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Herpes Simplex Virus Type 2 Enhances HIV-1 Susceptibility by Affecting Langerhans Cell Function

Marein A. W. P. de Jong,* Lot de Witte,§ Maureen E. Taylor,§ and Teunis B. H. Geijtenbeek*†

Genital herpes is the most prevalent viral sexually transmitted infection worldwide and is mainly caused by HSV type 2 (HSV-2). HSV-2 infection enhances HIV-1 susceptibility, even in the absence of clinical symptoms. In this study, we investigated the effect of HSV-2 on HIV-1 transmission by mucosal Langerhans cells (LCs). LCs are important in heterosexual transmission because they form a barrier against HIV-1 infection; LCs efficiently capture and degrade HIV-1 through the C-type lectin langerin, thereby preventing HIV-1 transmission. Notably, our data showed that HSV-2 enhanced HIV-1 infection of LCs and subsequent HIV-1 transmission to T cells. HSV-2 interfered with HIV-1 capture by langerin, which allowed efficient HIV-1 infection of LCs. HSV-2 inhibited the antiviral function of langerin at two levels; HSV-2 decreased langerin expression and competed with HIV-1 for langerin binding. HSV-2 replication was not required, because both UV-inactivated HSV-2 and TLR-3 agonist polyinosinic:polycytidylic acid similarly increased HIV-1 transmission by LCs. Therefore, we identified a mechanism by which HSV-2 enhances HIV-1 susceptibility, even in the absence of clinical symptoms. Our data demonstrated that viral coinfections, such as HSV-2, breach the protective function of LCs by abrogating langerin function, which increases HIV-1 susceptibility. These data reinforce the importance of preventing sexually transmitted infections, such as HSV-2, to reduce the transmission of HIV-1. The Journal of Immunology, 2010, 185: 1633–1641.

More than 30 million people worldwide are infected by HIV-1, the causative agent of AIDS, and heterosexual transmission of HIV-1 is the primary route of infection (1). Numerous studies demonstrated that sexually transmitted infections (STIs) enhance susceptibility to HIV-1 acquisition and transmission, even in the absence of clinical symptoms (1–5). The most common viral STI acquired during sexual contact in the developing world is genital herpes (6), which is primarily caused by HSV type 2 (HSV-2), although HSV type 1 infection can also lead to genital herpes (7). Pre-existing HSV-2 infection is a risk factor for acquiring HIV-1 infection through sexual contact (1–4). Although it remains unclear how HSV-2 enhances HIV-1 susceptibility, several mechanisms have been proposed. The formation of pustules and ulcers by genital herpes can facilitate HIV-1 entry into mucosal tissues (8). In addition, mucosal inflammation by HSV-2 can cause an influx of activated CD4+ T cells, which facilitates HIV-1 infection and subsequent dissemination (9).

Notably, HSV-2 can enhance HIV-1 susceptibility in the absence of apparent lesions (5). HSV-2 also induces inflammation, thereby including T cell influx, even in the absence of clinically apparent lesions. Furthermore, HIV-1–susceptible inflammatory cells can persist for a long period of time after HSV-2 infection has been cleared and the lesions have healed (10).

HSV-2 infection occurs via direct contact with infected lesions or body fluids and enters the body at mucosal tissues or through small lesions. Epithelial cells and keratinocytes are the primary target cells for HSV-2, but it also infects neuronal and immune cells (11). Once infected, the virus causes life-long infection of the host by establishing latency in the neurons of the sensitive ganglia. Reactivation occurs periodically, at times when the immune system is suboptimal, resulting in the formation of vesicles that break into ulcers at the genital mucosa (8, 12). The genital mucosa is crucial in sexual transmission of HIV-1, and coinfections with HSV-2 might affect the integrity of the genital mucosa and consequently increase HIV-1 susceptibility. Genital tissues contain Langerhans cells (LCs), the main dendritic cell (DC) subset present in stratified epithelia and mucosal tissues (13). LCs are involved in HIV-1 dissemination; HIV-1 can infect LCs, and infected LCs subsequently migrate to the lymphoid tissues where they transmit the virus efficiently to T cells and, thereby, mediate infection of the host (14). However, we recently showed that LCs have an anti–HIV-1 function (15). Under noninflammatory conditions, LCs prevent HIV-1 transmission by efficient capture of invading HIV-1 via the C-type lectin langerin, which targets the virus for degradation (15). Langerin prevents HIV-1 infection of LCs and, thereby, transmission of the virus to T cells in lymphoid tissues. Thus, LCs form a first line of defense against HIV-1 infection. However, inhibition of the antiviral function of langerin or inflammatory conditions allows infection of LCs by HIV-1, and infected LCs efficiently transmit HIV-1 to T cells (15, 16). Therefore, we hypothesized that viral STIs, such as HSV-2, increase HIV-1 susceptibility by affecting the protective function of LCs.

