

# HIV-1 Selection by Epidermal Dendritic Cells during Transmission across Human Skin

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## Summary

Macrophage tropic HIV-1 is predominant during the initial viremia after person to person transmission of HIV-1 (Zhu, T., H. Mo, N. Wang, D.S. Nam, Y. Cao, R.A. Koup, and D.D. Ho. 1993. *Science*. 261:1179–1181.), and this selection may occur during virus entry and carriage to the lymphoid tissue. Human skin explants were used to model HIV-1 selection that may occur at the skin or mucosal surface. Macrophage tropic, but not T cell line tropic strains of HIV-1 applied to the abraded epidermis were recovered from the cells emigrating from the skin explants. Dermis and epidermis were separated by dispase digestion after virus exposure to determine the site of viral selection within the skin. Uptake and transmission to T cells of all HIV-1 isolates was found with the dermal emigrant cells, but only macrophage tropic virus was transferred by emigrants from the epidermis exposed to HIV-1, indicating selection only within the epidermis. CD3<sup>+</sup>, CD4<sup>+</sup> T cells were found in both the dermal and epidermal emigrant cells. After cell sorting to exclude contaminating T cells, macrophage tropic HIV-1 was found in both the dermal emigrant dendritic cells and in dendritic cells sorted from the epidermal emigrants. These observations suggest that selective infection of the immature epidermal dendritic cells represents the cellular mechanism that limits the initial viremia to HIV-1 that can use the CCR5 coreceptor.

Key words: Langerhans cells • dendritic cells • skin • HIV • tropism

At least three members of the beta chemokine receptor family are used by different HIV-1 isolates as coreceptors for HIV-1 viral entry (1–4). This discovery has clarified some of the confusion of the earlier classifications of cellular tropism of HIV-1, based on their ability to infect macrophages or the human T lymphotropic virus type 1 transformed T cell line MT-2 (5). The differences in host cell preference can largely be attributed to the differing ability of HIV-1 isolates to use alternative coreceptors (6–8). CXCR4 is used by HIV-1 strains that preferentially enter T cell lines resulting in the syncytia-inducing (SI)<sup>1</sup> biological phenotype (4). CCR5 and, less commonly, CCR3 or CCR2b are used by HIV-1 that enters macrophages resulting in the macrophage tropic biological phenotype (9). Activated primary T cells and nontransformed T cell lines express both CXCR4 and CCR5 and can be infected by all isolates irrespective of the virus phenotype. Confusion in

classification has arisen because use of CCR5 or CXCR4 is not mutually exclusive and there is overlap between SI and macrophage tropism. The envelope of such dual tropic virus isolates that are SI and macrophage tropic can bind to both CCR5 and CXCR4 and infect T cell lines and macrophages (7, 9).

Preferential infection by macrophage tropic isolates that use the CCR5 coreceptor occurs during person to person transmission of HIV-1 (10–11). Later in disease, quasispecies of HIV-1 emerge that use CXCR4 (12) and have the ability to infect T cell lines reflected in the SI phenotype (5, 12, 13). We have shown that tonsil and thymic dendritic cells (DCs) can be infected by HIV-1 and that viral entry is preferential by macrophage tropic HIV-1 (14), suggesting non-CXCR4-dependent entry. Langerhans cells and DCs are the first cells exposed to infectious organisms or antigens and migrate with processed antigen. These cells migrate to draining lymph nodes where cognate T cell interactions initiate immune responses (15). Selective entry of HIV-1 into DCs and carriage of virus with selected phenotype to lymph nodes could therefore explain the restriction

<sup>1</sup>Abbreviations used in this paper: DC, dendritic cell; NSI, non-SI; SEB, staphylococcus enterotoxin B; SI, syncytia-inducing; TCID<sub>50</sub>, tissue culture infections dose 50%.

of virus phenotype during the initial viremia before seroconversion (10).

To study the cellular mechanisms involved in the earliest events in transmission, we have studied HIV-1 infection of skin explants. During *in vitro* culture of murine skin or ear halves (16) and human (17–19) split thickness skin, DCs migrate rapidly from the skin explants. We used this culture system as a model for the uptake of HIV-1 by DCs that are migrating from body surfaces *in vivo*, especially such surfaces as the vagina, cervix, and anal canal that are histologically so similar to skin, including their content of DCs. Isolates of HIV-1 that are macrophage tropic/non-SI (NSI; HIV-1<sub>Ba-L</sub>, HIV-1<sub>676</sub>) or T cell line tropic/SI (HIV-1<sub>228</sub>, HIV-1<sub>NL4.3</sub>) were added to these explants in two different ways: directly to the abraded surface of the epidermis to restrict the exposure to the epidermis, or to the culture medium allowing both dermal and epidermal exposure. We also studied skin that had been separated after virus exposure into epidermal and dermal components to determine which components of skin might be exerting selection pressure on viral phenotype.

## Materials and Methods

**Virus Culture and Infection.** Laboratory strains HIV-1<sub>Ba-L</sub> (macrophage tropic, NSI in MT-2 cells) and HIV-1<sub>NL4.3</sub> (SI in MT-2 cells) and low passage patient isolates HIV-1<sub>228</sub> (SI) and HIV-1<sub>676</sub> (macrophage tropic, NSI) were grown in PBMCs stimulated by staphylococcus enterotoxin B (SEB; 40 ng/ml; Sigma Chemical Co., St. Louis, MO). Virus stocks were filtered (0.2- $\mu$ m filter; Schleicher and Schuell, Keene, NH) and stored at  $-70^{\circ}\text{C}$  before use. HIV-1 was treated with RNase-free DNase I (50 U/ml; Boehringer Mannheim GmbH, Mannheim, Germany) for 15 min at room temperature. Tropism of the isolates was confirmed by infection of MT-2 and monocyte-derived macrophages. Infections with HIV-1 were performed overnight (16–18 h) using 10,000 TCID<sub>50</sub> (tissue culture infectious dose 50%) as determined by infection of activated PBMCs.

