JOURNAL OF VIROLOGY, Oct. 1997, p. 8008–8013 Vol. 71, No. 10
0022-538X/97/$04.00+0

Langerhans Cell Tropism of Human Immunodeficiency Virus Type 1 Subtype A through F Isolates Derived from Different Transmission Groups

MATTHIAS T. DITTMAR,[1]* GRAHAM SIMMONS,[1] SAM HIBBITTS,[1] MIKE O'HARE,[2] SUDA LOUISIRIROTCHANAKUL,[3] SIMON BEDDOWS,[3] JONATHAN WEBER,[3] PAUL R. CLAPHAM,[1] AND ROBIN A. WEISS[1]

Virology Laboratory, Chester Beatty Laboratories, Institute of Cancer Research, London SW3 6JB,[1] *Breast Cancer Laboratory, Ludwig Institute of Cancer Research, University College London, London W1,*[2] *and Department of Genito-Urinary and Communicable Diseases, Imperial College School of Medicine at St. Mary's Hospital, London W2 1PG,*[3] *United Kingdom*

Received 7 April 1997/Accepted 14 July 1997

To test the hypothesis that some subtypes of human immunodeficiency virus type 1 (HIV-1), especially subtype E, are more likely to infect mature Langerhans cells (mLC), we titrated a panel of 26 primary HIV-1 isolates of subtypes A through F on peripheral blood mononuclear cells (PBMC) and mLC. The majority of HIV-1 isolates from heterosexually infected patients did not show a preferred tropism for mLC compared to homosexually transmitted HIV-1 isolates. Only 6 of 26 isolates, 2 from patients infected by homosexual contact and 4 from patients infected by heterosexual contact, showed a higher infectivity for mLC than for PBMC. Both syncytium-inducing and non-syncytium-inducing isolates were able to infect mLC which express mRNA for the chemokine receptors CCR3, CCR5, and CXCR4.

The epidemic spread of human immunodeficiency virus type 1 (HIV-1) occurs primarily as a result of sexual transmission. The first surfaces that are exposed to HIV-1 consist of a stratified squamous epithelium of the mucosa in which dendritic cells are positioned above the basal layer (13, 46, 47). These dendritic cells, which express CD4 on their membranes, have been proposed as a possible primary target for HIV infection (4, 16, 36). There is increasing evidence that dendritic cells are infected in vivo (26, 39, 55, 69), and it has been found that dendritic cells are susceptible to HIV-1 in vitro (29, 40, 53, 59), although in some cases a productive infection was only detected after cocultivation with T cells (7, 29, 58). Many in vitro infection experiments with dendritic cells, however, were conducted with syncytium-inducing (SI), T-cell-line-adapted (TCLA) strains of HIV-1 and, to a lesser extent, with primary HIV-1 isolates, either SI or non-SI (NSI) (for a review, see reference 8). These studies revealed conflicting data regarding the susceptibility of dendritic cells to infection by TCLA, SI isolates (7, 53) and NSI isolates (8, 37, 38, 40, 54, 70).

Until recently, the majority of dendritic cell infectivity studies used subtype B HIV-1 isolates without taking into account the history of transmission. Soto-Ramirez et al. (67) claimed that HIV-1 subtype E viruses replicate in skin-derived dendritic cells (mature Langerhans cell [mLC] culture) better than HIV-1 subtype B viruses, whereas no significant difference in the replication capacity of either virus subtype on peripheral blood mononuclear cells (PBMC) was observed. Because of the distinct routes of transmission responsible for the spread of these virus subtypes in Thailand (90% of sexually transmitted viruses in Thailand are HIV-1 subtype E, whereas 75% of infected intravenous drug users [IVDU] in Thailand carry HIV-1 subtype B [72]), Soto-Ramirez et al. (67) concluded that dendritic cell tropism is associated with heterosexual transmission of HIV-1 subtype E in particular and may explain the differences seen in the epidemics of Africa and Asia as opposed to North America and Western Europe.

HIV-1 titration on PBMC and mLC reveals no subtype-specific differences. We collected a panel of primary HIV-1 isolates of subtypes A through F from different risk groups (see Table 1) and titrated them on phytohemagglutinin- and interleukin-2-stimulated PBMC and on mLC cultures. The 50% tissue culture infectious doses ($TCID_{50}$) for PBMC were determined according to the method of McKnight et al. (43). The virus stocks contained between 6×10^2 and 3×10^5 infectious units for PBMC (with the exception of isolate M23, with only 2.5×10^1 $TCID_{50}$/ml [Table 1]).

