should be acknowledged that TNF-α itself may contribute independently to the GC response.

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8. Mice were immunized intraperitoneally with 100 μl of phosphate-buffered saline containing 10% SRBCs (*22*). Ten days later, spleens were harvested, embedded in O.C.T. compound (Miles), and frozen in liquid nitrogen. Frozen tissue sections (6 to 10 μm thick) were fixed in cold acetone. Endogenous peroxidase was quenched with 0.2% H_2O_2 in methanol. Sections were stained by first incubating with PNA-biotin (Vector) and with rat antiserum produced to IgD (Southern Biotechnology). After washing, the sections were further incubated with streptavidin conjugated with alkaline phosphatase (AP) (Zymed) and rabbit antibody to rat IgG conjugated with horseradish peroxidase (HRP) (Southern Biotechnology). Color development for bound AP and HRP was with an AP reaction kit (Vector) and with diaminobenzidine. Sections were then counterstained with 1% methyl green.
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HIV-1 Langerhans' Cell Tropism Associated with Heterosexual Transmission of HIV

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Heterosexual transmission by vaginal intercourse accounts for most transmission of human immunodeficiency virus–type 1 (HIV-1) in Africa and Asia but is less important in the HIV-1 epidemics of the United States and Western Europe. Epithelial Langerhans' cells (LCs) represent a possible source of initial cell contact for vaginal infection. Fifteen primary isolates of HIV-1 from U.S. homosexuals and 18 HIV-1 isolates from Thailand heterosexuals were evaluated for growth in LCs of U.S. origin. All the viruses from the Thai heterosexuals, which were subtype E, grew more efficiently in the LCs than any of the viruses from the U.S. homosexuals, which are subtype B. These results suggest that LC tropism is associated with the efficiency of heterosexual transmission of HIV.

In Thailand, India, and sub-Saharan Africa, it has been estimated that 90% of HIV-1 infections are acquired through heterosexual exposure (*1*, *2*). In contrast, most transmissions of HIV in the United States and Western Europe are associated with anal intercourse among homosexual men or injection drug use (IDU), with less than 10% of infections attributed to heterosexual contact (*3*). HIV-1 subtypes or clades have been classified according to HIV envelope and *gag* gene sequences (*4*). HIV-1 subtypes A through I have been described (*2*, *5*), and subtypes A through E have been characterized for geographical distribution, with A, C, and D dominating in sub-Saharan Africa, C dominating in India, and E dominating in Thailand (*2*, *5*). HIV-1 B is the dominant subtype reported thus far from the United States or Western Europe (*4*). HIV-1 B has also been isolated occasionally in Africa, Thailand, and India but is unusual there except in individuals infected through IDU (*2*, *6*). HIV-1 B is also the dominant subtype in the Caribbean and in South America, where heterosexual transmission is more common than in the United States but less common than in Africa or Thailand (*1*). Factors known to alter the efficiency of heterosexual transmission include other venereal diseases, condom use, male circumcision, and anal intercourse (*7*).

The uneven expansion of HIV-1 subtypes raises the possibility that some viruses have less potential for heterosexual transmission in comparison to others. Such enhanced susceptibility of individuals in particular risk groups has been corroborated in Thailand by comparison of heterosexual transmission of HIV-1 subtypes between couples (*8*). For heterosexual contact, Langerhans' cells (LCs) have been proposed as a possible primary target for HIV infection (*9*–*11*). These cells, which express CD4 on their membranes, are located on the surfaces of the oral and genital mucosa and are particularly abundant in the cervix, but are absent from rectal mucosa (*12*). To evaluate LC infectivity in relation to heterosexual transmission, we inoculated primary LC cultures with primary HIV-1 isolates from U.S.

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homosexuals and Thai heterosexuals. The LCs were extracted from normal human skin and purified by density gradient centrifugation (*13*) with some modifications (*14*). The purity of the LC fraction ($CD1a^+$ cells) averaged 91% (range, 89 to 92%) without contamination of $CD3^+$ lymphocytes or $CD14^+$ monocytes by direct immunofluorescence.