**Virus Infection of Skin Explants.** Split thickness skin was prepared from the skin from healthy donors undergoing corrective surgery of breast or abdomen. The skin was stored at  $4^{\circ}\text{C}$  and used within 6 h of collection. Skin was incubated in RPMI 1640 with gentamicin (250  $\mu\text{g}/\text{ml}$ ) for 1 h before abrading the epidermal surface with a sterile wire brush or scalpel to disrupt or remove the corneal layer. Skin explants were prepared by cutting the abraded skin into pieces  $\sim 2\text{ cm} \times 2\text{ cm}$ . Virus was added to the epidermal surface of the skin after applying sterile petroleum jelly to the edges of the skin to produce a hydrophobic barrier confining the inoculum to the epidermis. After overnight virus exposure, the virus was aspirated from the skin surface and the skin within the reservoir was washed by repeated addition and aspiration of PBS (0.5–1 ml, 3–5 min between washes). In other experiments, virus was added directly to 5 ml of culture medium (RPMI 1640 supplemented with 10% fetal bovine serum (Commonwealth Serum Laboratories, Melbourne, Victoria, Australia), glutamine (2 mM), gentamicin (25  $\mu\text{g}/\text{ml}$ ), and Hepes (10 mM)). Infections were performed using 10,000 TCID<sub>50</sub> HIV-1, except as indicated. Virus was removed the next day (16–18 h after inoculation) by washing the skin three times with PBS without  $\text{Mg}^{2+}$  or  $\text{Ca}^{2+}$ . The skin was either returned directly to culture using fresh medium or treated with dispase before culture.

**Preparation of Epidermal Sheets.** The protocol used for dispase treatment was modified from the method described by Lenz et al. (17). Skin explants were washed three times with PBS to remove residual virus. The skin was incubated with dispase II (5–10 mg/ml; Boehringer Mannheim GmbH) in RPMI 1640 at  $4^{\circ}\text{C}$  with the dermal side facing up. After 4–6 h, the skin was washed three times with PBS to remove dispase, and using fine forceps, the epidermis was separated from the dermis and both layers washed in PBS. Epidermal and dermal sheets were floated on 5 ml of culture medium in 6-well plates for 3–4 d to allow migration of cells from the separated sheets. Cells were harvested from the medium after collagenase treatment (1 mg/ml collagenase type 2, for 2 h at  $37^{\circ}\text{C}$ ; Worthington, Lakewood, NJ) and washed before adding to T cells in coculture.

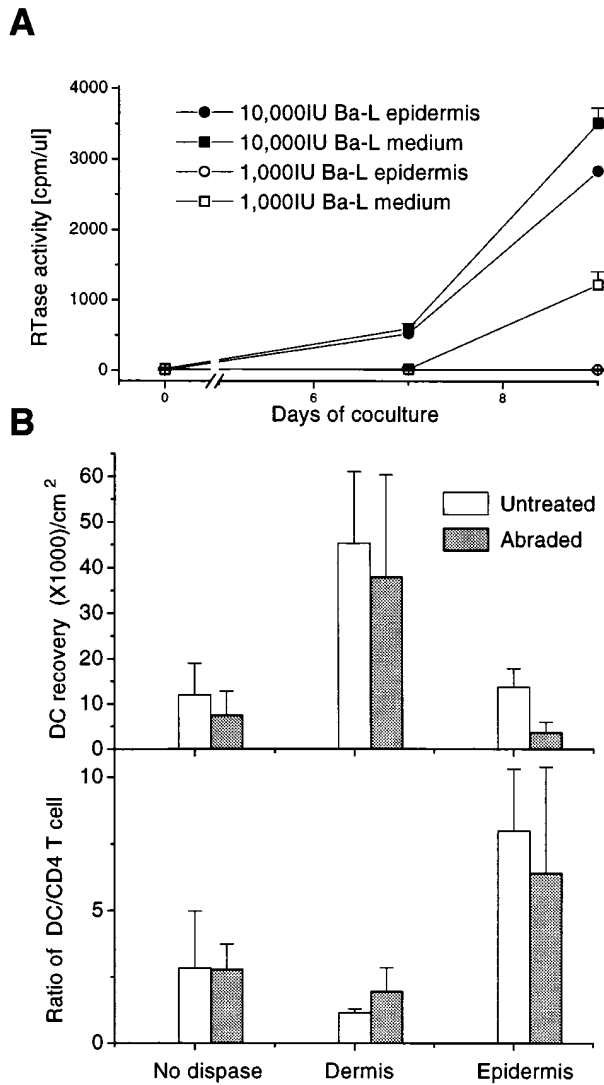
**Coculture of Emigrant Cells and DCs with T Cells.** After 3–4 d, the skin explants (total area: 3–4 cm<sup>2</sup>) were removed from each well and collagenase was added to the medium (1 mg/ml cultured for 2 h at  $37^{\circ}\text{C}$ ). The emigrant cells from each skin explant were pelleted, resuspended in fresh medium, and divided between wells containing  $10^6$  resting allogeneic PBMCs or activated PBMCs (activated for 3 d with 40 ng/ml SEB or 10  $\mu\text{g}/\text{ml}$  PHA) in 24-well microtiter plates. The small area of skin exposed to virus and the low numbers of cells emigrating from each explant made enumeration of DC numbers difficult. In most experiments, the number of DCs migrating from each individual skin explant was not directly determined and no correction was made for variation in the number of emigrant cells. Comparisons between different virus isolates was made using skin from the same donor and were standardized by area of skin explants, the skin area exposed to HIV-1, and the TCID<sub>50</sub> of the virus inocula.