The mLC were isolated as described previously (40, 48), with some modifications. Briefly, human skin was obtained as clinical specimens after corrective surgery (Stephen Kirby Skin Bank, London, United Kingdom) and subcutaneous fat was removed. Approximately 1-cm^2 skin pieces were incubated overnight at room temperature in Dispase (Boehringer Mannheim) (2 mg/ml in L15 medium), and the epidermis was peeled off with fine forceps. The epidermal sheets were cultured in 15-cm-diameter dishes containing 20 ml of RPMI 1640–10% fetal calf serum supplemented with 400 U of granulocyte-macrophage colony-stimulating factor (GM-CSF; Sandoz) per ml. After 3 days, migrating cells were collected and the low-density cell fraction was enriched by using a 13.4% metrizamide cushion (18, 53). The low-density cell fraction was then incubated for 30 min on ice with the dendritic-cell-specific antibody anti-CD83 (76), and after a second incubation with a magnetic-bead-conjugated anti-mouse immunoglobulin G2a antibody, the positive cells were separated by using the Minimacs system (Miltenyi Biotec, Camberley, United Kingdom). Flow cytometry was carried out as described previously (45), and it was shown by using specific conjugated antibodies from Sigma (St. Louis, Mo.) that there were less than 2% $CD3^+$ T

* Corresponding author. Mailing address: Chester Beatty Laboratories, Institute of Cancer Research, 237 Fulham Rd., London SW3 6JB, United Kingdom. Phone: 44 171-352 8133. Fax: 44 171-352 3299.

TABLE 1. Epidemiological information and infectivity of primary cultures of PBMC or mLC by study isolate

Sequence subtype[a]	Isolate[b]	Country	Sex of patient[c]	Risk factor[d]	Biological phenotype	Infectivity titer ($TCID_{50}$/ml) in:		PBMC/mLC infectivity ratio
						PBMC	mLC	
A	92UG31	Uganda	M	Het	NSI	3.2×10^3	6.3×10^1	50.0
	92RW20	Rwanda	F	Het	NSI	1.2×10^3	$<6.3 \times 10^1$	>20.0
B	M23	United Kingdom	M	Ho	NSI	2.5×10^1	6.3×10^1	0.4
	E80	United Kingdom	M	Ho	NSI	2.0×10^3	6.3×10^1	31.0
	BR92	Brazil	M	Ho	NSI	2.8×10^4	3.2×10^3	8.7
	BR49	Brazil	F	Het	NSI	2.8×10^4	6.3×10^1	444.0
	BR53	Brazil	F	Het	NSI	1.2×10^4	$<6.3 \times 10^1$	>190.0
	BR90	Brazil	M	Het	NSI	3.2×10^3	6.3×10^1	50.0
	BR65	Brazil	M	Ho	SI	2.9×10^3	3.2×10^2	9.0
	2028	United Kingdom	M	Ho	SI	6.3×10^4	6.3×10^2	100.0
	2076	United Kingdom	M	Ho	SI	3.0×10^5	6.3×10^2	476.0
C	BR25	Brazil	M	Hem	NSI	1.5×10^3	6.3×10^1	23.8
	BR28	Brazil	M	IVDU	NSI	1.3×10^4	3.2×10^3	4.0
	BR70	Brazil	F	IVDU	NSI	1.3×10^3	6.3×10^3	0.2
	JW1	Ethiopia	M	Het	NSI	2.7×10^3	6.3×10^1	42.8
	JW4	Zambia	M	Het	NSI	2.9×10^4	6.3×10^4	0.5
D	92UG24	Uganda	F	Het	SI	2.5×10^4	6.3×10^4	0.4
	JW5	Uganda	F	Het	SI	5.6×10^3	6.3×10^3	0.9
E	M53	Thailand	M	Ho	NSI	1.2×10^3	2.0×10^1	60.0
	92TH22	Thailand	M	Het	NSI	1.3×10^3	$<6.3 \times 10^1$	>20.0
	92TH23	Thailand	M	Het	NSI	2.5×10^4	6.3×10^2	39.0
	C2	Thailand	F	Het	NSI	6.3×10^2	6.3×10^3	0.1
	SL6	Thailand	F	Het	SI	1.9×10^3	6.3×10^2	3.0
	SL7	Thailand	F	Het	SI	3.8×10^3	6.3×10^2	6.0
	SL8	Thailand	M	Het	SI	1.2×10^3	6.3×10^1	19.0
F	BR58	Brazil	M	Het	SI	2.8×10^4	3.2×10^2	87.0

