

Langerin is a natural barrier to HIV-1 transmission by Langerhans cells

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Human immunodeficiency virus-1 (HIV-1) is primarily transmitted sexually. Dendritic cells (DCs) in the subepithelium transmit HIV-1 to T cells through the C-type lectin DC-specific intercellular adhesion molecule (ICAM)-3-grabbing nonintegrin (DC-SIGN). However, the epithelial Langerhans cells (LCs) are the first DC subset to encounter HIV-1. It has generally been assumed that LCs mediate the transmission of HIV-1 to T cells through the C-type lectin Langerin, similarly to transmission by DC-SIGN on dendritic cells (DCs). Here we show that in stark contrast to DC-SIGN, Langerin prevents HIV-1 transmission by LCs. HIV-1 captured by Langerin was internalized into Birbeck granules and degraded. Langerin inhibited LC infection and this mechanism kept LCs refractory to HIV-1 transmission; inhibition of Langerin allowed LC infection and subsequent HIV-1 transmission. Notably, LCs also inhibited T-cell infection by viral clearance through Langerin. Thus Langerin is a natural barrier to HIV-1 infection, and strategies to combat infection must enhance, preserve or, at the very least, not interfere with Langerin expression and function.

The most common route for HIV-1 infection is through sexual transmission across genital mucosa. There is a need for microbicides that prevent HIV-1 transmission¹. Progress is hindered because early events in HIV-1 entry remain unclear. Dendritic cell (DC) subsets in mucosal tissues are thought to transmit HIV-1 to T cells through C-type lectins^{2–5}. In mucosal tissues, DC subsets can be distinguished by their expression of C-type lectins⁶: Langerhans cells (LCs) specifically express Langerin and DCs express DC-SIGN (Fig. 1a). LCs reside in the epidermis of the skin and in most mucosal epithelia, such as the ectocervix, vagina and foreskin, whereas DC-SIGN⁺ DCs reside in the subepithelium⁶. Thus, LCs are the first DC subset to encounter HIV-1 (ref. 7) (Supplementary Fig. 1 online).

Several studies using *ex vivo* skin explants models have shown that LCs can be infected with HIV-1 and subsequently transmit HIV-1 to T cells^{7–9}. However, LC infection might be inefficient, as high virus concentrations were needed in these studies^{7–12}. The molecular mechanisms underlying HIV-1 transmission are difficult to identify using these *ex vivo* skin explant models. Therefore, we

used immature LCs isolated from fresh skin to investigate their ability to transmit HIV-1.

LCs were routinely over 90% pure, immature (Fig. 1b) and expressed CD4 and C-C chemokine receptor 5 (CCR5; Supplementary Fig. 2 online). First, we investigated whether LCs enhance T-cell infection in a manner similar to DCs (ref. 2). We infected T cells with HIV-1 in the presence of different DC subsets, but without washing to remove unbound virus. At low virus concentrations, T cells were efficiently infected in the presence of DCs but not LCs (Supplementary Fig. 2). At higher concentrations, T cells were efficiently infected even in the absence of DCs (Fig. 1c), but, notably, T-cell infection was significantly inhibited by LCs (Fig. 1c), suggesting that LCs, in contrast to DCs, do not enhance HIV-1 infection of T cells but rather prevent T-cell infection by viral clearance.

Therefore, we investigated whether LCs capture and transmit HIV-1 to T cells. We exposed DC subsets to low HIV-1 concentrations, washed the cells to remove unbound virus, and subsequently cocultured them with T cells. Immature LCs did not transmit HIV-1, in stark contrast to DCs (Fig. 1d). Both these behaviors were dependent on C-type lectins: the pretreatment of LCs with the mannan-specific C-type lectin inhibitor mannan resulted in HIV-1 transmission to T cells, and this was of a similar extent as that in non-mannan-exposed DCs; in contrast, mannan inhibited the ability of DCs to transmit virus (Fig. 1d). HIV-1 subverts the normal function of DC-SIGN⁺ DC for viral transmission to T cells in the lymphoid tissues^{2,13,14}. Our data suggest that LCs are resistant to this subversion and do not efficiently transmit virus to T cells, unless a C-type lectin is inhibited.

LCs efficiently bind HIV-1 gp120 through a calcium-dependent receptor with mannan specificity (Fig. 2a and ref. 4). To identify this receptor, we screened a LC-specific antibody library, created by immunizing mice with immature LCs (Supplementary Fig. 3 online), for the ability to block gp120 binding to LCs. We isolated the blocking antibody 10E2, and further analysis demonstrated that this antibody is specific to the C-type lectin Langerin (Fig. 2b). Thus, Langerin is a receptor for HIV-1 on LCs.