**Quantitation of Dendritic Cell Migration from Skin Explants.** Half of the skin explants were abraded and half left intact. Skin explants were exposed to different HIV-1 isolates by adding virus to the culture medium. After overnight exposure, the skin was washed, dispase treated, and epidermal and dermal sheets prepared. The emigrant cells were counted and emigrants labeled with direct antibodies against HLA-DR (HLA-DR PerCP or HLA-DR-FITC; Becton Dickinson, San Jose, CA), CD4 (leu3a FITC; Becton Dickinson), and CD8 (leu2a PE; Becton Dickinson) or CD3 (CD3-PE, Becton Dickinson). Cells were fixed and analyzed on a FACSort<sup>®</sup> (Becton Dickinson). The area of epidermal sheets was determined after harvesting the emigrants using a 2-mm grid overlay.

**DC Purification from Skin.** Emigrant cells were treated with 1 mg/ml collagenase for 2 h at  $37^{\circ}\text{C}$  and spun over a 1.040/1.070 g/ml step gradient of isoosmotic metrizamide (Nycomed, Oslo, Norway) to remove nonviable cells and most keratinocytes. Cells at the 1.040/1.070 interface were stained with FITC-conjugated anti-HLA-DR (Becton Dickinson) and PE-conjugated anti-CD3 (Becton Dickinson), and sorted using a closed cell sorter (FACS<sup>®</sup> Calibur; Becton Dickinson). The DCs were selected by size, high DR expression, and absence of CD3 expression.

**Reverse Transcriptase Assay.** Supernatants were collected from cultures every second day and stored at  $-20^{\circ}\text{C}$  until batch processed. The supernatants were assayed for reverse transcriptase activity as previously reported (20). Filters were counted using a micro-beta counter with cross-talk correction (Wallac, Turku, Finland) after applying Meltilex scintillant (Wallac).

**Semiquantitative PCR.** PCR was performed using *gag*-specific primers (SK38 and SK39) and HLA-DQ primers as controls for cell number and amplification efficiency by including in a multiplex amplification (primers: GH26 and GH27) as previously described (20).



**Figure 1.** Virus dose for infection and phenotype of emigrant cells from skin explants. (A) HIV-1<sub>Ba-L</sub> was added to the abraded surface of skin (*epidermis*) or to the culture medium (*medium*) at high (10,000 TCID<sub>50</sub>, *closed symbols*) or at low (1,000 TCID<sub>50</sub>, *open symbols*) concentrations. After overnight exposure, the explants were washed and returned to culture for 3 d. The emigrants were divided equally into triplicate cocultures with allogeneic T cells (see Materials and Methods). Reverse transcriptase activity was measured at 0, 7, and 9 d of culture. Transmission to T cells occurred after addition of 10,000 TCID<sub>50</sub>, but not 1,000 TCID<sub>50</sub>, of HIV-1 to the epidermal surface. Transmission occurred at both virus doses when exposure was via the culture medium. (B) Recovery and phenotype of cells from skin explants and disperse-treated skin. Skin explants with and without epidermal abrasion were cultured and the emigrant cells from skin explants and from the separated epidermal and dermal sheets enumerated and phenotype determined by flow cytometry. The areas of the skin explants and epidermal sheets were measured after culture using a 2-mm grid and the data plotted for cell recovery from triplicate cultures of 8–13 cm<sup>2</sup> of skin. Results are representative of three similar experiments.

## Results

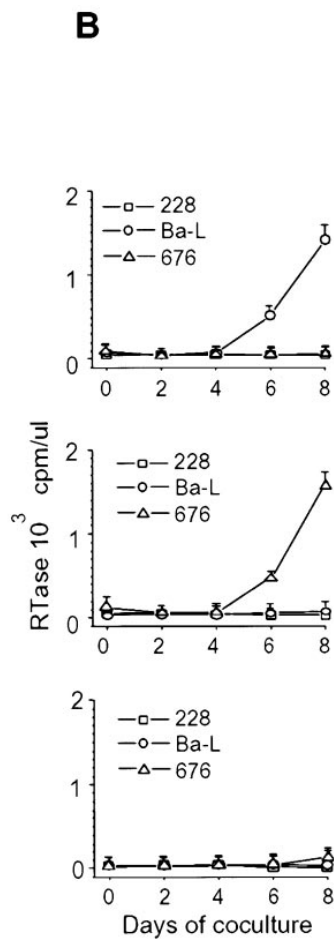
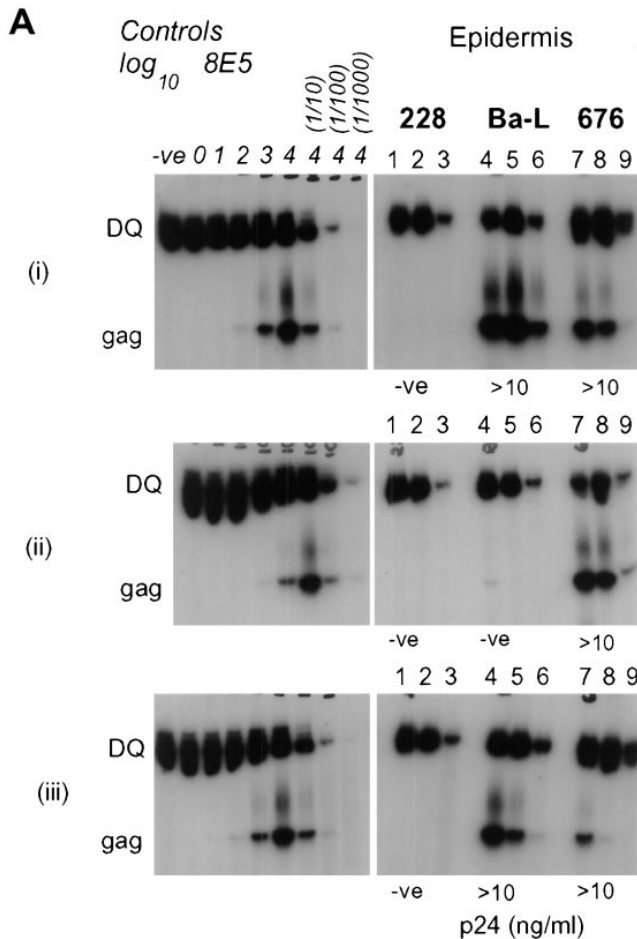
Different HIV-1 isolates of both NSI and SI phenotypes were carefully applied to the surface of abraded skin explants using petroleum jelly as a barrier to confine the virus to the epidermis. After 16–18 h, the virus was removed and