[a] Genetic subtype designations were made on the basis of sequence information from the *gag* gene, from gp120 or gp160, or from the C2-V5 region of gp120 or on the basis of heteroduplex mobility analysis (74). The E80 isolate was designated genetic subtype B based solely on the risk factor.

[b] Isolates 2028, 2076, M23, and E80 were described previously (65). Viruses designated by a code in the format exemplified by 92UG31 were provided by the World Health Organization Network for HIV Isolation and Characterization (2, 9, 27, 34, 56). Viruses designated by a code in the format exemplified by BR92 and SL8 were isolated at Imperial College School of Medicine (IC) at St. Mary's from samples obtained from the Infectious Disease Service, Porto Alegre, Brazil, and the Siriraj Hospital, Bangkok, Thailand, respectively. C2 virus was isolated at IC from PBMC obtained from an HIV-1-infected mother enrolled in the Bangkok Collaborative Perinatal HIV Transmission Study Group. M53 was isolated at the Institute of Cancer Research from the plasma of a male HIV-1-positive individual from Thailand. Virus JW5 was isolated at IC from a male Ugandan patient residing in the United Kingdom. Isolates were expanded in mitogen-stimulated seronegative PBMC (63).

[c] M, male; F, female.

[d] Het, heterosexual contact; Ho, homosexual contact; Hem, hemophilia patients.

cells and less than 1% each $CD14^+$ monocytes/macrophages and $CD19^+$ B cells as well as a strong HLA-DR response in these preparations (data not shown). The LC were cultured in RPMI 1640–10% fetal calf serum supplemented with 400 U of GM-CSF (Sandoz) per ml, 500 U of interleukin-4 per ml, and 800 U of tumor necrosis factor alpha (Genzyme, Cambridge, Mass.) per ml. The titrations with the HIV-1 isolates were done immediately after preparation (two titrations using mLC from two donors). After overnight incubation with serially diluted virus on 10^5 mLC in 100 μl of medium, the cells were washed four times and reverse transcriptase activity in cell culture supernatants was measured (Retrosys; Innovagen, Lund, Sweden) 7 days after infection. The HIV-1 titers were calculated as described previously (43).

The titration of the HIV-1 isolates on mLC revealed that some isolates were present at less than 6 infectious units per ml (TH22 and RW20) but that the majority were at concentrations of 6.3×10^2 to 6.3×10^4 $TCID_{50}$/ml (Table 1). No syncytia were observed after infection of mLC.

We then calculated the ratios between the $TCID_{50}$ values for PBMC and mLC for all isolates so that the virus isolates could be compared independently of the quantities of the virus stocks used. Figure 1 shows that there is no difference in cell tropism between the HIV-1 isolates obtained from patients infected by heterosexual contact and those obtained from patients infected by other routes (homosexual contact, IVDU, or clotting factor). The logarithmic mean ± the standard deviation (SD) for heterosexually transmitted isolates was 1.36 ± 0.62 versus 1.18 ± 0.52 for homosexually transmitted isolates. HIV-1 subgroup E isolates do not show a preferred tropism for mLC compared to HIV-1 subgroup B isolates (logarithmic means ± SDs for HIV-1 subgroup B and E isolates, 1.68 ± 0.65 and 1.18 ± 0.36, respectively). The ratios of the $TCID_{50}$s for PBMC and mLC show that in almost all cases infectivity is at least five times higher for PBMC, independent of the route of transmission for the primary infection. The exceptions are HIV-1 subtype B isolate M23 (homosexually transmitted); C2, a heterosexually transmitted HIV-1 subtype E virus; 92UG24 and JW5, two heterosexually transmitted HIV-1 subtype D isolates; one HIV-1 subtype C isolate, transmitted via drug abuse (BR70); and one heterosexually transmitted HIV-1 subtype C isolate (JW4). These six HIV-1 isolates infect mLC at 5 to 10 times the level of PBMC infection (Table 1).

