCs may thus be a site of initial infection by insect-produced virus, but DCs will likely not participate in large-scale virus replication during dengue infection. These results reveal that differential glycosylation of dengue virus envelope protein is highly dependent on cell state and suggest that studies of virus tropism using virus prepared in insect cells or tumor cell lines should be interpreted with caution.

The global prevalence of the dengue virus (DENV) has grown dramatically in recent decades, and it is now endemic in >100 countries, with some 2.5 billion people at risk of infection. Dengue is an arthropod-borne flavivirus that can be subdivided into the 4 major serotypes (DEN-1–DEN-4). Most dengue infections either are asymptomatic or lead to a self-limiting febrile illness, dengue fever. In some cases the illness is more severe, leading to dengue hemorrhagic fever (DHF) with severe plasma leakage and bleeding that can be life threatening.

DHF is more common in individuals undergoing secondary heterologous dengue infection than those suffering primary infections. There has been considerable work to understand the mechanism of severe disease, but the increased frequency during secondary infection and the occurrence of severe symptoms at a time when virus loads are falling sharply imply that it likely results from immunopathology driven by the acquired immune responses, rather than from direct viral cytopathology [1–4]. Levels of a number of cytokines, such as interferon γ and tumor necrosis factor α [5, 6], have been shown to correlate with disease severity, and a storm of inflammatory cytokine secretion has been proposed to lead to the vascular leak characteristic of DHF.

Recent reports have identified monocytes as a major cell target of viral replication, and heparin sulfates, DC-SIGN, mannose receptor, and other glycoproteins have been proposed as cellular receptors for DENV [7–18]. DC-SIGN polymorphism has been shown to be associated with the disease severity [19]. CLEC5A has also recently been described as a proinflammatory receptor for DENV that contributes to lethal disease in a mouse model [20].

As dengue virus circulates between 2 hosts, humans and insects, it has to be adapted to replicate and infect both

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species. The majority of studies of dengue infection and tropism use viruses produced in insect cell lines such as C6/36 or mammalian tumor cell lines such as Vero cells. Infection of humans occurs in a stepwise fashion: initial infection of cells with insect virus, followed by sequential infection of cells by mammalian-produced virus. We were interested to determine whether there were any differences in the tropism of viruses produced in primary non-transformed human cells.

MATERIALS AND METHODS

Viral Stocks and Cell Lines

The dengue-2 strain, 16681, was grown in C6/36 cells, Vero cells, and monocyte-derived dendritic cells (DCs). Cell-free supernatants were used either neat or after concentration by ultracentrifugation at 45,000 rpm for 4 h at 4°C, and the virus pellet was resuspended in 1.5% fetal bovine serum (FBS)/Leibovitz L-15. To concentrate large volumes of low-titer DENV supernatant, DENV were precipitated with 10% Polyethylene glycol 8000 (Sigma) before ultracentrifugation. U937 were maintained in 10% FBS/Roswell Park Memorial Institute medium (RPMI). NIH/3T3, and DC-SIGN and L-SIGN NIH/3T3 cell lines were obtained through the AIDS Research and Reference Reagent Program (Division of AIDS, National Institute of Allergy and Infectious Diseases, from Drs Thomas D. Martin and Vineet N. Kewal Ramani) and maintained in 10% FBS/Dulbecco's Modified Eagle's medium. All media were supplemented with 2 mmol/L L-glutamine, 100 U/mL penicillin, and 100 U/mL streptomycin.

The viral titers were determined by a focus-forming assay. Briefly, virus was serially diluted and incubated with Vero cells for 2 hours at 37°C. The monolayers were then overlaid with 1.5% carboxymethylcellulose and incubated at 37°C for 2 days. Virus foci were stained with anti-E antibody followed by peroxidase-conjugated anti-mouse immunoglobulin (Ig) and visualized by the addition of 3,3'-Diaminobenzidine tetrahydrochloride substrate.

Preparation of Primary Monocyte-Derived Dendritic Cells

DCs were cultured as described elsewhere [21]. Human CD14⁺ monocytes were cultured in RPMI 1640 with 20 ng/mL rhuGM-CSF (First Link) and 25 ng/mL rhuIL-4 (eBioscience). The appropriate phenotype of immature DC (ie, lacking CD14⁺ but expressing major histocompatibility complex class I and class II and DC-SIGN) was confirmed.