the skin was recultured for 3 d. The DC-enriched emigrating cells were harvested and cocultured with resting or activated allogeneic T cells. Virus production was measured in cocultures by reverse transcriptase activity in the supernatant or by semiquantitative PCR for HIV-1 *gag* sequences in the cocultured cells. In this system, some disruption of the corneal layer of the epidermis was necessary for infection, as no virus was recovered from the migrating cells when virus was applied to the surface of nonabraded skin (data not shown). A high number of infectious particles needed to be applied to the epidermal surface to recover virus from the emigrant cells during cocultures (Fig. 1 A). The transfer of infectious virus across the abraded epidermis was inefficient, as no virus transfer was demonstrated after the virus dose applied to the skin surface was reduced from 10,000 to 1,000 TCID<sub>50</sub>. The higher concentration of virus 10,000 TCID<sub>50</sub> was routinely used for infections.

Quantitatively, the recovery of DCs from the skin explants was similar to that obtained by others (17). There was a consistent reduction in cells migrating from the epidermal sheets prepared from abraded skin, but there was no significant difference in the DC numbers in the emigrants from dermis or abraded but otherwise untreated split skin (Fig. 1 B). By flow cytometric analysis, a higher ratio of DCs to CD4 T cells was found in epidermal emigrants, but abrasion of the epidermal surface had no detectable effect on the phenotype of the emigrant cells. Similarly virus exposure did not affect the recovery of DCs migrating from skin. In three experiments where total emigrant cell recovery, emigrant phenotype, and skin area were determined, the combined results for mean DC recovery from epidermal sheets ( $\pm$ SE) were 10,503 (3,294), 14,375 (7,825), 12,555 (8,493) cells/cm<sup>2</sup> for explants not exposed to HIV-1, exposed to T cell tropic (NL4.3), or exposed to macrophage tropic virus (ADA), respectively. The results for abraded skin explants exposed to the same viruses were 5,776 (3,508), 5,719 (2,197), and 5,260 (1,792).

Comparison of the virus recovery after application of different virus isolates to the epidermal surface showed selection of macrophage tropic virus during transmission across the skin explants (Fig. 2). Reverse transcriptase activity was present in the supernatants and provirus was detected in the cells of the cocultures containing cells migrating from skin exposed to macrophage tropic virus (Ba-L and 676; Fig. 2). The cells migrating from skin exposed to T cell line tropic isolates did not transmit virus (228; Fig. 2).

Next, we examined selection of different strains of HIV-1 by adding virus to the epidermal surface or directly to the medium in which the abraded skin explants were cultured. Addition of the virus to the medium allowed HIV-1 exposure of the cells mobilized within the dermis and is comparable to previous studies that have harvested the emigrant cells and used these cells as target populations (21–24). Our results were similar to previous studies using isolated emigrant cells exposed to HIV-1 in that there was transmission of all virus strains both macrophage and T cell tropic, from the HIV-1-exposed dermal emigrants to T cells. There was a clear difference in the pattern of virus transfer from emi-