In this article, we show that HSV-2 productively infects LCs and that LC activation by HSV-2 increases HIV-1 transmission by...
LCs to T cells. We identified two distinct effects of HSV-2 on the anti–HIV-1 function of LCs; HSV-2 induced downregulation of langerin expression and in addition HSV-2 virions inhibited HIV-1 binding to langerin by direct competition with HIV-1. The reduced langerin expression and function, decreased HIV-1 capture by LCs and allowed infection of LCs by HIV-1, which increased HIV-1 transmission to T cells. Notably, active HSV-2 replication was not required, because UV-inactivated HSV-2 (UV–HSV-2) similarly increased HIV-1 transmission by LCs. Furthermore, our data show that TLR-3 agonist polyinosinic-polycytidylic acid (poly[I:C]) also increased HIV-1 transmission. Thus, coinfection with HSV-2 is a major risk factor that increases HIV-1 susceptibility by affecting the anti–HIV-1 function of LCs. Our data suggest that dsRNA viruses might similarly increase HIV-1 susceptibility. These data further support an important role for LCs in HIV-1 transmission in the presence of subclinical genital herpes and other viral STIs.

**Materials and Methods**

**Abs and reagents**

The following Abs were used: PE-conjugated Abs against CD80, CD86 and CD4, CD1a-FITC (all from BD Pharmingen, San Diego, CA), CD1a-PE (Abcam, Cambridge, MA), HSV1/2 gBp (Novus Biologicals, Littleton, CO), goat anti-mouse–FITC (Zymed Laboratories, San Francisco, CA), langerin-PE (Beckman Coulter, Fullerton, CA), and CCR5-PE (Invitrogen, Breda, The Netherlands). Isotype-control Abs were used against mouse IgG1 (BD Pharmingen) and rat IgG1 (Invitrogen). The following stimuli or inhibitors were used: TLR agonists poly(I:C) (10 μg/ml; Sigma-Aldrich, St. Louis, MO), imiquimod (10 μg/ml; InvivoGen, San Diego, CA), Cpg-containing oligonucleotides (Cpg-ODN) 2216 (10 μg/ml; InvivoGen), Pam3CSK4 (5 μg/ml; InvivoGen), recombinant human TNF-α (0.1 μg/ml; Strathmann Biotec, Dengelsberg, Germany), and anti–TNF-α (20 μg/ml; Biovision, Mountain View, CA).

**Cell lines and viruses**

Jurkat T cells expressing CCR5 were generated by retroviral transduction, as previously described (17, 18). The HSV-2 strain 333 was grown on GMK cells and harvested after 24 h of infection. Supernatant was filtered over a 0.22-μm filter, and a plaque-titration assay was performed to determine viral titers (19). HSV-2 was inactivated by placing a Petri dish containing 3 ml viral suspension under a UV lamp for 10 min on ice. Virus inactivation was confirmed, and the virus stocks were stored at −80°C until further use. Medium from cultured GMK cells was treated similarly and used as a mock control.

HSV-1 viruses R5-tropic enhanced GFP (eGFP)-expressing HIV-1–NL4.3-Bal (referred to as HIV-1-eGFP) (20 ng/well) and replication-defective R5-tropic pseudotyped HIV-1 (HIV-1 BAL–pseudotyped NL4.3-eGFPΔENV) (50 ng/well) were produced in 293T cells, as described previously (16). Virus stocks were quantified by p24 ELISA (PerkinElmer, Wellesley, MA) and titrated using the indicator cells TZM-blue (16, 20).

**Isolation of epidermal single-cell suspension and LCs**

Human tissue was obtained from healthy donors undergoing corrective breast or abdominal surgery after informed consent, in accordance with institutional guidelines, and it was used within 3 h after surgery. An LC-enriched epidermal single-cell suspension was generated, as described previously (15, 16). In short, epidermis was separated after dispase II (1 mg/ml; Roche Diagnostics Systems, Somerville, NJ) treatment and digested in 0.05% trypsin solution. Cell suspension was purified over Lymphoprep (Miltenyi Biotec, Auburn, CA). This yielded a >90% pure CD1a+ LC population. Cells were cultured in IMDM supplemented with 10% FCS and 10 μg/ml gentamicin.

**Cell stimulation**

Cells (1 × 10^5) were preincubated with TLR stimuli, HSV-2 (1 × 10^5 PFU), UV–HSV-2 (1 × 10^5 PFU), or a mock control overnight. Flow cytometric analysis for cell-surface markers was performed. To determine productive HSV-2 infection of LCs, 1 × 10^5 cells were incubated with different viral input, and expression of HSV-2 gBp was measured by flow cytometry. A double staining for CD1a was performed to analyze infection of LCs. For TNF-α production, cells were incubated with different concentrations of HSV-2 or UV–HSV-2 for 6, 12, and 24 h or with poly(I:C). Supernatant was harvested, and TNF-α protein production was measured by ELISA (BioSource International, Camarillo, CA).