Langerin is specifically expressed by LCs and induces the formation of Birbeck granules¹⁵. These are LC-specific cytoplasmic organelles

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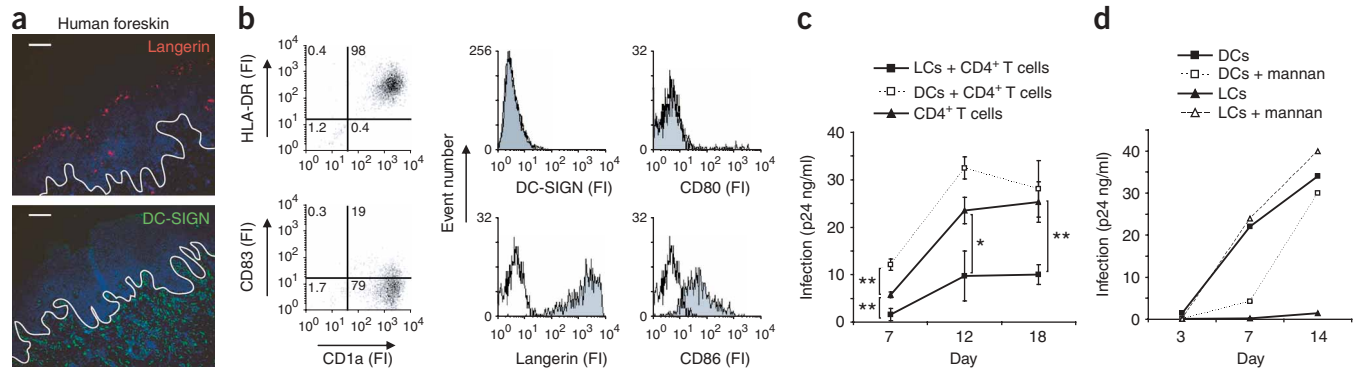


Figure 1 Immature LCs do not efficiently mediate HIV-1 transmission. **(a)** Immunofluorescence microscopy analysis of human foreskin. Scale bar, 50 μ m. Blue, nuclei; white, border epithelium and subepithelium. Top, epithelium; bottom, subepithelium. **(b)** Isolated LCs were immature, as determined by high expression levels of CD1a, Langerin and HLA-DR and low levels of costimulatory molecules. Open histograms, isotype control; filled histogram, specific antibody staining. FI, fluorescent intensity. **(c)** LCs inhibit T-cell infection. T cells were infected with HIV-1_{JR-CSF} in the presence of different DC subsets. Error bars represent s.d. of triplicates. $n = 3$ donors (#1–3). $**P < 0.01$ and $*P < 0.05$ by ANOVA. **(d)** LCs do not transmit HIV-1. DC subsets were pulsed with HIV-1_{JR-CSF} and T cells were added. Mannan was used to block C-type lectins. $n = 3$ donors (#3–5). Direct infection of DCs and LCs alone was below detection limit (< 2 ng/ml).

that are thought to be involved in antigen processing¹⁶. We observed that HIV-1 virions colocalized with Langerin at the cell surface and in intracellular vesicles (**Fig. 2c**). The virions were in close vicinity to Langerin-positive invaginations, representing different stages of Birbeck granule formation in both immature LCs and LC-like cells (ref. 17 and **Fig. 2c**). Thus Langerin on LCs captures HIV-1 and internalizes it into Birbeck granules. This LC-specific internalization pathway is probably central to the ability of Langerin to inhibit HIV-1 transmission and distinct from the internalization pathway of DC-SIGN, which partially protects the virus from degradation² and promotes viral transmission^{14,18}.