Monitoring DENV Infection

Cells were infected with DENV at a multiplicity of infection (MOI) of 1 for 24 h. Cells were washed twice in FACS wash (FW; Phosphate-Buffered Saline [PBS] containing .5% Bovine Serum Albumin, 2% FBS, 1% Human Serum, .01% NaN₃). Cells were fixed with 4% paraformaldehyde/PBS for 10 minutes and

permeabilized with .5% saponin in FW for 10 minutes. Cells were then incubated with anti-NS1 mAb or with anti-E mAb followed by anti-mouse IgG/PE (DakoCytomation) in .5% saponin/FW. Cells were resuspended in FW and analyzed by FACScan (Becton Dickinson). Data were analyzed by using Flowjo software (Tree Star). 3T3 and 293T were incubated with 20 U/mL heparin for 20 minutes at room temperature before being infected with DENV.

Cell Binding Assay

Cells were incubated with DENV produced from different cell types at equal amounts of E protein (measured by enzyme-linked immunosorbent assay) for 2 h at room temperature. After washing with ice-cold FW, cells were fixed with 4% paraformaldehyde/PBS, and surface-bound virus was detected with an anti-E mAb and anti-mouse IgG/PE followed by FACS analysis.

Transient Transfection of 293T With L-SIGN

The pcDNA3-LL-SIGN plasmids expressing 7 (N7) and 5 (N5) tandem neck region repeats or a mixture of N7 and N5 plasmids (ratio 1:1) were transfected into 293T cells using the Fugene6 (Roche). L-SIGN expression was verified with L-SIGN-specific antibody (clone 120604, R&D Systems).

Analysis of Glycan Residues on DENV Using Plant Lectins

DENV supernatant was precleared with protein A-agarose for one h at 4°C. Bead-free supernatant was incubated with 10 µg of 4G2 at 4°C for 2 h followed by protein A-agarose for 1 h. The beads were washed with .05% Tween/PBS 3 times and eluted with nonreducing loading buffer. The sample was run on non-reducing 10% Sodium dodecyl sulfate (SDS) polyacrylamide gels and electroblotted onto nitrocellulose membrane (Amersham). Glycan types on DENV proteins were determined using the DIG Glycan Differentiation Kit (Roche).

Endoglycosidase H (Endo H) or N-glycosidase F (PNGase F; New England Biolabs) was performed as described elsewhere [22]. Digested proteins were separated by 10% SDS polyacrylamide gels and analyzed by western blot. E protein was detected by anti-E mAb followed by peroxidase-conjugated anti-mouse IgG Ab.

RESULTS

DENV Produced in Insect Cells But Not in DCs Can Infect DCs

Following the bite from an infected mosquito, the host first encounters virus produced in the mosquito, and following this initial inoculation subsequent rounds of infection are driven by virus produced by host cells. To study these 2 distinct stages of pathogenesis, we compared viruses from C6/36 (insect cells) and virus produced from primary human monocyte-derived dendritic cells. Viral supernatants were