We obtained isolates of HIV-1 from 14 homosexual men at the Fenway Community Health Center (Boston, Massachusetts, USA), from 11 heterosexuals at the Chiang-Mai University Hospital in northern Thailand, and from 7 heterosexuals from Bangkok; all of the heterosexuals from Thailand had been infected with HIV through heterosexual contact. Five of the 18 Thai viruses were from patients with AIDS (acquired immunodeficiency syndrome) at CDC stage IV (*15*) and 13 were from patients who were asymptomatic or had limited clinical progression. None of the 15 U.S. viruses were from patients with CDC stage IV disease (Table 1).

Table 1. Phenotypic analysis of primary HIV-1 isolates from homosexuals in the United States and heterosexuals in Thailand. The p24 values are from day 21 after virus infection. Subtype was determined by nucleotide sequencing of envelope gp120 (*16*). NA, not available.

Virus sample	Subtype	p24 (ng/ml) values for	
		PBMCs	LCs
Isolates of U.S. homosexuals			
1	B	10.2	1.8
2	B	9.7	0.8
3	B	5.4	1.0
4	B	6.4	2.0
5	B	7.6	5.2
6*	B	3.3	3.1
7	NA	6.4	2.8
8	B	6.6	1.7
9	B	6.6	3.2
10	B	4.0	1.1
11	B	8.9	1.2
12	B	5.3	2.9
13	B	8.9	1.0
14	B	8.0	0.9
15	NA	4.4	6.2
Isolates of Thai heterosexuals			
16	E	6.2	13.3
17	E	4.3	20.5
18	E	10.4	14.1
19	E	5.2	8.8
20	E	5.4	13.9
21	E	4.0	10.6
22	E	9.2	14.9
23	E	9.2	14.9
24	E	3.4	15.8
25	E	2.7	21.1
26	E	5.6	15.6
27	E	7.0	17.2
28	E	4.5	22.6
29	E	8.8	17.6
30	E	5.7	14.2
31	E	4.0	13.7
32	E	3.8	22.1
33	E	4.8	18.3

*This virus was isolated 5 years later from the same patient as virus sample 4.

To determine the virus subtype, we sequenced the envelope gp120 of all of the Thai viruses and of 13 of the U.S. viruses as described (*16*). To evaluate their phenotypes, we inoculated all viruses into peripheral blood mononuclear cells (PBMCs) (*17*), monocyte-derived macrophages (MDMs) (*18*), and MT-2 cells (*19*). Virus replication was assessed by determination of p24 levels in the culture supernatant (*20*).

Table 1 summarizes the characteristics of each isolate and the results obtained for viral replication in LCs and PBMCs. All isolates replicated efficiently in PBMCs. Replications of the isolates in PBMCs, according to p24 levels, were not statistically different at days 7 and 21 in the two groups ($P = 0.19$ and 0.16, respectively; U–Mann Whitney), but the levels at day 14 were significantly higher for the U.S. viruses ($P = 0.001$; U–Mann Whitney) (Table 2). In contrast to the results obtained with PBMCs, the replication in LCs (Tables 1 and 2) was different for the U.S. and Thai isolates. The average p24 levels obtained at days 7, 14, and 21 were statistically higher for the Thai viruses ($P \leq 0.0005$ at all time intervals; U–Mann Whitney) (Tables 1 and 2). The Thai viruses also showed a continuous increase in viral replication during the length of the experiment. In contrast, the U.S. viruses showed a flat replication curve after day 14 with no difference in p24 levels between days 14 and 21 ($P < 0.3$). The lower replication of U.S. viruses was not related to cytopathic effects or to the death of LCs, because the average viability of LCs was comparable for all of the cultures.