Langerin-expressing cell lines bind HIV-1 gp120 (ref. 4). We used our blocking antibody to Langerin, 10E2, to investigate the function of Langerin on primary LCs in HIV-1 transmission to T cells. To exclude enzymatic cleavage of CD4 and coreceptors during the isolation

method¹⁹, we used purified emigrant LCs, which expressed high levels of Langerin, intermediate levels of CD4 and low levels of CCR5 (**Supplementary Fig. 4** online). LCs did not efficiently transmit HIV-1 (**Fig. 3a**), but preincubation of LCs with the blocking antibody 10E2 enhanced HIV-1 transmission to levels similar to those observed following preincubation with mannan. Although LCs from different donors remained refractory to HIV-1 transmission (50% tissue culture infectious dose (TCID) 10–1,000), LCs transmitted HIV-1 in the presence of mannan or at a very high viral load (TCID 10,000) (**Fig. 3b,c** and **Supplementary Fig. 4**). The effects observed were due to the inhibition of Langerin, as mannan did not induce LC maturation, cell death or upregulation of HIV-1 receptors (data not shown). We observed transmission at a very high viral load, strongly suggesting that Langerin is saturated at high HIV-1 concentrations. This might explain a recent finding: using a high viral load, LC-mediated

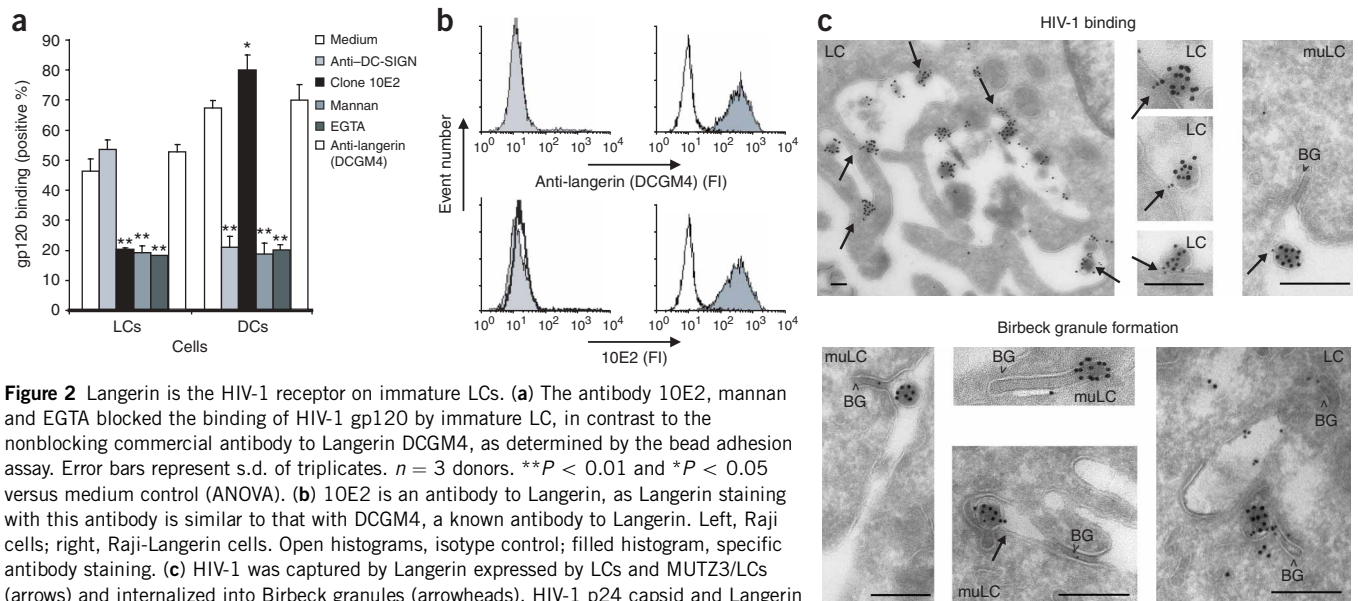


Figure 2 Langerin is the HIV-1 receptor on immature LCs. **(a)** The antibody 10E2, mannan and EGTA blocked the binding of HIV-1 gp120 by immature LC, in contrast to the nonblocking commercial antibody to Langerin DCGM4, as determined by the bead adhesion assay. Error bars represent s.d. of triplicates. $n = 3$ donors. $**P < 0.01$ and $*P < 0.05$ versus medium control (ANOVA). **(b)** 10E2 is an antibody to Langerin, as Langerin staining with this antibody is similar to that with DCGM4, a known antibody to Langerin. Left, Raji cells; right, Raji-Langerin cells. Open histograms, isotype control; filled histogram, specific antibody staining. **(c)** HIV-1 was captured by Langerin expressed by LCs and MUTZ3/LCs (arrows) and internalized into Birbeck granules (arrowheads). HIV-1 p24 capsid and Langerin staining are labeled by 15 nm and 10 nm gold, respectively. Scale bar, 100 nm.