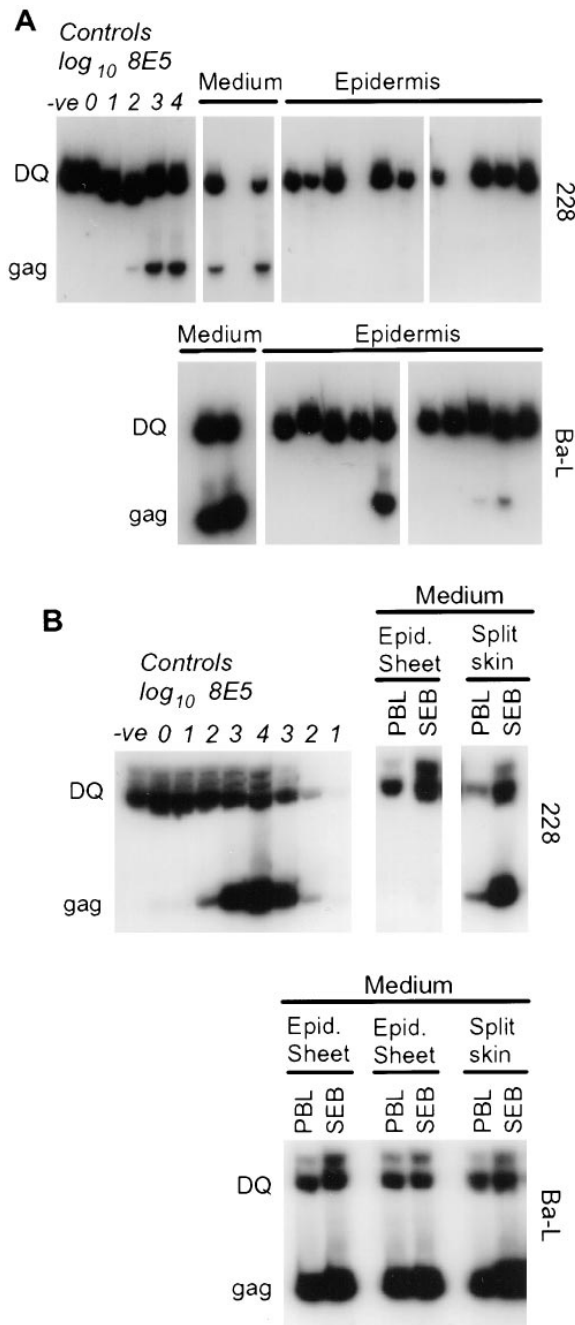


**Figure 2.** After epidermal exposure, macrophage tropic, but not T cell line tropic, HIV-1 is carried by the emigrant cells from the skin explants. (A) Skin explants were prepared from skin after abrading the epidermal surface (see Materials and Methods). 10,000 TCID<sub>50</sub> of virus was added to a contained area of the epidermal surface (*Epidermis*) of skin in 100  $\mu$ l of medium and incubated overnight at 37°C. The T cell tropic viral isolate HIV-1<sub>228</sub> (lanes 1–3) and macrophage tropic isolates HIV-1<sub>Ba-L</sub> (lanes 4–6) and HIV-1<sub>676</sub> (lanes 7–9) are shown. The epidermal surface was washed free of virus and floated on 5 ml of medium for 3 d at 37°C. The emigrant cells were washed and cocultured in 24-well plates with 10<sup>6</sup> unstimulated peripheral blood lymphocytes. At day 8, coculture cells were lysed and analyzed by semiquantitative PCR at three dilutions (lanes 1, 4, and 7: neat lysate; lanes 2, 5, and 8: 1/10 lysate; lanes 3, 6, and 9: 1/100 lysate). PCR controls were the latently infected cell line 8E5 diluted in uninfected 10<sup>5</sup> PBLs; 4, 10<sup>4</sup>; 3, 10<sup>3</sup>; 2, 10<sup>2</sup>; 1, 10<sup>1</sup>; 0, 10<sup>0</sup>; and -ve, 0 8E5 cells/reaction. HIV-1 p24 antigen concentrations in culture supernatants collected on day 8 were measured by enzyme-linked immuno-

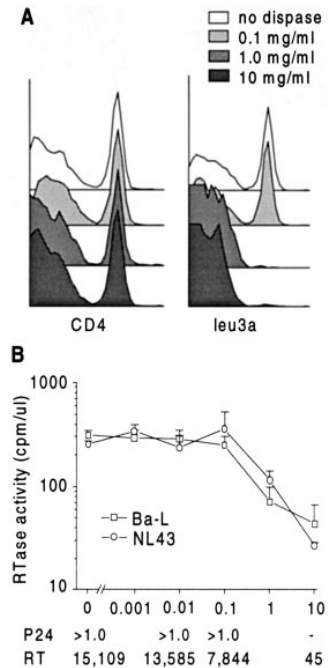
absorbant assay (HIV EIA; Abbott, Abbott Park, IL). (B) Viral replication in cocultures was detected by RTase activity in culture supernatants taken on days 0, 2, 4, 6, and 8. Results shown are representative of four separate experiments.

grant cells to T cells after epidermal virus exposure, compared with simultaneous epidermal and dermal exposure (Fig. 3 A). The cells migrating from dermis exposed to virus, transferred all of the virus isolates we tested whether they were T cell line (228; Fig. 3 A) or macrophage tropic (Ba-L; Fig. 3 A). For similar viral inocula added to the epidermal surface of the skin (Fig. 3 A, *Epidermis*), macrophage but not T cell line tropic virus was recovered from emigrant cells. Not all cocultures showed transmission of macrophage tropic virus to T cells, indicating that only a small proportion of the cells migrating from the skin explants carried infectious virus. Activated as well as resting T cells were used to maximize the amplification of virus carried by the emigrant cells. As expected from previous work with blood DCs (20, 25) coculture with mitogen-activated cells rather than allogeneic cells resulted in more frequent recovery of virus during coculture (Fig. 3 B, see also Fig. 5). Under conditions optimal for recovery of emigrant cells from the epidermis, where the dermis was removed after epidermal virus exposure and the emigrant cells from the epidermis examined for virus carriage, T cell line tropic virus was not transferred by the emigrant cells (Fig. 3 B).