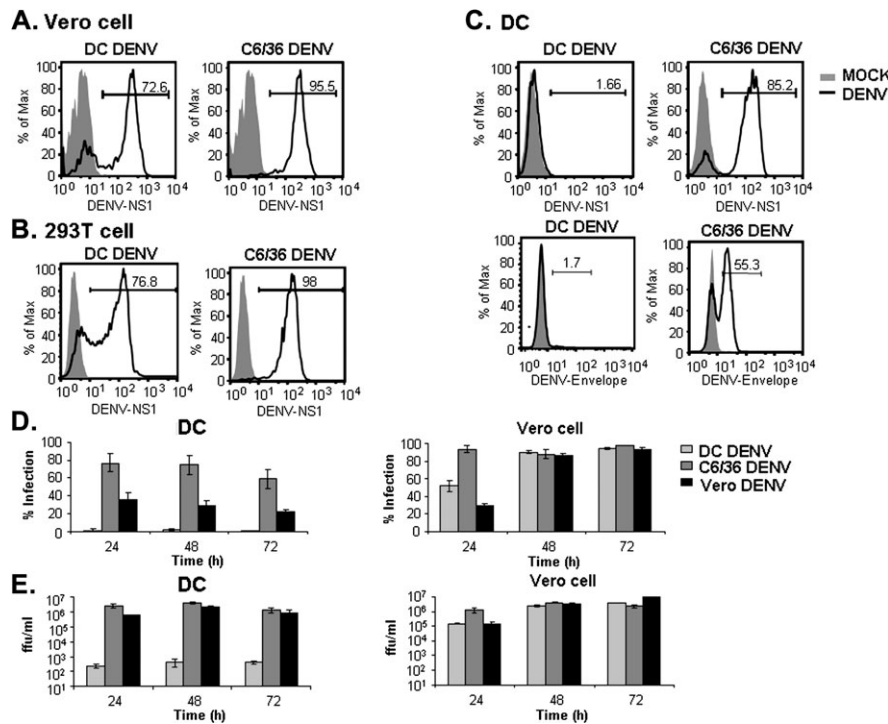


Figure 1. DC-produced dengue virus cannot re-infect DCs. *A*, Vero cells; *B*, 293T cells; and *C*, DCs were infected with C6/36-produced or DC-produced DEN-2 (16681) at MOI = 1. After 24 h, cells were intracellularly stained with anti-DEN-2 NS1 mAb (2G6) or anti-E mAb (4G2) and analyzed by flow cytometry. *D*, DCs and Vero cells were infected with DC-produced, C6/36-produced, or Vero-produced DEN-2. At 24, 48, and 72 h, cells were intracellularly stained with anti-NS1 mAb (2G6) and analyzed by flow cytometry. *E*, Cell supernatants were measured for viral progeny by focus-forming assay. The results are shown as mean \pm SE from 4 independent experiments.

titered using a focus-forming assay on Vero cells, and equal amounts of titered virus were used to infect Vero, 293T, or DCs at an MOI of 1. The percentage of infected cells was monitored by cytofluorometry staining of the intracellular nonstructural dengue antigen NS1, which is produced only following productive infection. Insect-derived virus was equally competent at infecting the 3 cell types with high efficiency (Figure 1A–C). Surprisingly, DC-produced virus was not able to re-infect DCs but was nevertheless fully competent at infecting 293T and Vero cells.

The lack of infectivity of DC-produced virus on DCs was also shown using a different mAb, 4G2, which reacts with an epitope on dengue envelope protein (Figure 1C, lower panel). A time course of infection was performed where DCs or Vero cells were infected with virus produced in C6/36, Vero, or DCs, and infection was monitored by FACS or using a focus-forming assay to measure infectious virus harvested from the supernatants of infected cells (Figure 1D–E). At 24, 48, and 72 h the DC-produced virus showed a much reduced ability to infect DCs when compared with virus produced in C6/36 or Vero cells.

The experiments we have described above used the dengue serotype 2 strain 16681. To see whether these results could be generalized, we checked infection of both DCs and Vero cells

with dengue serotype 2 strain New Guinea C, serotype 1 strain Hawaii, serotype 3 strain H87, and serotype 4 strain H241. Infection efficiency was measured by FACS at 24 and 48 hours following infection with virus produced in either C6/36 cells or DCs (Figure 2A–B). In all cases DC-produced virus was much less infectious for DCs than insect-produced virus, whereas infection of Vero cells was roughly equal.

It is known that mature DCs are relatively resistant to dengue infection, so to rule out the possibility of any DC-produced cofactors that might induce maturation or any other form of resistance to infection we performed a mixing experiment whereby DC-produced and C6/36-produced viruses were used to infect DCs. In these experiments using mixed viruses the DCs were again susceptible to infection, presumably by the C6/36-produced virus fraction (Figure 2C).

The lack of reinfection of DCs by DC-produced virus may reflect a difference in the surface binding interaction of DC- versus insect-produced viruses to DCs. To test this Vero cells and DCs were incubated with DENV produced from the different sources, and binding to the cells was then assessed by staining with the anti-E mAb 4G2. Virus produced in C6/36 cells could bind to DCs, while binding of DC virus back onto DCs was almost completely absent (Figure 2D). Conversely, both DC- and insect-produced viruses were able to bind to Vero cells.