The replication kinetics observed in PBMCs for all the viruses used here also diminish the possibility that an inoculum effect was responsible for the differences in replication obtained in LCs. Indeed, the U.S. viruses that showed poor replication in LCs replicated at faster, but similar, kinetics in comparison to those of subtype E viruses in PBMCs. High replication in LCs (p24 levels higher than 10,000 pg/ml) was significantly associated with the geographical origin of the HIV-1 virus ($P < 0.00001$, Fisher's exact test).

When we compared p24 values for subtype B isolates to those of subtype E isolates, the subtype E viruses showed higher replication levels in LCs at days 7, 14, and 21 ($P = 0.001$, 0.0002, and 0.0002, respectively; U–Mann Whitney), whereas the levels in PBMCs were statistically different at day 14 ($P = 0.001$; U–Mann Whitney), with higher levels for subtype B viruses. All subtype E viruses showed higher levels of replication in LCs than any of the subtype B isolates tested. In addition, virus replication in LCs was associated with the detection of intracellular p24 by protein immunoblotting.

Eight of the 15 U.S. viruses grew in monocytes, as did 11 of the 18 Thai viruses used. LC tropism was independent of macrophage tropism and of MT-2 cell growth and syncytia formation (*21*). Two HIV-1 B viruses from healthy U.S. heterosexual women and two HIV-1 B viruses from healthy Thai injection drug users were also tested for replication on LCs. These four HIV-1 viruses grew at minimal levels, within the range seen for the 15 type B viruses from the U.S. homosexual men (Table 1). Because the LCs used in this measurement were of U.S. origin, to rule out the unlikely possibility that LCs of Thai origin may be refractory to HIV infection, we also tested a subset of six viruses on LCs of Thai origin. This subset included two HIV-1 B viruses from U.S. homosexuals (Table 1, samples 1 and 2), two HIV-1 E viruses from Thai heterosexuals (Table 1, samples 28 and 32), and two HIV-1 B viruses from Thai injection drug users. The two HIV-1 E viruses also grew threefold better than any of the four HIV-1 B viruses on the LCs of Thai origin.

The LC cultures used here were from an epidermal suspension with a high content of LCs. Ten percent of the suspensions used were composed of epidermal cells, but these cells are not very susceptible to infection by HIV-1 (*11*, *22*, *23*).

Table 2. HIV-1 replication in PMBCs and LCs of U.S. origin. The columns represent the mean of p24 values obtained for 15 U.S. isolates and 18 Thai isolates plus or minus one standard deviation.

HIV origin	Average values for p24 (ng/ml) ± SD on day		
	7	14	21
	Replication in PBMCs		
USA	1.01 ± 0.45	5.8 ± 2.6	6.78 ± 2.09
Thailand	0.78 ± 0.57	2.77 ± 2.59	5.78 ± 2.25
	Replication in LCs		
USA	0.76 ± 0.79	2.7 ± 2.26	2.32 ± 1.6
Thailand	2.35 ± 0.64	9.8 ± 2.57	16.06 ± 3.78

The possibility of contamination of our preparations with monocytes or lymphocytes ($CD3^+$ and $CD14^+$) seems remote. Most T cells and macrophages reside in the dermis, and these cells migrate at different densities in the gradient used. In addition, we did not use cytokine stimulation in the LC cultures. The absence of interleukin-2, and the minimal viral replication seen with macrophage cultures for some viruses, suggest that the degree of HIV-1 replication seen in LCs cannot be explained by contamination with other cells. Finally, in agreement with previous observations, HIV-1 infection in LCs was noncytopathic and did not induce syncytia formation (*9*, *24*).