**Fluorescent bead adhesion assay**

Strepavidin-coated beads (TransFluorSpheres, Molecular Probes) were coated with HIV-1 gp120, as described previously (21). The adhesion assay (Fig. 3A) was performed as follows (19, 22, 23): 1 × 10^5 cells were incubated overnight with TLR agonists or different concentrations of HSV-2 and UV–HSV-2. Cells were washed extensively with PBS and stained with CD1a-FITC. Next, cells were washed in Tris buffer (20 mM Tris-HCl [pH 7.5], 150 mM NaCl, 1 mM CaCl2, 2 mM MgCl2, supplemented with 0.5% BSA) before adding HIV-1 gp120 beads for 45 min at 37°C. Cells were washed and binding was measured by flow cytometry. Freshly isolated LCs were incubated with HSV-2 or mannann for 15 min prior to adding HIV-1 gp120 beads to investigate competition with langerin. After 45 min incubation at 37°C, cells were washed and binding was measured by flow cytometry.

**Langerin-binding ELISA**

Different concentrations of HSV-2 were coated onto ELISA plates overnight at room temperature. Nonspecific binding was blocked by incubating the plate with Tris buffer for 1 h at 37°C. Recombinant human langerin (24) (2 μg/ml) was added for 1 h at 37°C. Unbound langerin was washed away, and binding was determined using an anti-langerin Ab (DCGM4; Beckman Coulter), followed by peroxidase-conjugated goat anti-mouse IgG Ab (Jackson Immunoresearch Laboratories, West Grove, PA). Specificity was determined in the presence of mannan (1 mg/ml) or EGTA (10 mM/ml). Tetramethylbenzidine was used as a substrate for peroxidase, and absorbance was measured at 450 nm.

**HSV-2 mRNA transcription**

A total of 1 × 10^5 CD1a+ LCs (>95% pure) were infected with different concentrations of HSV-2 or UV–HSV-2. After 6, 24, or 48 h, the cells were washed extensively with PBS, and mRNA was isolated with the mRNA Capture Kit (Roche Diagnostics Systems). cDNA was synthesized with the Reverse Transcriptase Kit (Promega, Madison, WI). For quantitative real-time PCR analysis, PCR amplification was performed in the presence of SYBR green, as previously described (16, 25). Specific primers for HSV-2 thymidine kinase and GAPDH were designed by Primer Express 2.0 (Applied Biosystems, Foster City, CA); forward primer sequence, 5′-GCA-GCCGATGACTTACTGC-3′; reverse primer sequence, 5′-GCCTGGTGTTT-GTAGATTTGC-3′. Transcription was adjusted for GAPDH transcription.

**HSV-2 production by LCs**

A total of 1 × 10^5 LCs (>95% pure) were infected with different concentrations of HSV-2 or UV–HSV-2. After 1 h, cells were washed extensively and incubated in a total volume of 100 μl. After 3, 18, or 40 h, supernatant was harvested and stored at −20°C until analysis. To measure productive infection, supernatant was filtered to remove cell-debris, and 10 μl was added to GMK cells. After 24 h, infection of GMK cells was measured by flow cytometry for the expression of HSV-2 gBp. Pure HSV-2 (1 × 10^5 PFU/ml) was treated similarly as the supernatant as a control.

**Viability assay**

A total of 1 × 10^5 LCs (>95% pure) were infected with different concentrations of HSV-2 or UV–HSV-2 at multiplicity of infection (MOI) of 1. Cells were collected at 18, 18, and 40 h postinfection and analyzed for apoptosis using Annexin V and propidium iodide (PI) (BD Pharmingen). Cells were incubated with Annexin V-FITC and 0.1 μg PI for 15 min at room temperature in the dark. Cells treated with 3% formaldehyde for 30 min on ice were used as a positive control (data not shown). Cells were analyzed by flow cytometry. PI− cells were considered necrotic or late apoptotic; Annexin V− cells were considered apoptotic.

**Immunofluorescence microscopy**

CD1a+ LCs (1 × 10^5; >95% pure) were incubated with 1 × 10^5 PFU HSV-2 or UV–HSV-2 for 24 h. Cells were washed and stained intracellularly for HSV-2–ICP27 or HSV-2–ICP5, in combination with CD1a (all from Santa Cruz Biotechnology, Santa Cruz, CA). Cells were analyzed by confocal microscopy (Leica AOB5 SP2 CSLM system).

**HIV-1 infection**

Cells stimulated overnight were washed extensively and incubated with R5-tropic HIV-1–NL4.3-Bal (20 ng/well). For mRNA analysis, after 6 and 24 h, cells were washed extensively with PBS, and host and viral mRNA were...
isolated with the mRNA capture kit (Roche Diagnostic Systems). For real-time PCR analysis, PCR amplification was performed in the presence of SYBR green, as previously described (16, 25). HIV-1 Tat/Rev transcripts were measured by using specific primers that detect Tat and Rev mRNA across an intron (for sequence see Ref. 16). Transcription was adjusted for GAPDH transcription, and relative mRNA expression of HIV-1–infected control samples was set at 1.