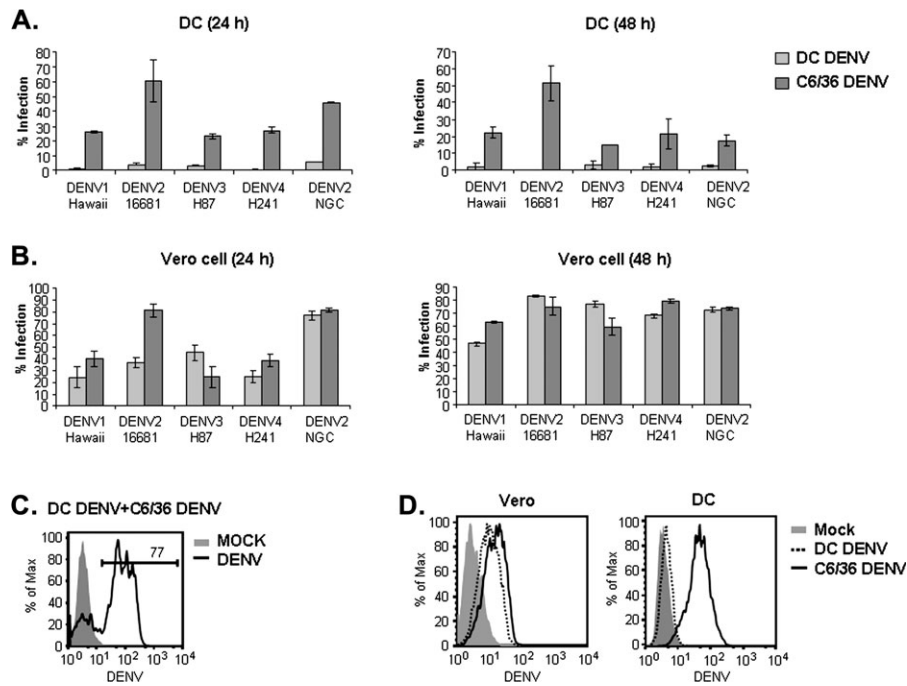


Figure 2. DC-derived virus from all serotypes have reduced ability to reinfect DCs. (A–B) DCs and Vero cells were infected with DEN-1 Hawaii, DEN-2 16681, DEN-3 H87, DEN-4 H241, and DEN-2 NGC derived from either DCs or C6/36 cells. At 24 and 48 h, cells were intracellularly stained with anti-NS1 mAb specific to all DENV serotypes (1F11) and analyzed by flow cytometry. C, DCs were incubated with a mixture of C6/36-produced and DC-produced virus at MOI = 1 and stained for infection with 2G6. D, Vero cells and DCs were incubated with C6/36-produced or DC-produced DENV, surface stained with anti-E mAb (4G2), and analyzed by flow cytometry. The results are shown as mean \pm SE from 2–3 independent experiments.

DC-Produced DENV Preferentially Infects via L-SIGN

To formally prove that the loss of infection of DCs was a result of the loss of affinity of DC-produced virus for DC-SIGN, we went on to test infection on 3T3 cells expressing DC-SIGN and included in these assays the related C-type lectin L-SIGN (Figure 3A), which has also been reported to be a receptor for dengue virus. Insect-derived virus could efficiently infect both DC-SIGN– and L-SIGN–expressing cells when compared with control 3T3 cells (Figure 3B). Similar to the lack of observable infection of DCs, the DC-derived virus showed a much lower level of infection on DC-SIGN–expressing 3T3 cells, with <2% infection. Surprisingly, however, this virus progeny was still able to infect cells expressing L-SIGN, with up to 13% infection observed.

Previous reports with dengue virus have suggested that the virus shows equal tropism for both DC-SIGN and L-SIGN. These reports were from virus produced in tumor cell lines and not from primary cells. We tested the infectivity of virus produced in Vero cells, and in agreement with these previous reports dengue produced in this tumor cell line was able to efficiently infect 3T3 cells via either DC-SIGN or L-SIGN (Figure 3C). Finally, we tested the binding of insect- and DC-produced virus to the 3T3 transfectants (Figure 3D). In agreement with the DC binding experiments DC-produced virus was unable to bind to DC-SIGN–expressing DCs, whereas

both insect- and DC-derived viruses could bind to L-SIGN–expressing cells.

DC-Produced DENV Contains Complex Glycan

To gain insight into the glycosylation profiles of DENV produced in C6/36, Vero, and DC, purified virus was tested against a panel of lectins with differing carbohydrate-binding specificities by western blot (Figure 4A). Among the 5 plant lectins tested, GNA is the only one that selectively interacts with high- and pauci-mannose-type *N*-glycans. SNA and MAA bind to sialic acid terminally linked to galactose by α 2,6 linkage and α 2,3 linkage, respectively, which may occur on complex-type *N*-glycans. DSA recognizes repeating *N*-acetylglucosamine (galactosyl β 1,4 *N*-acetylglucosamine) sequences; these may also occur on complex-type *N*-glycans. PNA, unlike DSA, binds preferentially to β 1,3-linked terminal galactose, such as galactosyl β 1,3 *N*-acetylgalactosamine, a sequence commonly occurring on *O*-glycosylated proteins and gangliosides.