The discovery of differences in the epidemic spread of

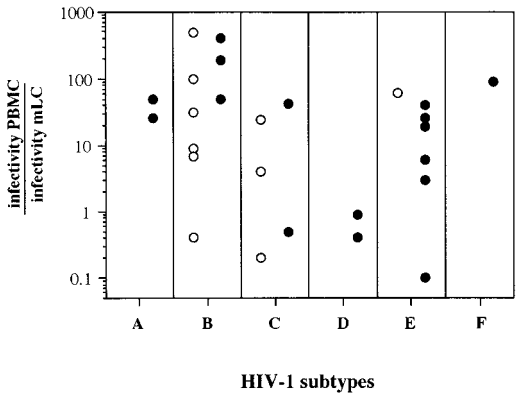

FIG. 1. PBMC/mLC infectivity ratios for primary HIV-1 isolates used in this study. HIV-1 infectivity titers for PBMC and mLC cultures (taken from Table 1) were used to calculate the ratios, which are plotted for each HIV-1 subtype separately. Risk groups: ○, homosexual transmission route, IVDU, or hemophilia patients; ●, heterosexual transmission route.

HIV-1 subtypes in different countries led to questions as to whether the cause is due to differences in the properties of the virus or host, to social behavior, or to population founder effects. Some have postulated that HIV-1 subtype E (as well as HIV-1 subtype C) viruses are transmitted with greater efficiency through heterosexual sex than other HIV-1 subtypes, thereby accounting for the rapid spread of the epidemic in Asia (52, 67, 71). In Africa, HIV-1 subtype E is less prevalent and less widely distributed geographically than HIV-1 subtypes A, C, and D (6), although it probably spread from the Central African Republic or Cameroon to Thailand (28, 42, 49, 51). However, there are no data available indicating a segregated pattern of HIV-1 subtypes in African countries. Nevertheless, HIV-1 subtype B, which is predominant among homosexuals and IVDU in the Americas and Europe, has caused serious epidemics of heterosexually transmitted infection in Honduras and elsewhere in the Caribbean and Latin America (73).

To address a possible virus-related factor for greater efficiency of transmission through heterosexual sex, we tested dendritic cells (mLC) derived from skin epidermis for their susceptibility for different HIV-1 subtypes. Our data show that mLC cultured in the presence of certain cytokines support active replication of HIV-1 in vitro when the T cells represent <2% of the culture population. In particular, GM-CSF and tumor necrosis factor alpha (TNF-α) enhance HIV-1 replication (25, 35) and may account for the active replication in purified mLC reported here and by others (37, 38, 40, 53, 67). Although the very small proportion of T cells present in the cultures may play some role in interacting with mLC to render them permissive to HIV, the virus clearly replicates in mLC, and six isolates (M23, 92UG24, BR70, JW4, JW5, and C2) propagated better in mLC cultures than in PBMC.

In vivo, replication of HIV-1 has been shown to occur in dendritic-cell-derived syncytia in the adenoid (26), probably as well as in the mucosal surfaces of the vagina, as shown for experimental simian immunodeficiency virus infections (68).

The results of our titrations of 26 primary HIV-1 isolates with known histories of transmission do not support the correlation between dendritic cell tropism and heterosexual transmission. Although some HIV-1 subtype E isolates infect mLC more readily than some HIV-1 subtype B isolates, this phenotype is independent of the route of transmission reported for the primary infection (Table 1). Moreover, the inclusion of HIV-1 subtype A, C, D, and F isolates shows that high-level infectivity of mLC is not restricted to any particular HIV-1 subtype. Neither was there any difference in tropism between HIV-1 subtype B isolates obtained from patients infected via homosexual contact and those from patients infected via heterosexual contact.