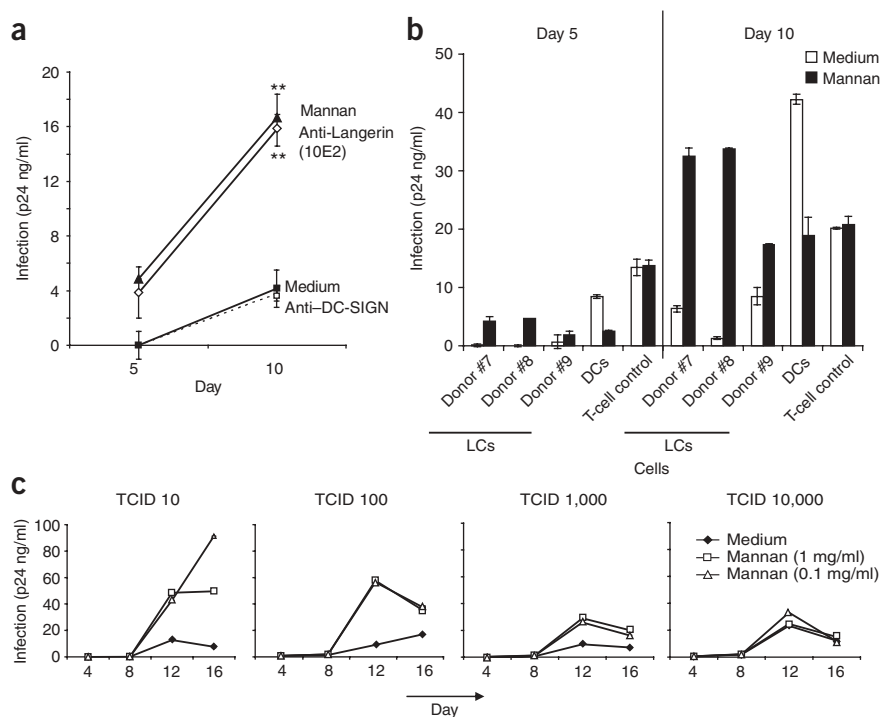


Figure 3 Langerin restricts HIV-1 transmission to T cells by LCs. (**a–c**) Emigrant LCs (donors #6–10) and moDCs were pulsed with HIV-1_{JR-CSF} and activated CD4⁺ T cells were added. Mannan and 10E2 were used to determine the function of DC-SIGN and Langerin. Activated CD4⁺ T cells were infected separately to control T-cell infectivity, and are depicted as T-cell control. Error bars represent s.d. of triplicates. ***P* < 0.01 versus medium control by ANOVA. The donor variability (#7–9) is depicted in **b**. The responses to different viral inoculums and different concentration of mannin are shown in **c**.

transmission was neither inhibited nor increased by mannin¹⁰. Thus, inhibition of Langerin confers upon LCs the ability to efficiently transmit HIV-1 to T cells.

It is assumed that Langerin mediates HIV-1 transmission in a similar manner to DC-SIGN and mannose receptor^{2,20}. We compared the functions of Langerin and DC-SIGN using transfected cell lines. Although Raji-Langerin cells efficiently bound HIV-1, the cells did not transfer HIV-1 to T cells, in contrast to Raji-DC-SIGN cells (Fig. 4a). At very high virus concentrations, mock control Raji cells transmitted HIV-1; in contrast, Langerin diminished HIV-1 infection (Supplementary Fig. 5 online), probably by binding to and degrading the virus, a process referred to as viral clearance. Indeed, HIV-1 captured by Raji-Langerin cells was rapidly degraded (Fig. 4b). To measure the efficacy of this viral clearance, we infected T cells with HIV-1 in the presence of Langerin⁺ and DC-SIGN⁺ cells. T-cell infection was significantly diminished when Raji-Langerin cells were present, compared to when they were absent or when T cells were cocultured with mock- or DC-SIGN-transfected Raji cells (Fig. 4c). These data demonstrate that, in contrast to DC-SIGN, Langerin functions as a scavenger receptor rather than as a *trans*-receptor, restricting T-cell infection by clearing and degrading infectious viral particles (Figs. 1c and 4c).