Virus produced in C6/36 cells bound exclusively to GNA, implying that it contained predominantly high- or pauci-mannose *N*-glycans consistent with the glycosylation patterns seen in insect cells. Vero-produced virus was bound by 3 of the lectins tested—GNA, SNA, and DSA—suggesting that the virus contained a variety of high-mannose and complex- or hybrid-type *N*-glycans

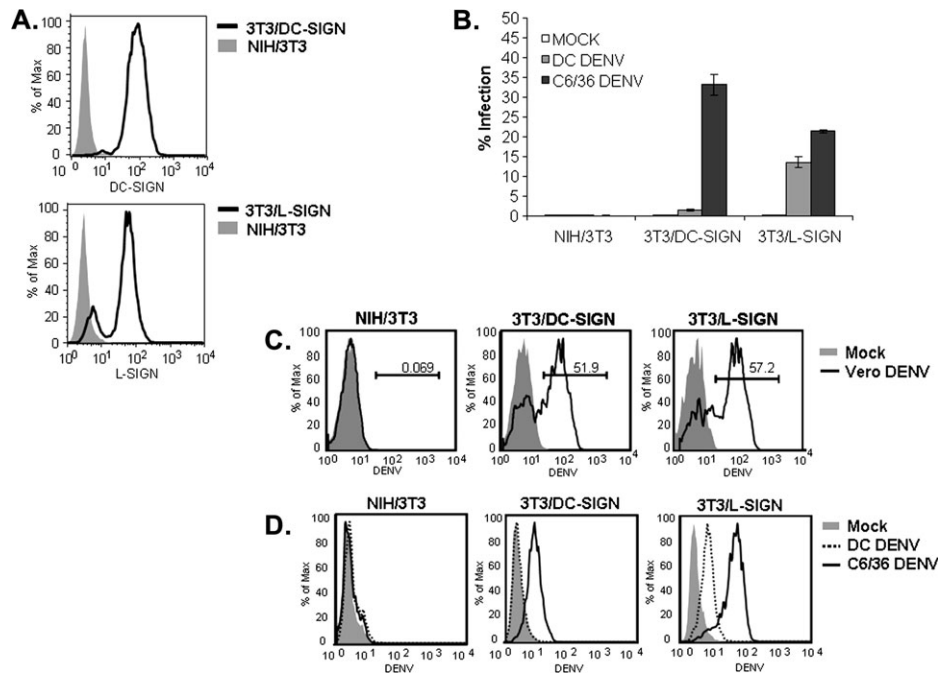


Figure 3. DC-produced virus selectively infects via L-SIGN. (A) DC-SIGN and L-SIGN expression was verified by immunostaining with DC-SIGN- and L-SIGN-specific antibodies. (B) Dengue produced in DCs or C6/36 were used to infect DC-SIGN-expressing, L-SIGN-expressing, and wild-type 3T3 cells. After 24 h, cells were checked for infection by intracellular staining with anti-NS1 mAb (2G6). The results are shown as mean \pm SE from 2 independent experiments. (C) Dengue virus produced in Vero cells was used to infect DC-SIGN-expressing, L-SIGN-expressing, and wild-type 3T3. After 24 hours, infected cells were revealed by intracellular staining with anti-NS1 mAb (2G6). The profiles are examples of 3 independent experiments. (D) DC-SIGN-expressing, L-SIGN-expressing, and wild-type 3T3 were used to detect the binding with DC- and C6/36-produced virus in the presence of heparin. Dengue infection and binding data shown in panels B–D for L-SIGN were derived by gating the L-SIGN-positive population.

with or without α 2,6-linked sialic acids, possibly on polyN-acetylglucosamine outer chains. The DC-produced virus was bound only by SNA and DSA, indicating that there was a lack of high-mannose or hybrid N-glycans and that only complex-type N-glycans were present. All of the lectin binding signals occurred at the same position on the gels as E protein in the region of 60 kDa; we did not see any signal at 19 kDa where prM would be expected to migrate. However, this could be due to a low level of prM on these viruses.

Finally, we assessed the N-linked glycan on the envelope protein by digestion with either Endo H, which will remove high-mannose simple glycans containing 3 or more terminal mannose residues, or PNGase F, which removes all simple or complex N-linked glycan (Figure 4B). DC-produced virus showed a mobility shift of around 3 or 6 kDa corresponding to the addition of 1 or 2 complex carbohydrates at positions 67 and 153. This was completely resistant to digestion with Endo H, indicating the presence of complex low-mannose carbohydrate.

For both insect- and Vero-produced virus, envelope protein migrating at \sim 2 or 4 kDa higher than the PNGase F-digested material indicated, as with DC-produced virus, that envelope had either 1 or 2 added sugars. Following Endo H digestion a complex pattern of bands was revealed, indicating that some of

the added sugars were Endo H sensitive and therefore contained $>$ 3 terminal mannose residues, whereas others were Endo H resistant and contained more heavily processed structures, which is in agreement with a recent report [23].