HIV-1 proviral DNA and mRNA have been detected previously in LCs from the skin of infected patients (*10*, *25*). Viral particles budding from LCs also suggest that these cells are productively infected (*10*, *24*). LCs have been proposed as a primary target cell in heterosexual transmission (*9–11*), and related supporting evidence has been presented (*26*, *27*). As a primary target cell for heterosexual infection by HIV-1, LCs offer several characteristics that are not fulfilled by other types of cells. They are located at the tissue surface where they can contact free virus from secretions directly. On the other hand, mononuclear cells are located in the dermis, so the virus would need to cross the epithelium to infect them.

The differences we observed in LC tropism between different virus subtypes could help to explain differences in the frequency of heterosexual transmission of different subtypes of HIV-1. Even though subtype B viruses transmitted heterosexually could also replicate locally in genital LCs, smaller amounts of replication might decrease the risk of B viruses being transmitted. This model fits the results obtained by Kunanusont *et al.* (*8*) in Thailand, where HIV-1 heterosexual transmission in couples where one partner is HIV^+ and the other is HIV^- was less efficient when the index case harbored a B subtype (IDU), compared to that in couples where subtype E viruses were involved. This observation, along with the large numbers of heterosexually transmitted HIV-1 infections seen in Africa and Southeast Asia related to nonclade B viruses, would support the possibility of adaptation or maintenance of some HIV-1 subtypes like E, and probably C, for heterosexual transmission. Although there is no reason to expect that classification by subtypes will necessarily parallel sexual transmission efficiency, it is possible that strains of subtype B in the United States, transmitted primarily by injection drug users sharing needles and microscopic abrasions in receptive anal sex, may have lost—or never had—viral sequences needed to be efficiently transmitted heterosexually. Although present in Asia and Africa, HIV-1 B is not associated with heterosexual epidemics there.

Differences in the rates of HIV-1 heterosexual transmission may also be attributable to sexual behavior practices and perhaps even to host genetic susceptibility. Cofactors that may increase the efficiency of heterosexual transmission of HIV include certain sexual behaviors and the presence of other sexually transmitted diseases (*7*, *28*). However, in Asia or Africa none of the factors studied have fully explained the heterosexual epidemics there (*1*).

We have described here a specific tropism of HIV-1 for LCs that correlates with the geographical distribution of the HIV isolates and with the HIV-1 subtype. Our findings support a possible role for LCs as primary targets for heterosexual infection and offer a possible explanation for the major differences seen in the epidemics of Asia and Africa, as opposed to those of the United States and Western Europe. If introduced in the West, viruses such as HIV-1 E might pose a greater threat for heterosexual transmission than does HIV-1 B.

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18. MDMs were isolated by the plastic adherence method with the use of 4×10^6 PBMCs per cubic centimeter and RPMI 1640 plus 5% (v/v) human serum and 20% fetal bovine serum. After 5 days, cells were washed twice with PBS and maintained with RPMI 1640 with 20% fetal bovine serum, 100 U/ml of penicillin, and 100 μg/ml of streptomycin.
19. MT-2 cells were obtained from the AIDS Research and Reference Reagent Program, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, USA, and were maintained at a density of $<1 \times 10^6$ cells/ml in the same media as for MDMs and PMBCs.
20. 10^5 LCs, 10^6 PBMCs, 10^6 MDMs, and 10^6 MT-2 cells were challenged with 1 ng/ml of p24 from each isolate on day 1. All the LC assays were done 24 hours after the extraction and enrichment of the cells. Assays with MDMs were started 4 days after the removal of nonadherent cells. Cell cultures were inoculated with each isolate for 18 hours at 37°C, washed with PBS, and resuspended in fresh complete media. Infections with PBMCs, MDMs, and MT-2 cells were done twice, and LC infections were done in duplicate with at least two different LC preparations. To determine p24 levels, we obtained culture supernatants by removing cells and cell debris after centrifugation at 3500 rpm for 10 min from LCs, PBMCs, and MDMs on days 7, 14, and 21. Standard dilutions were done in each sample, and the procedure was performed according to the manufacturer's protocol (NEN-Dupont Core profile enzyme-linked immunosorbent assay).
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