Previous studies have demonstrated that direct infection of LCs by high concentrations of CCR5-tropic HIV-1 is necessary for transmission to T cells^{9,11}. Therefore, we hypothesized that Langerin protects LCs from HIV-1 infection and subsequent transmission to T cells. LCs did not transmit CXCR4 tropic single-round pseudotyped HIV-1 to T cells, either in the absence or presence of inhibitors, in contrast to DC-SIGN⁺

DCs (Fig. 4d). This strongly suggests that direct infection of LCs is necessary for transmission. Next, we infected LCs with different concentrations of CCR5-tropic HIV-1. Notably, LCs were only infected efficiently when Langerin was inhibited by either mannin or the blocking antibody to Langerin (Fig. 4e and Supplementary Fig. 5). Langerin inhibited the infection of LCs even at high virus concentrations (Fig. 4f). When Langerin was not blocked, we observed LC infection only at very high virus concentrations (10,000 TCID), indicating that Langerin is saturated at these concentrations and is not able to protect against infection. At later time points (day 11), we observed donor differences between Langerin saturation at different viral inputs (1,000 compared to 10,000 TCID; Supplementary Fig. 5), suggesting that Langerin efficacy differs between donors. Mature LCs are more efficiently infected²¹ and our data suggest that this might be due to the downregulation of Langerin. Thus, Langerin prevents LC infection and this mechanism keeps the LCs refractory to HIV-1 transmission. Inhibition of Langerin by inhibitors or through saturation enables HIV-1 binding to CD4 and CCR5, and subsequent LC infection and transmission to T cells.

The protective function of Langerin has implications for the design of anti-HIV-1 microbicides, as suggested inhibitors such as mannin⁵ have an unwanted and completely

counteractive effect on LCs—namely, they negate the protective function of LCs and enable transmission by LCs. A microbicide candidate is the Lewis X antigen that inhibits the binding of DC-SIGN, but not Langerin, to HIV-1 (Supplementary Fig. 6 online). It should be noted, however, that the physiological importance of LC transmission to T cells *in vivo* is not yet known. The simian immunodeficiency virus-1 (SIV-1) macaque model is a good model to investigate the efficacy of potential microbicides. Studies have shown that macaque LCs are infected by SIV-1 (ref. 22), and further research will show whether this is due to viral concentrations or to a differential function of macaque Langerin.

Here we show that Langerin on LCs functions as a protective mechanism in intact mucosa by scavenging invading HIV-1 and preventing LC infection and viral dissemination (Supplementary Fig. 1). Differences in Langerin function due to genetic, cellular or infectious factors might influence susceptibility to HIV-1 infection. We observed this protective function of Langerin in 12 different donors. Some differences in efficiency were observed, and in one donor (out of 13) we observed efficient transmission by LCs (Supplementary Table 1 online). It is possible that Langerin polymorphisms contribute to these differences²³. Our data can also explain differences in HIV-1 susceptibility, as the risk of acquiring HIV-1 is severely increased by inflammation, ulceration and coinfection with sexually transmitted diseases²⁴. These conditions might hamper the protective function of Langerin by either decreasing its expression due to LC maturation or by facilitating the access of HIV-1 to the subepithelial layer of DCs (by breaching the protective layer of LCs; Supplementary Fig. 1).