These observations implicated cells in the epidermis as the selectors for macrophage tropic virus. To test this further, HIV-1 was added to the epidermal surface (epidermis) or to the entire abraded skin explants (medium), and after culture, the dermal and epidermal layers were separated by dispase treatment. The effect of dispase digestion at 4°C on free virus and activated PBMCs was tested. Viral p24 was reduced and reverse transcriptase undetectable in dispase-treated virus and dispase treatment markedly reduced the infectivity of virus stocks and the expression of the leu3a epitope of CD4 that is expressed on T cells and is involved in viral entry (Fig. 4). The dispase treatment during separation of dermis and epidermis is, therefore, likely to markedly reduce any subsequent infection of the migrating cells through an effect on the target cells and probably also on virus infectivity. After epidermal virus exposure, HIV-1 was not recovered from the dermal sheets if the separation was performed within 18 h, indicating some delay in the movement into the dermis of cells carrying virus from the site of HIV-1 exposure in the superficial epidermis. In abraded skin, explants exposed to virus by adding virus to the culture medium (Fig. 5, *Medium*) before dispase treatment, both



**Figure 3.** Epidermal, but not dermal, exposure to HIV-1 results in selective carriage of macrophage tropic virus by emigrant cells. (A) Culture conditions were as described in Fig. 2. HIV-1<sub>228</sub> or HIV-1<sub>Ba-L</sub> (10,000 TCID<sub>50</sub>) was added directly to the underlying culture medium (Medium) and to the surface (Epidermis) of abraded skin explants (3–4 cm<sup>2</sup>). After 16 h, the virus was removed and the skin placed back in culture for 4 d. Migrating cells were treated with collagenase, washed, and added to 10<sup>6</sup> unstimulated PBLs. On day 10, coculture cells were lysed and analyzed for gag sequences by semiquantitative PCR. Results are for nine replicate skin explants exposed to 228 and 10 exposed to Ba-L via epidermis, and for duplicate cultures exposed via the culture medium. (B) Similar culture conditions to A. Abraded skin explants were exposed to 10,000 TCID<sub>50</sub> of HIV-1<sub>Ba-L</sub> or HIV-1<sub>228</sub> by adding virus to the culture medium (Medium). After 16 h, the explants were washed and half were left as explants and half were treated with dispase to separate epidermal and dermal layers. Skin explants (Split skin) and epidermal sheets (Epid. Sheet) were placed back

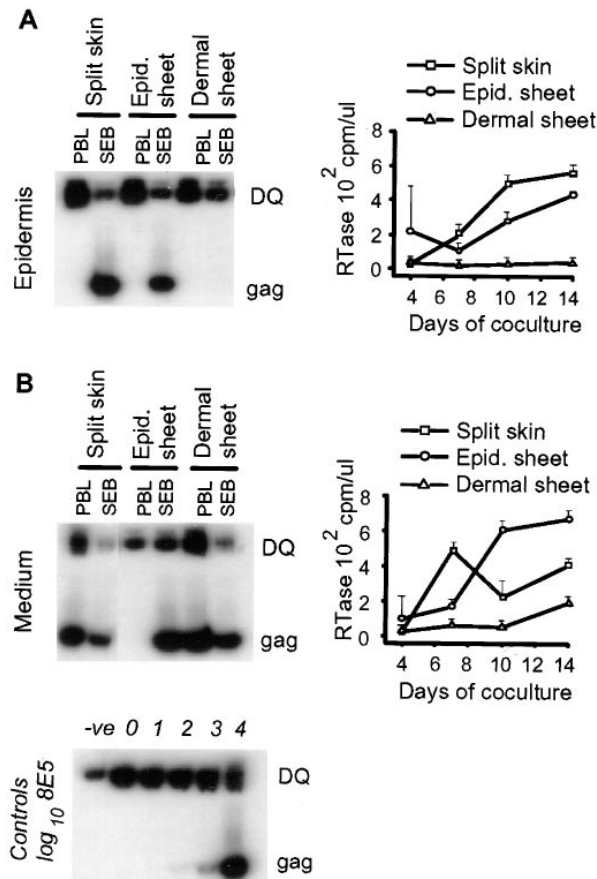


**Figure 4.** Effects of dispase on HIV and target cells. (A) CD4 T cells from peripheral blood were cultured for 2 h at 4°C in dispase concentrations between 0.1 and 10 mg/ml. Cells were washed and labeled with CD4 (OKT4) and leu3a. The leu3a, but not OKT4 (CD4), epitope of the CD4 receptor recognized by HIV-1 was removed at concentrations of 1 mg/ml or greater. (B) Virus production from SEB (40 ng/ml)-stimulated PBLs was reduced when dispase-treated virus was used to infect the cells. Dispace treatment for 2 h at 4°C at concentrations of dispase >0.1 mg/ml removed RTase activity (RT cpm/μl) and p24 (ng/ml) activity from the HIV-1<sub>Ba-L</sub> virus stocks.

the epidermal and dermal emigrants carried macrophage tropic HIV-1.

The differences in the ability of emigrant cells from dermis and epidermis to carry T cell line tropic virus may be due to differences in phenotype of the DCs in the dermis and epidermis (17), to the different composition of the emigrant cells from the two skin layers, or to exposure of DCs to HIV-1 at different stages of cellular maturation. We found emigrants from dermis and epidermis differed in the proportion of T cells and DC-T cell conjugates (Figs. 1, and 6 A). In a series of seven experiments in which the emigrant cells were sorted, analysis of the low density dermal emigrant cells showed a mean (±SD) of 13.2 (11.2)% CD3<sup>+</sup> T cells, 9.7 (15.2)% DC-T cell conjugates, and 9.9 (7.0)% DCs compared with 3.2 (3.6)%, 1.5 (2.3)%, and 5.2 (4.4)%, respectively, for epidermis. A higher proportion of DCs in the epidermis were found as single unconjugated DCs (70% compared with 50% for dermal DCs), but by flow cytometric analysis there was no significant difference in the distribution of these populations in skin after exposure to T cell or macrophage tropic virus. To confirm that DCs rather than other cell populations were responsible for virus selection and carriage, DCs from the dermis and epidermis were sorted by flow cytometry and added to allogeneic PBMCs in limiting dilution. The T cell line tropic virus isolate HIV-1<sub>NL4.3</sub> (Fig. 6 B) was recovered during coculture from dermal, but not from epidermal, DCs. In contrast, macrophage tropic virus HIV-1<sub>Ba-L</sub> (Fig. 6 B) was recovered