For HIV-1 p24 protein production, CD1a+ LCs (1 × 10^5; >95% pure) were incubated with 1 × 10^5 PFU UV–HSV-2 or poly(I:C) overnight. Cells were washed extensively and inoculated with HIV-1–NL4.3-BaL (20 ng/well). After 24 h, cells were washed extensively and incubated in 100 μl medium. At day 6, HIV-1 p24 was measured by using retro-tek HIV-1 p24 detection ELISA (Zeptometrix, Buffalo, NY), according to the manufacturer’s protocol.

**HIV-1 transmission**

Cells were washed extensively and inoculated with HIV-1–eGFP (20 ng/well) or replication-defective R5-tropic pseudotyped HIV-1–eGFPΔENV (50 ng/well). After 2 and 40 h, cells were washed, and CCR5+ Jurkat

![Figure 1](http://www.jimmunol.org/)

**FIGURE 1.** HSV-2 productively infects LCs. A and B, Epidermal LC fraction was infected with different concentrations of HSV-2 for 18 h. HSV-2 infection was determined by measuring HSV-2 gB expression on CD1a+ LCs by flow cytometry. A, Numbers represent the percentage of the total population. B, Percentage of LCs that were infected with HSV-2. Error bars represent Sds of duplicates. These results were observed for three donors. C and D, CD1a+ LCs isolated by CD1a MACS were infected with HSV-2 or UV–HSV-2. mRNA expression of HSV-2 thymidine kinase gene was measured at different viral inputs (C) after 24 h or at different time points (D) at MOI of 1. Transcription was adjusted for GAPDH expression. E, CD1a+ LCs were incubated with HSV-2 (MOI 1) for 24 h and stained for HSV-2 ICP5 and ICP27 (red) and CD1a (green). Images were taken using confocal microscopy at ×630 with ×4 zoom. F, CD1a+ LCs were incubated with HSV-2 at different concentrations, and supernatant was harvested at different time points. Productive infection was determined by titrating supernatant on susceptible GMK cells. Infection of GMK cells was analyzed by flow cytometry for HSV-2 gB expression. As a control, GMK cells were directly infected with HSV-2 (pure HSV-2 condition). G, Viability of LCs infected with HSV-2 or UV–HSV-2 at MOI of 1 was determined 3, 18, and 40 h postinfection by staining for Annexin V and PI. Results are representative of at least two separate experiments.
T cells \( (2 \times 10^5) \) were added. Transmission to CCR5+ Jurkat T cells was measured over time by flow cytometry.

**Statistical analysis**

One-way ANOVA was used to compare the means of multiple groups. When the overall F-test was significant, differences were investigated further using a post hoc Bonferroni test using GraphPad Prism software. A \( p \)-value \( <0.05 \) was considered statistically significant.

**Results**

**HSV-2 productively infects human LCs**

HSV-2 is one of the most common viral STIs (7) and is a risk factor for enhanced HIV-1 susceptibility (1–4). Therefore, we investigated whether HSV-2 infects LCs and, thereby, affects HIV-1 transmission. Infectious HSV-2 and UV–HSV-2 were used to distinguish between effects caused by viral replication or through direct activation by pattern-recognition receptors. An epidermal LC fraction, containing 10–15% CD1a+ LCs, was infected with different concentrations of HSV-2 for 18 h, and cell-surface expression of HSV-2 gpB was analyzed to determine HSV-2 infection. gpB is an HSV-2 envelope glycoprotein that is expressed as a late viral gene product (26). We used CD1a as an LC marker to distinguish LCs from other cells in the epidermal LC fraction [i.e., keratinocytes, T cells (16), and melanocytes]. LCs and CD1a− keratinocytes were productively infected with HSV-2 in a concentration-dependent manner (Fig. 1A, 1B). HSV-2 infection of LCs increased with the viral load; at \( 1 \times 10^5 \) PFU, \( \sim 10\% \) of LCs were infected, whereas at a higher viral load (\( 10^5 \) PFU), up to 45% of LCs were infected (Fig. 1B). We never observed >3% gpB+ cells when LCs were incubated with similar concentrations of UV–HSV-2 (data not shown), demonstrating that gpB staining is due to productive infection of LCs and not to uptake of HSV-2 particles.

To confirm productive HSV-2 infection, LCs were purified from the epidermal LC fraction and subsequently infected with HSV-2 or UV–HSV-2, and expression of HSV-2 thymidine kinase mRNA was measured by quantitative real-time PCR. In contrast to UV–HSV-2, HSV-2 infection induced transcription of thymidine kinase, which increased over time and was dose dependent (Fig. 1C, 1D). In addition, intracellular expression of HSV-2 immediate early protein ICP27 and late viral protein ICP5 was analyzed by confocal microscopy. ICP27 and ICP5 were expressed by LCs after 24 h of infection with HSV-2 but not with UV–HSV-2 (Fig. 1E, data not shown). Furthermore, LCs produced infectious HSV-2 particles, because supernatant from infected LCs caused HSV-2 infection of a susceptible cell line (Fig. 1F). Together, these data demonstrated that LCs are productively infected by HSV-2.