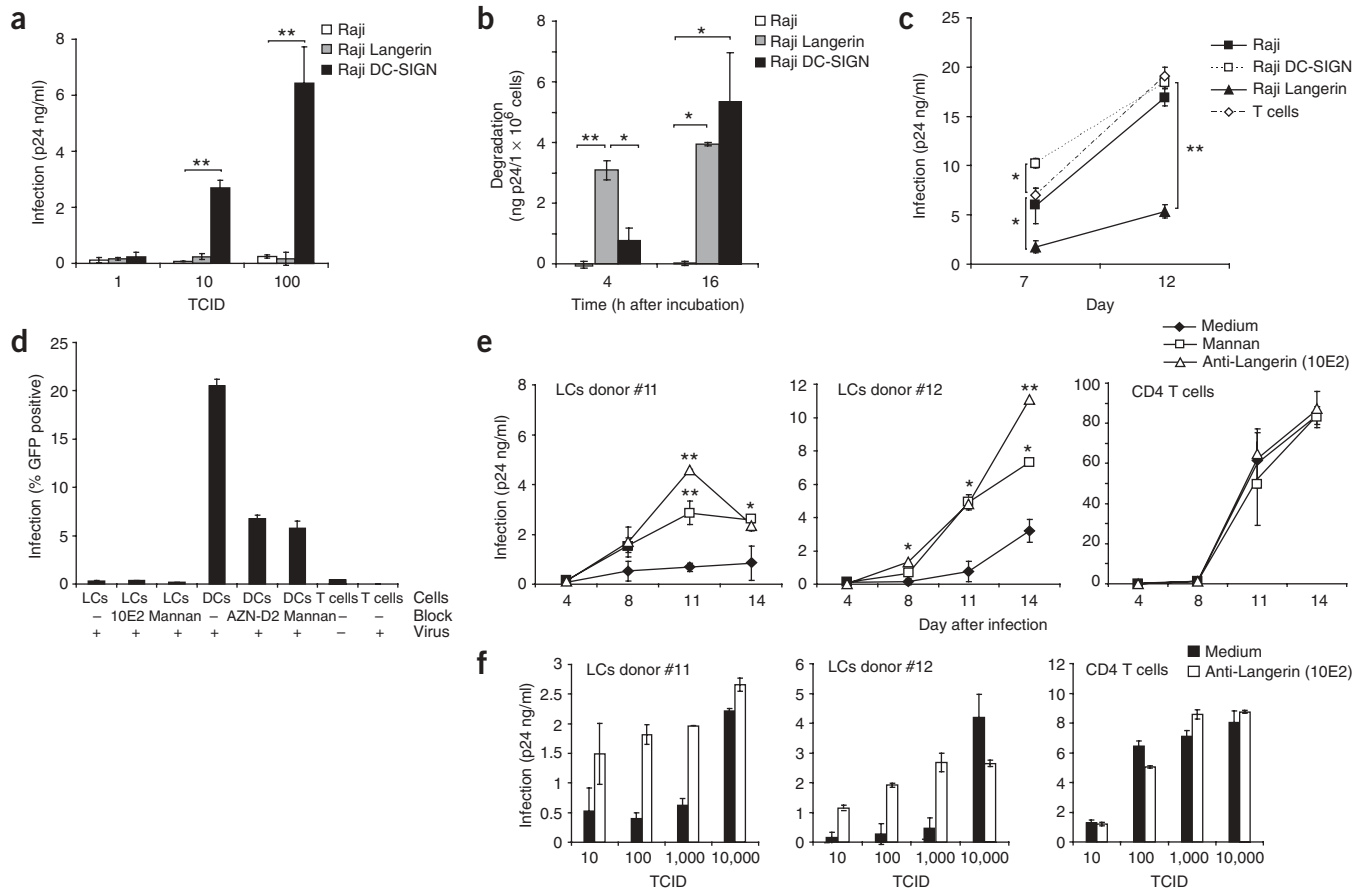


Figure 4 Langerin inhibits LC infection and subsequently transmission to T cells. **(a)** Raji-transfectants were pulsed with HIV-1_{JR-CSF} and their ability to transmit virus to T cells was measured at day 7. ND = not detected; $n = 3$. $**P < 0.01$ versus Raji. **(b)** Langerin rapidly degrades HIV-1. Raji transfectants were pulsed with HIV-1_{JR-CSF} and degradation was measured. Results are presented as the amount of p24 degraded in time (p24 uptake at 0 h was 1.2 ng (Raji), 7.6 ng (Raji-Langerin) and 13.2 ng (Raji-DC-SIGN)). Error bars represent s.d. of triplicates. **(c)** Langerin inhibits T-cell infection through viral clearance. T cells were infected with HIV-1_{JR-CSF} in the presence of Raji transfectants, $n = 3$, $**P < 0.01$; $*P < 0.05$ versus Raji by ANOVA. **(d)** DC subsets were pulsed with pseudotyped HIV-1_{NL4.3} and cocultured with activated T cells. The role of C-type lectins was determined by preincubation with mannan, antibodies to Langerin (10E2) or DC-SIGN (AZN-D2). Viral replication was monitored by measuring GFP expression by flow cytometry and shown as percentage GFP positive of the total CD3⁺ population. Error bars represent s.d. of duplicates and $n = 3$ donors (#14–16). **(e, f)** Langerin prevents HIV-1 infection of LCs. LCs (donors #11, 12) were infected with different inoculum of HIV-1_{JR-CSF} (TCID 10 in **e**) in presence of mannan and the antibody to Langerin 10E2. Error bars represent s.d. of triplicates. $**P < 0.01$; $*P < 0.05$ versus medium control.

METHODS

Antibodies, virus, cell lines and reagents. We generated the blocking antibody to Langerin, 10E2, by immunizing Balb/c mice with immature LCs (**Supplementary Fig. 3**). We used the fluorescent bead adhesion assay to screen hybridoma supernatants for antibodies that blocked the binding of HIV-1 gp120 to immature LCs. The monoclonal antibodies, plasmids, HIV-1 viruses and cell lines used are described in **Supplementary Methods** online. We calculated the multiplicity of infection (moi) as viral TCID divided by the number of input cells.