We conclude from these experiments that in C6/36, Vero cells, and DCs both N-linked glycosylation sites can be used but that a single site is used in \sim 50% of cases. Envelope from DC-produced virus contains complex, highly processed Endo H-resistant carbohydrate. However, in both C6/36 and Vero, a proportion of the sugar is high mannose and therefore will allow interaction with DC-SIGN, which is consistent with the results from lectin blotting shown above.

Heterogeneity of L-SIGN Neck Length Has No Effect on DENV Infection

L-SIGN contains a tandem repeat in its neck region, N-terminal to the carbohydrate recognition domain, which can be of variable length between 3 and 9 repeats. L-SIGN exists as tetramers, and previous studies have suggested that heterogeneity in the tandem repeat region of the L-SIGN neck region may contribute to severe acute respiratory syndrome (SARS) susceptibility by altering the viral Env-receptor affinity [24]. To determine whether heterogeneous L-SIGN neck lengths affect DENV infection levels, we examined the effects of

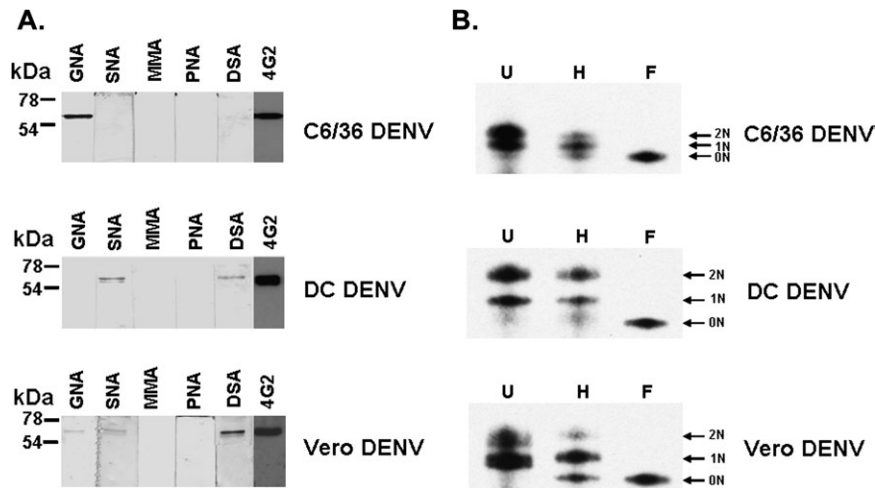


Figure 4. Dengue produced in DC contained complex glycans. (A) Immunoprecipitated DC-, Vero-, and C6/36-produced DENV were run on 10% SDS Sodium dodecyl sulfate polyacrylamide gel electrophoresis followed by western blotting. Viral strips were incubated with a set of lectins, GNA, SNA, MMA, PNA, and DSA, as a company's instruction. Anti-E mAb (4G2) was used to indicate the position of the dengue E protein. (B) C6/36-, DC-, and Vero-derived DENV were undigested (U) or digested with Endo H (H) or PNGase F (F) and separated on 10% SDS PAGE followed by western blotting and detected by 4G2.

a single length repeat and compared it with cells expressing 2 different-length L-SIGN alleles simultaneously. We examined this by transient transfection, and 293T cells were used for these assays as they can be transiently transfected to a high level. 293T were transfected with L-SIGN containing 5 or 7 repeats singly or together in equal amounts. L-SIGN expression was confirmed by surface staining and flow cytometry. Equal expression of alternate-length L-SIGN constructs was

also confirmed by western blotting. Transfectants were then infected with dengue virus, and infection was monitored by intracellular staining together with an antibody specific to L-SIGN to reveal transfected cells. All transfectants were equally infected with no reduction in cells expressing both L-SIGN alleles, suggesting that heterotetrameric L-SIGN was as effective as homotetrameric L-SIGN at promoting infection (Figure 5).