No selection of virus phenotype by mLC in vitro. Recently, the second components of the receptor complex, or coreceptors, have been identified (17, 22, 24), and they belong to the seven-transmembrane G-protein-coupled chemokine receptor family. As predicted (5, 20, 21, 30, 44), the requirements of SI and NSI isolates are different: the CXCR4 chemokine receptor functions as an HIV-1 coreceptor for TCLA (24) and primary SI (65) isolates, whereas primary NSI or macrophage-tropic strains use the CCR5 chemokine receptor (1, 17, 22) as the main coreceptor with CD4. Some other members of the chemokine receptor family can also be used by a number of primary HIV-1 isolates, among them CCR2b and CCR3 (10, 12, 19, 31). However, CXCR4 and CCR5 seem to be the main coreceptors used by HIV-1 in vivo irrespective of the viral genetic subtype (12, 75). In vivo, NSI viruses are detected soon after seroconversion even when a transmitter harbors a mixture of viral phenotypes (62, 77). It is possible that the first cell type infected, likely dendritic cells, is responsible for this observed restricted phenotype (4, 46).

With our panel of 26 primary HIV-1 isolates, we determined the virus phenotype by cell-free infection of MT2 cells and scored it as SI when syncytia developed during the observation period (20 days) (32, 33) (Table 1). To determine the coreceptor usage of the study isolates, U87 cells stably expressing human CD4 and either human CXCR4, CCR5, or CCR3 (17) (kindly provided by D. Littman) were challenged and infection was monitored after 4 to 5 days by immunocytostaining as described previously (11). As Table 2 shows, the majority of the primary HIV-1 isolates tested use CCR5 as the main coreceptor for virus entry. All HIV-1 isolates that scored as SI viruses in the MT2 assay use CXCR4 as a coreceptor in the U87-CD4 assay regardless of HIV-1 subtype. As some of our isolates infecting mLC use only CCR5 and others use only CXCR4, it seemed possible that mLC express both chemokine coreceptors. This was confirmed by reverse transcription (RT)-PCR analysis using primer pairs to detect CXCR4, CCR5, and CCR3 mRNA in mLC and blood dendritic cells (18) (Fig. 2) and also by HIV-1 infectivity inhibition studies using either AOP-RANTES, a potent CCR5 antagonist (66), or SDF-1, the natural ligand for CXCR4 (Fig. 3). Primary HIV-1 isolates with a broad use of CCR5, CXCR4, and CCR3 as coreceptor show an 87- to 476-fold higher level of infectivity for PBMC than for mLC. Viruses capable of using only CCR5 as the coreceptor showed no significant differences in their capacities to infect mLC compared to those using only CXCR4. The HIV-1 isolates that infect mLC the best use either CCR5 (M23, BR70, and C2) or CXCR4 (UG24 and JW5). Thus, mLC in short-term culture are permissive to SI and NSI primary HIV-1 isolates via either CXCR4 or CCR5.

Although the viral phenotype at the time of virus isolation may not precisely reflect the phenotype at the time of transmission, our in vitro results do not support the proposed selection of the NSI, CCR5-dependent viral phenotype during the primary infection with respect to mLC infection. However, the described resistance to NSI variants due to a homozygous deletion of functional CCR5 strongly indicates the existence of such selection (15, 41, 64). There is a possibility that upregulation of chemokine receptors during in vitro culture in the presence of cytokines (4 days for mLC and 1 day for blood dendritic cells) affects coreceptor expression, as is seen for PBMC (3), although the mLC studied show the expected im-

TABLE 2. HIV-1 coreceptor usage by study isolates[a]

Sequence subtype	Isolate	Biological phenotype	Focus-forming units of HIV-1 with coreceptor		
			CXCR4	CCR5	CCR3
A	92UG31	NSI	−[b]	8.5×10^1	−
	92RW20	NSI	−	4.0×10^1	−
B	M23	NSI	−	4.3×10^3	−
	E80	NSI	−	4.3×10^4	2.3×10^1
	BR92	NSI	−	1.2×10^4	−
	BR49	NSI	−	2.0×10^4	−
	BR53	NSI	−	1.5×10^2	−
	BR90	NSI	−	4.0×10^2	−
	BR65	SI	3.1×10^2	−	−
	2028	SI	4.4×10^3	1.2×10^3	3.0×10^2
	2076	SI	1.0×10^4	9.3×10^3	1.0×10^2
C	BR25	NSI	−	2.6×10^3	−
	BR28	NSI	−	1.0×10^3	−
	BR70	NSI	−	1.5×10^3	−
	JW1	NSI	−	9.4×10^3	−
	JW4	NSI	−	1.7×10^4	−
D	92UG24	SI	3.0×10^3	−	−
	JW5	SI	3.6×10^3	−	−
E	M53	NSI	−	2.5×10^1	−
	92TH22	NSI	−	2.0×10^2	−
	92TH23	NSI	−	5.0×10^1	−
	C2	NSI	−	1.2×10^3	−
	SL6	SI	8.0×10^2	−	−
	SL7	SI	4.5×10^2	−	−
	SL8	SI	1.8×10^3	−	−
F	BR58	SI	7.8×10^3	9.0×10^3	6.4×10^3

[a] U87-CD4 cells expressing one of the chemokine receptors were immunocytostained 4 to 5 days after challenge with the described virus stocks. All challenge experiments were done three times.