HSV-2 infection affects the viability of monocyte-derived DCs (27). Therefore, we investigated the viability of LCs after HSV-2 or UV–HSV-2 stimulation. LCs were infected with HSV-2 at an MOI of 1 and analyzed apoptosis and necrosis at 3, 18, and 40 h postinfection. Annexin V and PI were used to distinguish viable cells (Annexin V−/PI−), early apoptotic cells (Annexin V+/PI−), and late apoptotic cells (Annexin V+/PI+). After 18 h, viability was 60% for HSV-2–infected cells compared with 70% for noninfected LCs (Fig. 1G). After 40 h, viability had decreased to \( \sim 40\% \) compared with 70% for untreated LCs. These results showed that HSV-2 decreased the viability of LCs after prolonged culture. In contrast, UV–HSV-2 had no effect on LC viability.
HSV-2 and viral TLR agonists induce LC maturation

Next, we investigated whether HSV-2 infection or viral TLR agonists induce LC maturation. Viral recognition is mainly mediated by TLR-3 for dsRNA, TLR-7 and -8 for ssRNA, and TLR-9 for unmethylated DNA. Immature LCs express TLR-3 and -7 but little TLR-8 and no TLR-9 (28, 29). Epidermal LC fraction was infected with HSV-2/UV–HSV-2 and TLR-3, -7, and -9 agonists poly(I:C), imiquimod, and bacterial CpG-ODN, respectively (Fig. 2A). LC maturation was determined by measuring costimulatory molecules CD80 and CD86 after 18 h by flow cytometry. In contrast to a mock infection control, HSV-2 infection of LCs induced upregulation of the costimulatory molecules CD80 and CD86 after 18 h by flow cytometry. In contrast to a mock infection control, HSV-2 infection of LCs induced upregulation of the costimulatory molecules CD80 and CD86 (Fig. 2B, data not shown). Notably, UV–HSV-2 induced upregulation of the costimulatory molecules to a similar extent as infectious HSV-2 (Fig. 2B).

Poly(I:C) and, to a lesser extent, imiquimod induced expression of CD80 and CD86, in contrast to CpG-ODN (Fig. 2B). Thus, HSV-2 and viral TLR agonists induce LC maturation.

HSV-2 and viral TLR agonists decrease langerin expression and increase HIV-1 infection

HIV-1 receptors CD4 and CCR5 are crucial for the infection of LCs with R5-tropic HIV-1, whereas, in contrast, langerin expression prevents infection of LCs (15). Therefore, we investigated the effect of HSV-2 and viral TLR agonists on the expression of CD4, CCR5, and langerin. The expression of HIV-1Rs CD4 and CCR5 was neither affected by HSV-2 nor any of the TLR agonists (Fig. 2B). Notably, HSV-2 infection, UV–HSV-2, and poly(I:C) strongly decreased langerin expression, whereas imiquimod caused only a marginal decrease (Fig. 2B).

Next, we investigated whether HSV-2 and poly(I:C) decreases HIV-1 capture. Primary LCs were treated with HSV-2, UV–HSV-2, and different TLR agonists overnight and washed extensively before binding to HIV-1 gp120 was determined. As shown previously (15), HIV-1 gp120 interacted efficiently with immature LCs, and the interaction was blocked by the competitive inhibitor of langerin, mannan (Fig. 3A), strongly suggesting that langerin is the primary receptor for HIV-1. Notably, HSV-2 infection and incubation with UV–HSV-2 significantly decreased HIV-1 binding to LCs in a concentration-dependent manner (Fig. 3A). Poly(I:C) also decreased HIV-1 binding to LCs in contrast to imiquimod or CpG-ODN (Fig. 3A). Subsequent incubation with mannan reduced the binding to background levels for all conditions (Fig. 3A, data not shown). These data demonstrated that LC maturation by HSV-2 infection, UV–HSV-2, and TLR-3 agonists decreased HIV-1 capture by LCs, which might be due to downregulation of langerin expression.

Next, we investigated whether the decreased langerin expression by HSV-2 and TLR-3 agonist increases HIV-1 infection. LCs were incubated overnight with HSV-2, UV–HSV-2, and viral TLR agonists, washed, and infected with R5-tropic HIV-1. HIV-1 replication was determined after 6 and 24 h by measuring Tat/Rev transcripts using quantitative real-time PCR (16). Notably, HSV-2, UV–HSV-2, and poly(I:C) strongly enhanced HIV-1 transcription, in contrast to imiquimod and CpG-ODN (Fig. 3B–D). In addition, UV–HSV-2 or poly(I:C) stimulation of CD1a+ LCs increased HIV-1 p24 protein production (Fig. 3E). Thus, HSV-2 infection, as well as stimulation with UV–HSV-2 and viral TLR-3 agonists, enhances HIV-1 infection of LCs, which might be due to LC maturation and downregulation of langerin expression.