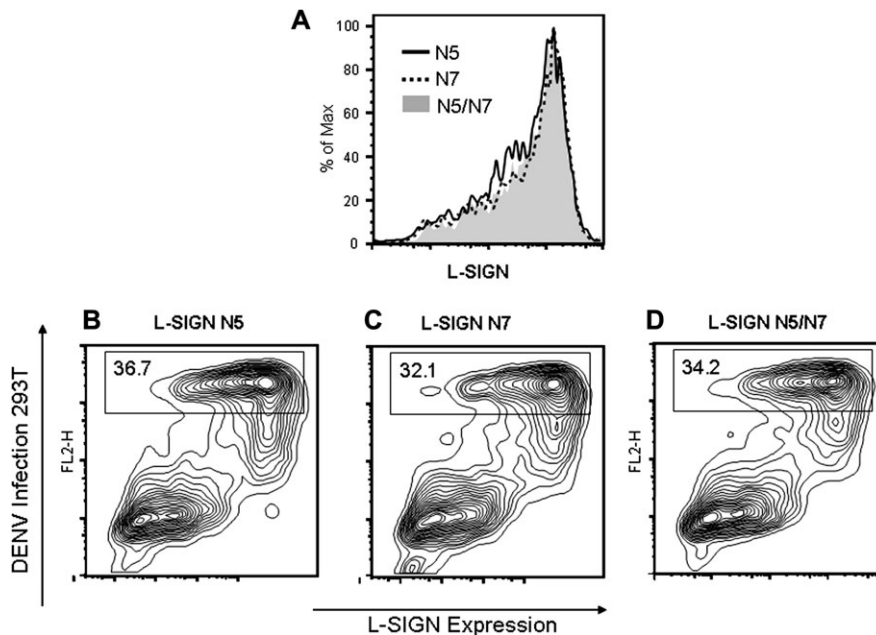


Figure 5. Infection via L-SIGN with heterologous and homologous neck tandem repeat regions. 293T cells were transfected with L-SIGN constructs expressing 5 or 7 repeats or both 5 and 7 in combination. Cells were first stained with anti-L-SIGN to show equal levels of expression of the 2 constructs (A). Cells were then stained with anti-L-SIGN and 4G2 to reveal dengue-infected cells (B–D). The data are representative of 2 independent experiments.

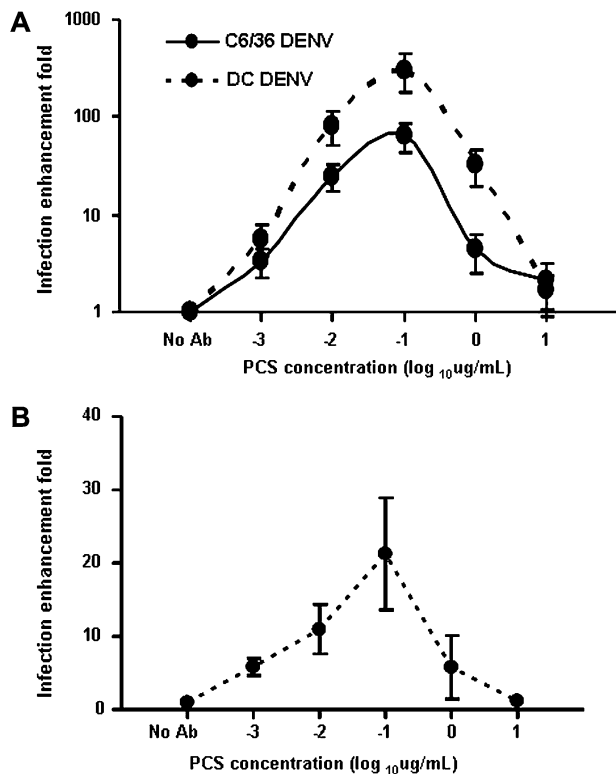


Figure 6. Anti-dengue antibodies enhance infectivity of DC-produced DENV. *A*, U937 were infected with C6/36-produced and DC-produced virus (DEN-2 16681) in the presence of an increasing concentration of pooled convalescent dengue immune serum. Infection was detected by intracellular staining with anti-NS1 mAb (2G6). *B*, ADE of DCs infected with DC-produced virus. The results are shown as mean \pm SE from 3 independent experiments.

Infectivity of DC-Produced DENV Is Enhanced by Anti-Dengue Antibodies

Finally, although DC-produced virus cannot reinfect DCs efficiently, we were interested to determine whether DC-produced virus was still able to infect cells by antibody-dependent enhancement (ADE), which would allow it to replicate by infection of Fc receptor-expressing cells. C6/36- and DC-derived viruses were incubated with increasing levels of pooled convalescent dengue immune serum and subsequently used to infect U937, a monocyte cell line that expresses the Fc receptor and which shows relatively low infectivity without the presence of enhancing antibodies. Viruses produced in both DCs and insect cells were susceptible to enhancement, over the same range of antibody concentrations, showing that DC-produced virus could exploit ADE to replicate in individuals undergoing a secondary dengue infection (Figure 6A). In a final series of experiments we investigated whether DC-produced virus could be induced to infect primary human DCs by ADE (Figure 6B). DC-produced virus could be enhanced to infection by >20-fold, whereas the already high level of infection of DCs by insect-produced virus was not enhanced further by dengue serum.