[b] −, no immunocytostained cells were detected after 4 to 5 days (<10 focus-forming units/ml).

munological properties when cultured under these conditions (61). Little is known about the level of chemokines (especially SDF-1, the natural ligand for CXCR4) in the mucosal surfaces, which conceivably might account for the inhibition of infection by SI variants during vaginal transmission. However, IVDU and accidentally infected persons with hemophilia also harbor

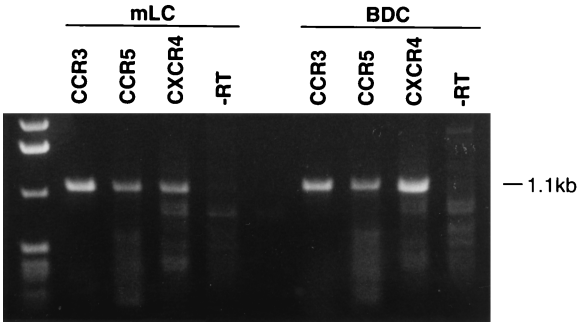

FIG. 2. RT-PCR analysis of mRNA expression of the chemokine receptor genes CCR3, CCR5, and CXCR4. Equal amounts of cDNA were used to detect the expression of CCR3 (14, 57), CCR5 (60), and CXCR4 (50) in mLC and blood dendritic cells (BDC) (18). −RT, RT-PCR for CXCR4 was performed without reverse transcriptase.

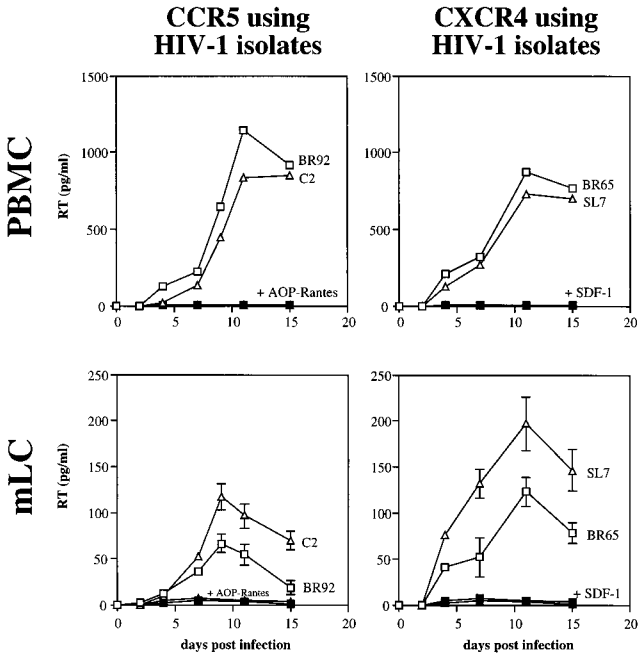

FIG. 3. Replication kinetics and HIV-1 infectivity inhibition by SDF-1 and AOP-Rantes. PBMC or mLC (5×10^4) were challenged with 500 $TCID_{50}$ (multiplicity of infection, 0.01) of HIV-1 subtype B isolate BR92 or BR65 or subtype E isolate C2 or SL7 with or without preincubation with SDF-1 (2,000 ng/ml) or AOP-RANTES (800 ng/ml), respectively. After 12 h, the cells were washed four times and then cultured in the presence or absence of SDF-1 and AOP-RANTES. Reverse transcriptase activities in the cell culture supernatants were monitored for up to 15 days (graphs represent mean values of reverse transcriptase activities determined in two independent experiments).

NSI viruses early in infection, indicating that the mucosal membrane is not necessarily the selective barrier.