![FIGURE 3.](http://www.jimmunol.org/) HSV-2 and TLR stimulation decrease HIV-1 capture and enhance HIV-1 infection of LCs. A, The epidermal LC fraction was stimulated for 18 h with different stimuli, and LCs were analyzed for HIV-1 gp120 binding. Specificity was determined by inhibiting langerin using mannan and EGTA. Bar graph depicts percentages of LCs that captured HIV-1 gp120. Error bars represent SDs of triplicates. These results are representative of three donors. The epidermal LC fraction (B, C) or CD1a+ LCs (D) were stimulated for 20 h with different stimuli. Next, stimulated cells were incubated with R5-tropic HIV-1-NL4.3-BaL for 6 h (B–D) or 24 h (C, D). HIV-1 replication was determined by measuring Tat/Rev transcripts by quantitative real-time PCR and normalized for GAPDH expression. Medium condition was set at 1. Error bars represent SDs of duplicates. These results were observed for at least two donors. E, CD1a+ LCs were stimulated for 20 h with different stimuli. Next, stimulated cells were incubated with R5-tropic HIV-1-NL4.3-BaL for 24 h. Cells were washed extensively; at day 6, HIV-1 p24 was determined in the cultures by ELISA. Results are representative of two donors. *p < 0.05; **p < 0.01; ***p < 0.001.
HSV-2 competes with HIV-1 for langerin binding

Langerin interacts with different pathogens, such as HIV-1, Candida albicans, and Mycobacterium (15, 30–33). Therefore, we investigated whether HSV-2 interacts with langerin and, thereby, competes with HIV-1 for the carbohydrate-recognition domain of langerin. Recombinant langerin strongly interacted with HSV-2 in a concentration-dependent manner (Fig. 4A), as determined by the langerin-binding ELISA (24). The binding was specific for the carbohydrate-recognition domain of langerin, because it was blocked by mannan and calcium-chelator EGTA. These data indicated that HSV-2 competes with HIV-1 for the langerin-binding site. To investigate this further, LCs were preincubated with HSV-2 for 15 min, and HIV-1 gp120 capture was measured by flow cytometry. The short incubation time excludes LC maturation and infection. Notably, preincubation of LCs with HSV-2 strongly reduced binding of HIV-1 gp120 to LCs (Fig. 4B, 4C), demonstrating that HSV-2 blocks HIV-1 capture by directly competing with HIV-1 for langerin. Thus, these data showed that HSV-2 prevents HIV-1 capture by langerin by downregulation of langerin expression, as well as by competition for the langerin-binding site.

HSV-2 and poly(I:C) enhance HIV-1 transmission by LCs to T cells

Under steady-state conditions, LCs are refractory to HIV-1 infection through the protective function of langerin (15). However, HSV-2 and TLR-3 agonist poly(I:C) increased HIV-1 infection of LCs by inducing LC maturation and affecting langerin function. Therefore, we investigated whether HSV-2 and TLR agonists increased HIV-1 transmission to T cells. Infectious HSV-2 could not be used in the transmission assays because it also infects T cells and induces cell death in the coculture after several days, even in the presence of HSV-2 inhibitor acyclovir (data not shown). The epidermal LC fraction was treated with viral TLR agonists and UV–HSV-2 for 20 h, washed extensively, pulsed with R5-tropic HIV-1–eGFP for 2 h, and subsequently cocultured with CCR5+ Jurkat T cells (16). Viral transmission was assessed over time by flow cytometry (16). LCs did not efficiently transmit HIV-1 to T cells (Fig. 5A, 5B) (15, 16). Strikingly, UV–HSV-2 and poly(I:C) significantly enhanced HIV-1 transmission by LCs obtained from different donors (Fig. 5A, 5B). Imiquimod marginally enhanced HIV-1 transmission, but this was not statistically significant, whereas CpG-ODN did not affect transmission.

To investigate whether the increased transmission by HSV-2 and poly(I:C) was a direct effect on LC function, a bystander effect through activation of keratinocytes or caused by contaminating T cells, LCs were purified from the epidermal single-cell suspension by CD1a isolation. CD1a+ LCs and the CD1a− fraction were stimulated with UV–HSV-2 or poly(I:C) overnight and subsequently infected with R5-tropic HIV-1–eGFP for 40 h. Cells were washed extensively, CCR5+ Jurkat T cells were added, and

FIGURE 4. HSV-2 competes with HIV-1 for langerin binding. A, HSV-2 viral particles were coated onto ELISA plates, and binding was detected using recombinant langerin. Mannan and EGTA preincubation demonstrate the specificity for langerin. Error bars represent SDs of triplicates. These results were observed in three individual experiments. B and C, LCs were preincubated with different concentrations of HSV-2 for 15 min before HIV-1 gp120 beads were added. The percentages of LCs that captured HIV-1 gp120 are depicted. Error bars represent SDs of duplicates. The results are representative of three donors.