DISCUSSION

During natural dengue infection, mosquito saliva containing DENV particles is injected into the skin during a blood meal. Skin-resident immature DCs have been proposed to be the primary site of infection for insect-derived DENV [25]. The primary receptor on DCs for dengue virus is believed to be DC-SIGN. DC-SIGN binds N-linked high-mannose oligosaccharides, including glycans with terminal fucose residues that include the blood group Lewis^x and Lewis^a epitopes [26].

L-SIGN, like DC-SIGN, binds to intercellular adhesion molecule 3 (ICAM-3) and is thought to establish cellular interactions with ICAM-3-expressing T cells. L-SIGN is able to capture a variety of viruses (including the related Flaviviridae hepatitis C virus [HCV] and West Nile virus [WNV] as well as human immunodeficiency virus [HIV] type 1, cytomegalovirus, and SARS) [27–31]. DC-SIGN and L-SIGN preferentially bind pyranose sugars, in particular mannose. A study comparing L-SIGN- and DC-SIGN-specific ligands using glycan arrays revealed a restricted repertoire of glycan ligands for L-SIGN, namely, the high-mannose-type *N*-glycans. In contrast, DC-SIGN bound to fucosylated glycans in addition to high-mannose-type *N*-glycans [32].

The dengue E protein has 2 potential N-linked glycosylation sites at positions 67 and 153. Cryo-electron microscopy reconstructions of dengue virus complexed to soluble DC-SIGN show interaction with the glycan at position 67 [33]. There have been several reports on the N-linked glycosylation of dengue envelope. Some reports suggest the use of both sites, whereas others suggest that only one is used [23, 34–36]. We show that in C6/36, Vero cells, and DCs either one or both sites are used for the dengue 2 serotype 16681. Although both glycosylation sites can play important roles in infectivity and viral replication, functional studies have confirmed the importance of Asn-67 for infection of DC-SIGN-expressing cells [33]. WNV E protein contains a single N-linked glycosylation site at position 154 that is absent from some virus strains [37]. Both dengue and WNV contain a single N-linked site in prM, and although prM is cleaved by furin during viral maturation, a substantial fraction of uncleaved prM is found on some dengue viruses, particularly that produced in insect cells, and such partially cleaved viruses can still be infectious. For WNV the N-linked site on prM can also mediate interaction with L-SIGN, and in common with glycan at position 154 this also showed differential specificity for L-SIGN when expressed in mammalian cell lines, perhaps mediated by the presence of N-acetylglucosamine-terminated structures [29].

The difference in the binding specificities of dengue viruses produced in Vero versus primary dendritic cells was somewhat surprising and likely related to the expression of high-mannose moieties in Vero. A number of tumor cell lines express high-mannose sugars, and we have previously described the

generation of a monoclonal antibody that recognizes a variety of tumor cell types and which binds to high-mannose moieties [38]. The differential affinity of DC-SIGN for ligands expressed by tumor cell lines versus primary cells has been observed before and led some to speculate that DC-SIGN may participate in tumor surveillance [39]. There appears to be a further added level of complexity as the glycosylation profiles may vary in a given cell line depending on the exact position of the N-linked site within the polypeptide chain [29].

In humans, L-SIGN expression is restricted to endothelial cells beneath the subcapsular sinus in lymph nodes [40], sinusoidal endothelial cells in the liver [27, 41], alveolar and endothelial cells in the lung [42], and capillaries in the villous lamina propria of terminal ileum and Peyer patches [43]. Dengue antigens have been demonstrated in the sinusoidal tissue of the liver and vascular endothelium of the lung and spleen and may provide an explanation for the unique pathology observed in these organs [44, 45]. The accumulation of antigen in L-SIGN-expressing tissue may also result in an increase in localized transfection in a similar way that L-SIGN is thought to be responsible for the capture of HCV from the blood and transmission to hepatocytes or in the case of HIV, to CD4⁺ T cells [28, 41].

We conclude that skin-resident DCs are a likely target for initial infection by dengue virus injected by the infecting mosquito; however, subsequent dissemination of the virus to monocytes and other cell types will no longer use DC-SIGN as a primary receptor and may rely in part on a shift to L-SIGN as the primary lectin receptor. The differential glycosylation of the DENV E protein during replication in primary mammalian cells suggests that studies of virus tropism using virus prepared in insect cells or tumor cell lines should be interpreted with caution.

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