One possible explanation for the events taking place after sexual and nonsexual transmission could be the inhibition of SI strains after the primary target cells (mLC or blood dendritic cells) encounter the antigen (NSI and SI variants) and migrate to the lymph nodes. SDF-1 produced by stromal cells in the lymph nodes may inhibit further dissemination of SI variants so that only NSI variants are detectable soon after infection (23). The recently described finding that memory T cells express higher levels of CCR5 than CXCR4 and might therefore be responsible for the selection of CCR5-dependent NSI isolates after nonsexual and sexual transmission (3) could also explain early events after transmission, again underscoring the importance of CCR5 as the main coreceptor for HIV-1, independent of the route of transmission.

We thank A. McKnight, L. Picard, and J. Reeves for helpful discussions and R. Cheingsong-Popov, A. Bobkov, and M. Garaev, Imperial College (IC), for their help in the designation of genetic subtypes. We are grateful to the Stephen Kirby Skin Bank for providing fresh skin samples and to D. R. Littman (Howard Hughes Medical Institute, New York University) for providing the U87/CD4 cells stably expressing the chemokine receptors CCR3, CCR5, and CXCR4. We thank our collaborators who have generously provided virus stocks or blood samples on which virus isolations were performed. We are especially indebted to S. Osmanov (WHO) and H. Holmes (National Institute for Biological Standards and Control, Potters Bar, United Kingdom) for the 92UG31, 92RW20, 92UG24, 92TH22, and 92TH23 isolates and Breno Riegel Santos (Infectious Diseases Service, Porto Alegre, Brazil) for the blood samples from which the BR92, BR65, BR49, BR53, BR90, and BR58 viruses were isolated. We also thank the following persons for their contributions: N. Shaffer and T. Mastro and staff at the

Bangkok Collaborative Perinatal HIV Transmission Study Group, Siriraj Hospital, Bangkok, Thailand (patient C2); G. Carnegie, U. Desselberger, and the United Kingdom HIV Molecular Epidemiology Group, Addenbrooke's Hospital, Cambridge (isolates 2028 and 2076); P. Kaleebu, Uganda Virus Research Institute, Entebbe (isolate JW5); and C. Wasi and P. Auewarakul, Siriraj Hospital, Bangkok, Thailand. We thank A. E. I. Proudfoot, T. N. C. Wells (both of Glaxo Welcome, Geneva, Switzerland) and R. Offord (Centre Medicale Universitaire, Geneva, Switzerland) for providing AOP-Rantes.

This work was supported by the Medical Research Council.