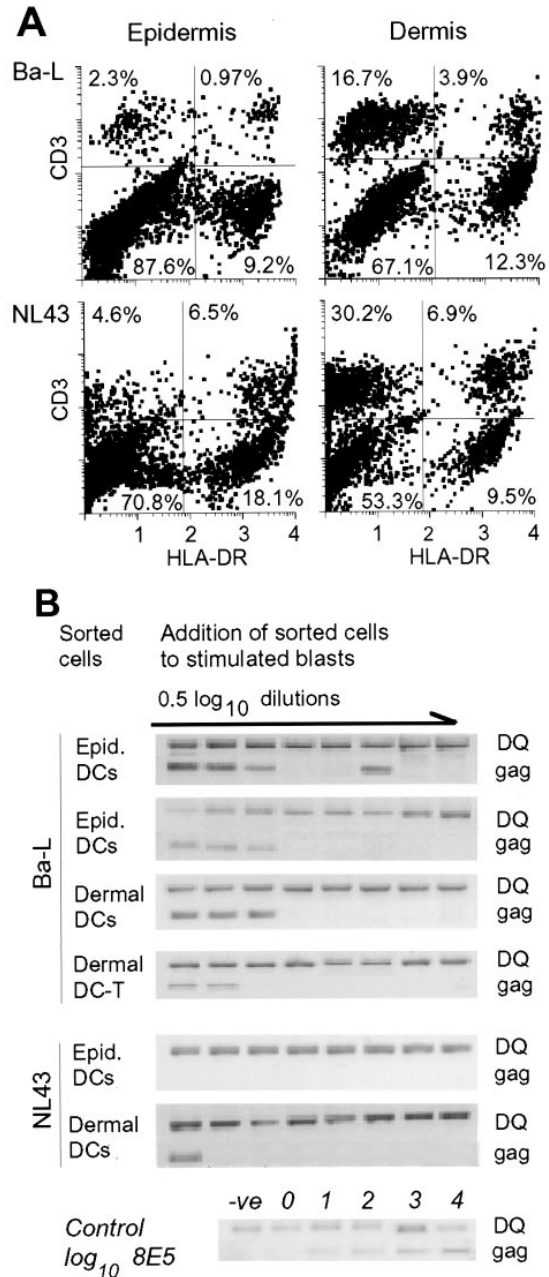
in culture. Emigrating cells were harvested on day 4 and cocultured with 10<sup>6</sup>/well unstimulated or SEB (40 ng/ml)-stimulated PBLs. PCR for HLA-DQ and gag sequences were performed on lysates of cocultures on day 10.



**Figure 5.** Emigrant cells from epidermal, but not dermal, sheets selectively carry macrophage tropic virus. Abraded skin explants were exposed to 10,000 TCID<sub>50</sub> of HIV-1<sub>Ba-L</sub> by adding virus to the culture medium. After overnight incubation, the virus was removed, half the treated skin explants were left intact, and the remainder were treated with dispase to enable the epidermis to be separated from the dermis. (A) The skin explants (*Split skin*), and epidermal and dermal sheets were cultured by floating on medium in 6-well plates. After 4 d, migrating cells from the individual wells were harvested and added to unstimulated (*PBL*) and stimulated PBLs ( $10^6$  SEB activated blasts/well) in IL-2 supplemented medium. Cells were lysed on day 14 and analyzed for HIV-1 *gag* sequences by PCR. (B) Virus production in cocultures of stimulated PBLs and emigrant cells from epidermis and dermis. HIV-1<sub>Ba-L</sub> was added only to epidermal surface (*top*) and dispase treated after the overnight virus pulse. Virus production was measured by RTase in culture supernatants from intact skin explants (*Split skin*), epidermal sheets (*Epid. sheet*), and the dermal sheets (*Dermal sheet*) and plotted against time in culture.

from both the sorted epidermal and dermal DCs at a frequency higher than from the dermal DC-T cell conjugates. In keeping with the results obtained using bulk emigrant cell populations, only a small proportion of sorted DCs carried virus. After exposure to macrophage tropic virus <0.5% of the DCs sorted from the cells emigrating from the epidermis could transmit infection to T cells (Fig. 6 B).

**Figure 6.** Dendritic cells in the epidermis select for macrophage tropic virus. Abraded skin explants were pulsed with 1,000 TCID<sub>50</sub>/ml (~5,000 TCID<sub>50</sub>) of HIV-1<sub>Ba-L</sub> by adding virus to the medium overnight at 37°C.