FIGURE 5. HSV-2 and poly(I:C) stimulation of LCs enhances HIV-1 transmission. A and B, The epidermal LC fraction was stimulated for 18 h. Cells were washed extensively and incubated with R5-tropic HIV-1–eGFP for 2 h. Cells were washed, and CCR5+ Jurkat cells were added. Transmission of HIV-1 was assessed over time by flow cytometry. A, Time course of HIV-1 transmission for two different donors. B, Combined results for five donors are depicted. C, CD1a+ LCs and CD1a− fraction were stimulated for 18 h with poly(I:C) or UV–HSV-2. Cells were washed and incubated with R5-tropic HIV-1–eGFP for 40 h. CCR5+ Jurkat cells were added, and transmission of HIV-1 was assessed over time by flow cytometry. Results are representative for two independent donors. Error bars represent SDs of duplicates. ***p < 0.01; ****p < 0.001.
HIV-1 transmission to T cells was measured at day 6. Strikingly, poly(I:C) and UV–HSV-2 strongly enhanced HIV-1 transmission by CD1a⁺ LCs, whereas no transmission was observed with the CD1a⁻ fraction containing keratinocytes and T cells (Fig. 5C). These data strongly suggest that LCs, but neither keratinocytes nor T cells, mediate HIV-1 transmission (16) and that HSV-2 and TLR3 agonist poly(I:C) affect LC function and, thereby, increase HIV-1 transmission to T cells.

**TNF-α production and trans-infection are not involved in enhanced HIV-1 transmission by HSV-2 and poly(I:C)**

The proinflammatory cytokine TNF-α enhances HIV-1 replication (16, 34, 35). Therefore, we investigated whether TNF-α production was involved in the enhanced HIV-1 infection of LCs after HSV-2 and poly(I:C) incubation. The epidermal LC fraction was stimulated with HSV-2, UV–HSV-2, or poly(I:C), and the production of TNF-α was measured at different time points. HSV-2 and UV–HSV-2 produced low levels of TNF-α, which increased over time and was concentration dependent. Poly(I:C) induced low levels of TNF-α production (Fig. 6A). To investigate whether TNF-α production was involved in the enhanced HIV-1 transmission by HSV-2 and poly(I:C), a neutralizing Ab against TNF-α was added during the transmission assay (16). Anti–TNF-α Ab did not significantly affect HIV-1 transmission induced by UV–HSV-2 and poly(I:C), whereas it abrogated TNF-α–induced HIV-1 transmission (Fig. 6B). These data show that TNF-α is not responsible for the observed enhanced HIV-1 transmission by LCs after poly(I:C) or HSV-2 stimulation.

Our data strongly suggest that HSV-2 and TLR3 ligands increase transmission of HIV-1 by de novo production of HIV-1 by LCs. It was demonstrated previously that Pam3CSK4-activated LCs (16) and LPS/TNF-α–activated LC-like cells (36) enhance HIV-1 transmission without LC infection, a mechanism known as trans-infection. Although we observed an enhanced replication of HIV-1 in LCs in response to HSV-2 and poly(I:C), it was demonstrated previously that Pam3CSK4-activated LCs (16, 34, 35). Therefore, we investigated whether TNF-α production is involved in the enhanced HIV-1 transmission by HSV-2 and poly(I:C). The proinflammatory cytokine TNF-α enhances HIV-1 replication (29). These data strongly suggest that HSV-2, as well as other

**Discussion**

Genital HSV-2 infection is highly prevalent in sexually active people worldwide, with a prevalence in the general population ∼20% in the United States and 60% in sub-Saharan Africa (37). Epidemiological studies demonstrated that HSV-2 infection increases the risk for HIV-1 acquisition, even in the absence of clinical symptoms (1–5). In this study, we demonstrated that HSV-2 strongly increases HIV-1 transmission by LCs. Immature LCs form a protective barrier against HIV-1 infection through the C-type lectin langerin (15, 30–32). However, our data suggest that HSV-2 interferes with this anti-HIV-1 function by decreasing langerin expression and saturating langerin activity, leading to increased HIV-1 infection of LCs and, consequently, increased transmission to T cells. These data show that, in addition to the previously described mechanisms, viral STIs enhance HIV-1 susceptibility by altering LC function.

Although epithelial cells and keratinocytes are the primary target cells for HSV-2, we demonstrate that LCs are productively infected by HSV-2. Infection with HSV-2 induced LC maturation, with increased expression of costimulatory molecules but decreased expression of langerin, whereas expression of CD4 and CCR5 was not affected. Maturation was independent of viral replication because UV–HSV-2 induced a similar maturation. HSV-2 was shown to interact with TLR-2, which is expressed by LCs and may be involved in LC maturation (38). However, we previously showed that TLR-1/2 ligand Pam3CSK4 does not affect langerin expression on LCs (16). Thus, HSV-2 might also trigger other receptors that induce LC maturation.

A comparison of different viral TLR agonists showed that TLR-3 agonist poly(I:C) was the most potent in inducing LC maturation, as well as decreasing langerin expression, whereas TLR-7/8 agonist imiquimod had a marginal effect on LC maturation and langerin expression. As expected, TLR-9 agonist CpG did not affect LC function because TLR-9 is not expressed by LCs (29). These data strongly suggest that HSV-2, as well as other
viruses containing TLR-3 ligands, induce LC maturation and affect langerin expression.