Donors. We obtained normal healthy skin samples following plastic surgery, after obtaining informed consent from all donors. For the coculture infection and transmission studies, we used immature LCs from five donors (labeled #1–5 in the figures). For the infection and transmission studies, we used emigrant LCs from eight donors (#6–13). For the pseudotyped HIV-1 transmission experiments, we used immature LCs from three donors (#14–16).

Cells. We isolated monocytes, LCs and T cells from different donors. We cultured monocyte-derived immature DCs (referred to as iDCs) as previously described². We isolated primary LCs as previously described^{18,25}, with slight modifications. Normal healthy adult skin obtained from plastic surgery was

used within 3 h after the operation. We cut 3-mm-thick slices of skin, containing the epidermis and dermis, using a dermatome. The slices were incubated with Dispase II (1 mg/ml, Roche Diagnostics) in Iscoves Modified Dulbecco's Medium (IMDM), 10% FCS and gentamycin (10 μ g/ml) for either 2 h at 37 °C or overnight at 4 °C. We mechanically separated the epidermis, washed it in medium and cut it into 1-cm² pieces. We generated emigrant LCs by floating the epidermis on IMDM, 10% FCS, 10 μ g/ml gentamycin and 800 units/ml granulocyte-macrophage colony-stimulating factor (GM-CSF). After 3 d, we layered the migrated cells on a Ficoll gradient and cultured them at 0.5×10^6 /ml in IMDM, 10% FCS, 10 μ g/ml gentamycin and 800 units/ml GM-CSF. We isolated immature LCs by incubating epidermal sheets in PBS containing DNase I (20 units/ml; Roche Applied Science) and either trypsin (0.05% Beckton Dickinson) or collagenase blend F (0.25%, Sigma Chemicals) for 30 min at 20–22 °C. We used FCS to inactivate trypsin digestion and generated a single-cell suspension. We then layered cells on a Ficoll gradient and selected LCs using CD1a-labeled immunomagnetic microbeads (Miltenyi Biotec). Isolated LCs expressed functional active Langerin as determined by gp120 binding (**Fig. 2a**), suggesting that Langerin function in skin is not impaired by soluble mannose structures. Peripheral blood mononuclear cells were isolated from buffy coats, activated with phytohemagglutinin (3 μ g/ml) and cultured in RPMI with 10% FCS. On day 3, the CD4⁺ T cells were enriched

by negative selection using MACS beads (Miltenyi Biotec) and cultured with interleukin-2 (IL-2; 100 units/ml).

Fluorescent bead adhesion assay. We performed the fluorescent bead adhesion assay as already described². For carbohydrate profiling, we coated streptavidin beads with 5 µg of the biotinylated carbohydrate structures. For specific receptor blocking, we preincubated cells for 30 min with 20 µg/ml of blocking antibodies, 1 mg/ml of mannan or 50 µg/ml of biotinylated carbohydrate structures.

HIV-1 transmission, infection and coculture infection. We plated cells at 20,000, 100,000 or 300,000 cells/well in 96-well plates for transmission, coculture and infection experiments, respectively. We preincubated cells with the blocking antibodies AZN-D1 or 10E2 (20 µg/ml) or with mannan (0.1 or 1 mg/ml) for 1 h at 37 °C, before incubating them with HIV-1_{JR-CSF}. For the HIV-1 transmission experiments, we incubated cells with HIV-1 TCID 100 (moi 5 × 10⁻³ (1.1 ng p24)) unless stated otherwise, and we washed cells three times with warm medium after incubation with virus. We used HIV-1 TCID 100 (moi 1 × 10⁻³ (1.1 ng p24)) in coculture infection experiments unless stated otherwise. For both transmission and coculture infection assay, we added 200,000 activated CD4⁺ T cells after 2 h and cocultured these in a flat-bottom 96-well plate. For HIV-1 infection experiments, we washed cells three times with medium after 1 d and replated them in a flat-bottom 96-well plate. We collected the supernatant at different time points and monitored viral replication using the p24 ELISA.

HIV-1 degradation assay. We pulsed Raji cells and transfectants (1 × 10⁶) with HIV-1_{JR-CSF} (50 ng, moi 4.8 × 10⁻³) for 30 min at 20–22 °C, washed them three times with medium, and replated them in 96-well plates at 37 °C. We harvested cells and supernatants after 0 h, 4 h and 16 h, and lysed them in a mixture of 0.1% empigem, PBS and 1,000 units/ml DNaseI. We measured viral content using the p24 ELISA and calculated the amount of degraded p24 by subtracting the p24 content of cell lysates at different time points from that at time 0.