After dispase treatment, epidermal and dermal sheets were floated in medium for 3 d. Migrating cells were treated with collagenase and separated over a step metrizamide gradient, stained with FITC-HLA-DR and PE-CD3, and sorted for DCs using a cell sorter. (A) As previously shown (17-19), cells migrating from the dermis contained DCs, T cells, and DC-T cell conjugates, whereas cells from the epidermis contained DCs and DC-T cell conjugates, but few CD3<sup>+</sup> cells. (B) Sorted cells were analyzed for their ability to transmit infection by diluting into coculture with T cells. Half log<sub>10</sub> dilutions of the sorted emigrant cells ( $10^4$ ) were performed in 96-well plates with SEB-stimulated PBLs ( $10^5$ /well) in medium containing 5% IL-2. Virus replication in cocultures was assessed by lysing cocultures on day 10 and analyzing for *gag* sequences using PCR. At the highest cell concentration, the PCR reactions contained the equivalent of 2,500 sorted cells. Duplicate dilution series for DCs sorted from epidermal emigrants and DCs, and DC-T cell conjugates from dermal emigrants from Ba-L exposed skin are shown. Dilution series for DCs sorted from epidermis and dermis of NL43 exposed skin are shown in the lower two panels.

## Discussion

Although HIV-1 macrophage tropism has been described in terms of the ability to infect macrophages, it is not clear that this is due to viral targeting of monocytes or macrophages *in vivo*. Macrophage tropism of HIV-1 isolates is measured using monocyte-derived macrophages, usually after 5–7 d of culture. Different HIV-1 isolates vary widely in their ability to use CCR5 and enter peripheral blood-derived macrophages (26, 27) or tissue macrophages, but entry into freshly isolated monocytes is blocked before reverse transcription (28) and viral load in macrophages from lymphoid tissue is low (29). A requirement for infection of macrophages for the entry and subsequent expansion of HIV-1 has nevertheless been inferred from the observation of initial infection with macrophage tropic virus (11). Infection of macrophages in uterine cervical tissue explants by macrophage tropic, but not by nonmacrophage tropic, HIV-1 isolates has been found (30). Recent data on coreceptor usage by macrophage tropic virus, however, suggests macrophages may not be strong selectors for CCR5 usage, as they allow entry by a range of coreceptors (31). Although primary infection with virus of SI phenotype has been reported (32–34) subjects homozygous for a deletion within CCR5, but with intact CCR2b, CCR3, and CXCR4 genes have nearly complete protection from all HIV-1 infection (35, 36) arguing both for dual tropism of any SI isolates found in primary infection and for infection of cells that select for viral usage of CCR5 as an early event in the establishment of HIV-1. DCs have been considered important in carriage of virus from skin to the lymph node during transmission of HIV-1 and have been implicated as the cells carrying virus from the skin or mucosa to lymph nodes in primate models (37, 38), but their role in selecting for macrophage tropism or CCR5 coreceptor usage has so far been unclear.

Cultured blood and skin DCs efficiently activate T cells and induce productive infection (22, 25, 39), even when only low levels of infection are present in the DCs (21). The emigrant cells from skin explants may also allow virus production in migrating DC–T cell conjugates in the absence of cognate interactions or T cell activation (22, 40). The formation of such DC–T cell conjugates during migration from skin may allow efficient transfer of virus from Langerhans cells and be critical in the initial transfer of virus to responding T cells. The observation of DC-derived syncytia in the T cell-rich areas near the crypts of the oropharyngeal lymphoid tissue (41) also implies that the formation of DC–T cell conjugates represents sites of virus production *in vivo*. DCs clearly can provide an efficient pathway for introducing infection to T cells. Any virus selection by DCs during this process would result in predominance of “DC tropic” viral phenotypes during the subsequent viremia.

Infection of cultured blood DCs *in vitro* has up to now not shown selective infection of DCs by virus with a specific biological phenotype (25, 42, 43). Monocyte-derived DCs (23, 44), DCs cultured from precursors (43), or emigrant DCs from skin (21–24) show little (45) or no selectivity of viral entry. Both emigrant DCs from skin and monocyte-derived DCs express CXCR4 and CCR5 receptors (23, 43). In contrast, we have now shown selective transmission of macrophage tropic virus by the emigrants from the epidermis.

A differential effect of HIV-1 on DC migration such that T cell tropic virus, but not macrophage tropic HIV-1-inhibited DC migration, could result in such preferential carriage of macrophage tropic virus. We did not observe this in the dermis where dermal DCs, including sorted DCs, could carry both viral phenotypes. If this mechanism operates, it must be specific for Langerhans cells.

DC maturation could account for differences between our observations and the previously reported nonselective HIV-1 transmission by DCs and the transmission of all isolates by dermal DCs. The resident immature epidermal DCs are likely to be the cells initially exposed to virus during epidermal exposure. The DCs themselves carried HIV-1 since the DCs sorted to exclude T cells and DC–T cell conjugates were at least as effective as the highly susceptible DC–T cell conjugates in transmitting virus to allogeneic T cells. All strains of HIV-1 probably bind to the mature and immature DCs (40), and differences in carriage by epidermal DCs *in situ* compared with the cultured DCs and dermal DCs may reflect more efficient antigen uptake and processing pathways in the immature DCs (46, 47). Virus carriage without infection may be important in DC–T cell transmission from mature DCs (43, 48), but whether this mechanism of carriage is present in immature DCs is unclear. We hypothesize that virus that binds to immature DCs but lacks the ability to bind or enter by CCR5 may reach the degradative pathways and be destroyed before cognate interactions and infection of T cells can occur. Introduction of virus into the dermis where more mature emigrating DCs expressing the alternative coreceptors are present may allow infection of activated dermal DCs or DC–T cell conjugates. This will partially abrogate the requirement for CCR5 usage and allow infection with a wide range of viral phenotypes. It may also contribute to the infrequent infection of subjects homozygous for the CCR5 deletion (49). During transmission and viral entry, a requirement for HIV-1 to infect resident DCs, rather than a requirement to infect macrophages, may be critical in the selection of the macrophage tropic virus that can efficiently use the CCR5 coreceptor.

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