Notably, our data show that HSV-2 interacts with langerin. These data support a function for langerin as a pattern-recognition receptor (39) for different pathogens, including viruses, such as HIV-1, HSV type 1 (data not shown), and HSV-2, fungi, and bacteria (15, 30–33). The function of langerin as a pattern-recognition receptor is unclear. HSV binding to langerin might result in more efficient Ag processing and presentation (31, 40). Previously, we showed that langerin prevents HIV-1 infection of LCs by capturing HIV-1 for degradation (15). Thus, langerin might perform a similar function for HSV-2. However, LCs express high levels of proteoglycans, such as syndecan-3 (41), which function as attachment receptors for HSV-2 and might facilitate HSV-2 infection, even in the presence of langerin. Further studies are necessary to identify the function of langerin in HSV infection.

Langerin is an important C-type lectin on LCs that prevents HIV-1 infection by degrading HIV-1 (15, 42). Previous studies showed that inhibition of langerin by a blocking Ab or a carbohydrate ligand increased HIV-1 infection of LCs (15). Notably, our data show that HSV-2 and TLR-3 agonist poly(I:C) decreased langerin expression, which prevented efficient HIV-1 binding by LCs. Moreover, HSV-2 competed with HIV-1 for langerin binding, further hampering the protective function of langerin. Thus, HSV-2 affects langerin function at different levels, and this prevents HIV-1 capture, leading to increased HIV-1 infection of LCs and, consequently, enhanced transmission to T cells. Notably, HSV-2 replication is not required for the observed increase in HIV-1 transmission, suggesting that virus particles present at lesions might already affect LC function. Various studies showed that HSV can increase HIV-1 replication at several levels (43–45), and this might also affect HIV-1 infection of LCs. However, our data showed that HSV-2 replication is not required, because UV–HSV-2 induced HIV-1 infection and transmission to a similar level as replication-competent HSV-2. These data suggest that aside from competition of HSV-2 with HIV-1 for langerin binding, innate signaling by pattern-recognition receptors induces LC maturation, which might also affect HIV-1 replication. Similarly, we cannot exclude that TLR-3 triggering by poly(I:C) also affected HIV-1 replication by activating NF-κB and other transcription factors that might exert an additive effect on HIV-1 replication.

A recent study showed that TLR-3 ligand poly(I:C) does not enhance HIV-1 transmission (46), whereas our data showed that poly(I:C) is a potent inducer of LC maturation and HIV-1 transmission. The differences might be due to the model used, because the investigators obtained LCs with a mature phenotype after migration and TLR-3 triggering by poly(I:C) also affected HIV-1 replication by activating NF-κB and other transcription factors that might exert an additive effect on HIV-1 replication.

Immune activation is an important determinant in HIV-1 replication and infection. Proinflammatory cytokines, such as TNF-α and IL-1β, enhance HIV-1 replication (16, 34, 35). We previously demonstrated that viral pathogens induce the production of TNF-α in mucosal tissue, which increases HIV-1 transmission by LCs (16). However, HSV-2 infection induced low TNF-α and no IL-1β production (data not shown); in addition, anti-TNF-α did not abrogate the enhanced HIV-1 transmission observed in the presence of HSV-2 or poly(I:C). These data strongly suggest that TNF-α is not involved in the HSV-2– or poly(I:C)-enhanced HIV-1 infection of LCs. Previously, we showed that TNF-α and TLR-2 agonist Pam3CSK4 increased HIV-1 transmission by enhancing HIV-1 replication and HIV-1 capture, respectively (16). In this study, we identified another mechanism that affects HIV-1 infection of LCs: viral pathogens or viral TLR agonists hamper langerin expression and function, which increases HIV-1 infection of LCs (15, 16, 36). A recent study demonstrated that immune infiltrates persist well after the HSV-2 lesion has healed. The increased expression of DC-SIGN (DC-specific ICAM-3 graving non-integrin) on DCs in these infiltrates and T cell influx might contribute to HIV-1 susceptibility (10). Further studies are necessary to investigate the expression of langerin on LCs present in the healed HSV-2 lesions, because prolonged immune activation might lead to a decrease in the antiviral function of LCs.

Collectively, our data support a role for LCs in the enhanced susceptibility to HIV-1 in the presence of HSV-2. Infectious HSV-2 and noninfectious particles abrogate the anti–HIV-1 function of LCs by inducing LC activation and decreasing langerin expression as well as function. Continuous HSV-2 shedding by mucosal tissue maintains the presence of HSV-2, which further blocks langerin function and induces the activation of LCs. Furthermore, dsRNA viruses that are mimicked by poly(I:C) might also increase HIV-1 susceptibility. Our data emphasize the importance of STI prevention, because it is a risk factor for the acquisition of HIV-1, even in the absence of clinical symptoms.

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Disclosures

The authors have no financial conflicts of interest.

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