Microscopy. Fixation and preparation are described in **Supplementary Methods**. For electron microscopy, LCs or MUTZ-derived LC-like cells (**Supplementary Methods**) (2 × 10⁶; ref. 17) were incubated with 1 µg p24 (TCID 9.5 × 10⁴, moi 4.8 × 10⁻²) of HIV-1_{JR-CSF} for 4 h and processed for visualization by electron microscopy. Cells were fixed and sections were prepared according to standard procedures.

Statistical analysis. We assessed significant differences using a one-way analysis of variance (ANOVA). When the overall *F*-test was significant, we further investigated differences among the groups using the *post-hoc* Bonferroni test (Graphpad Prism software). *P* < 0.05 was considered statistically significant.

Note: Supplementary information is available on the Nature Medicine website.

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AUTHOR CONTRIBUTIONS

L.d.W. designed, executed and interpreted most experiments and prepared the manuscript. A.N. generated viruses and helped with several experiments. M.P. generated the Langerin lentiviral construct under supervision of V.P., who also helped with the manuscript preparation. D.F. executed and interpreted the electron microscopy analysis. M.A.W.P.d.J. helped with LC isolations. T.d.G. contributed reagents and knowledge on LC isolation. Y.v.K. provided supervision and helped with the manuscript preparation. T.B.H.G. supervised all aspects of this study including study design, execution and interpretation, and manuscript preparation.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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- Lederman, M.M., Offord, R.E. & Hartley, O. *Nat. Rev. Immunol.* **6**, 371–382 (2006).
- Geijtenbeek, T.B. *et al. Cell* **100**, 587–597 (2000).
- Turville, S.G. *et al. Blood* **103**, 2170–2179 (2004).
- Turville, S.G. *et al. Nat. Immunol.* **3**, 975–983 (2002).
- Veazey, R.S. *et al. Nature* **438**, 99–102 (2005).
- Patterson, B.K. *et al. Am. J. Pathol.* **161**, 867–873 (2002).
- Kawamura, T., Kurtz, S.E., Blauvelt, A. & Shimada, S. *J. Dermatol. Sci.* **40**, 147–155 (2005).
- Pope, M. *et al. Cell* **78**, 389–398 (1994).
- Reece, J.C. *et al. J. Exp. Med.* **187**, 1623–1631 (1998).
- Kawamura, T. *et al. Proc. Natl. Acad. Sci. USA* **100**, 8401–8406 (2003).
- Kawamura, T. *et al. J. Exp. Med.* **192**, 1491–1500 (2000).
- Collins, K.B., Patterson, B.K., Naus, G.J., Landers, D.V. & Gupta, P. *Nat. Med.* **6**, 475–479 (2000).
- Banchereau, J. & Steinman, R.M. *Nature* **392**, 245–252 (1998).
- McDonald, D. *et al. Science* **300**, 1295–1297 (2003).
- Valladeau, J. *et al. Immunity* **12**, 71–81 (2000).
- Hunger, R.E. *et al. J. Clin. Invest.* **113**, 701–708 (2004).
- Masterson, A.J. *et al. Blood* **100**, 701–703 (2002).
- Garcia, E. *et al. Traffic* **6**, 488–501 (2005).
- Richters, C.D. *et al. Clin. Exp. Immunol.* **98**, 330–336 (1994).
- Nguyen, D.G. & Hildreth, J.E. *Eur. J. Immunol.* **33**, 483–493 (2003).
- Kawamura, T., Qualbani, M., Thomas, E.K., Orenstein, J.M. & Blauvelt, A. *Eur. J. Immunol.* **31**, 360–368 (2001).
- Miller, C.J. & Hu, J. *J. Infect. Dis.* **179** (suppl. 3), 413–417 (1999).
- Ward, E.M., Stambach, N.S., Drickamer, K. & Taylor, M.E. *J. Biol. Chem.* **281**, 15450–15456 (2006).
- Cohn, M.A. *et al. J. Infect. Dis.* **184**, 410–417 (2001).
- Picut, C.A., Lee, C.S., Dougherty, E.P., Andersen, K.L. & Lewis, R.M. *J. Histochem. Cytochem.* **35**, 745–753 (1987).