REFERENCES

1. **Alkhatib, G., C. Combadiere, C. C. Broder, Y. Feng, P. E. Kennedy, P. M. Murphy, and E. A. Berger.** 1996. CC CKR5: a RANTES, MIP-1α, MIP-1β receptor as fusion cofactor for macrophage-tropic HIV-1. Science **272:**1955–1958.
2. **Bachmann, M. H., E. L. Delwart, E. G. Shpaer, P. Lingenfelter, R. Singal, and J. I. Mullins.** 1994. Rapid genetic characterization of HIV type 1 strains from four World Health Organization-sponsored vaccine evaluation sites using a heteroduplex mobility assay. AIDS Res. Hum. Retroviruses **10:**1345–1353.
3. **Bleul, C. C., L. Wu, J. A. Hoxie, T. A. Springer, and C. R. Mackay.** 1997. The HIV coreceptors CXCR4 and CCR5 are differentially expressed and regulated on human T lymphocytes. Proc. Natl. Acad. Sci. USA **94:**1925–1930.
4. **Braathen, L. R., G. Ramirez, R. O. Kunze, and H. Gelderblom.** 1987. Langerhans cells as primary target cells for HIV infection. Lancet **ii:**1094.
5. **Broder, C. C., D. S. Dimitrov, R. Blumenthal, and E. A. Berger.** 1993. The block to HIV-1 envelope glycoprotein-mediated membrane fusion in animal cells expressing human CD4 can be overcome by a human cell component(s). Virology **193:**483–491.
6. **Burke, D. S., and F. E. McCutchan.** 1996. Global distribution of HIV-1 clades, p. 253–278. *In* V. T. DeVita, Jr., S. Hellman, S. A. Rosenberg, M. E. Essex, A. S. Fauci, and J. S. Freeman (ed.), AIDS: etiology, diagnosis, treatment and prevention, 4th ed. J. B. Lippincott Co., Philadelphia, Pa.
7. **Cameron, P. U., P. S. Freudenthal, J. M. Barker, S. Gezelter, K. Inaba, and R. M. Steinman.** 1992. Dendritic cells exposed to human immunodeficiency virus type-1 transmit a vigorous cytopathic infection to CD4+ T cells. Science **257:**383–387.
8. **Cameron, P. U., M. G. Lowe, S. M. Crowe, U. O'Doherty, M. Pope, S. Gezelter, and R. M. Steinman.** 1994. Susceptibility of dendritic cells to HIV-1 infection in vitro. J. Leukocyte Biol. **56:**257–265.
9. **Cheingsong Popov, R., S. Lister, D. Callow, P. Kaleebu, S. Beddows, and J. Weber.** 1994. Serotyping HIV type 1 by antibody binding to the V3 loop: relationship to viral genotype. AIDS Res. Hum. Retroviruses **10:**1379–1386.
10. **Choe, H., M. Farzan, Y. Sun, N. Sullivan, B. Rollins, P. D. Ponath, L. Wu, C. R. Mackay, G. LaRosa, W. Newman, N. Gerard, C. Gerard, and J. Sodroski.** 1996. The beta-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. Cell **85:**1135–1148.
11. **Clapham, P. R., D. Blanc, and R. A. Weiss.** 1991. Specific cell surface requirements for the infection of CD4-positive cells by human immunodeficiency virus types 1 and 2 and by simian immunodeficiency virus. Virology **181:**703–715.
12. **Connor, R. I., K. E. Heridan, D. Ceradini, S. Choe, and N. R. Landau.** 1997. Change in coreceptor use correlates with disease progression in HIV-1-infected individuals. J. Exp. Med. **185:**621–628.
13. **Daniels, T. E.** 1984. Human mucosal Langerhans cells: postmortem identification of regional variations in oral mucosa. J. Invest. Dermatol. **82:**21–24.
14. **Daugherty, B., S. Siciliano, J. DeMartino, L. Malkowitz, A. Sirotina, and M. Springer.** 1996. Cloning, expression, and characterization of the human eosinophil eotaxin receptor. J. Exp. Med. **183:**2349–2354.
15. **Dean, M., M. Carrington, C. Winkler, G. A. Huttley, M. W. Smith, R. Allikmets, J. J. Goedert, S. P. Buchbinder, E. Vittinghoff, E. Gomperts, S. Donfield, D. Vlahov, R. Kaslow, A. Saah, C. Rinaldo, R. Detels, Hemophilia Growth and Development Study, Multicenter AIDS Cohort Study, Multicenter Hemophilia Cohort Study, San Francisco Cohort Study, ALIVE Study, and S. O'Brien.** 1996. Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of CKR5 structural gene. Science **273:**1856–1862.
16. **Delorme, P., C. Dezutter Dambuyant, A. Ebersold, C. Desgranges, J. Thivolet, and D. Schmitt.** 1993. In vitro infection of epidermal Langerhans cells with human immunodeficiency virus type 1 (HTLV-IIIB isolate). Res. Virol. **144:**53–58.
17. **Deng, H., R. Liu, W. Elmeier, S. Choe, D. Unutmaz, M. Burkhart, P. Di Marzio, S. Marmon, R. E. Sutton, C. M. Hill, C. B. Davis, S. C. Peiper, T. J. Schall, D. R. Littman, and N. R. Landau.** 1996. Identification of a major co-receptor for primary isolates of HIV-1. Nature **381:**661–666.
18. **Dittmar, M. T., G. Simmons, Y. Donaldson, P. Simmonds, P. R. Clapham, T. F. Schulz, and R. A. Weiss.** 1997. Biological characterization of human immunodeficiency virus type 1 clones derived from different organs of an AIDS patient by long-range PCR. J. Virol. **71:**5140–5147.
19. **Doranz, B. J., J. Rucker, Y. Yi, R. J. Smyth, M. Samson, S. C. Peiper, M. Parmentier, R. G. Collman, and R. W. Doms.** 1996. A dual-tropic primary HIV-1 isolate that uses fusin and the beta-chemokine receptors CKR-5, CKR-3, and CKR-2b as fusion cofactors. Cell **85:**1149–1158.
20. **Dragic, T., and M. Alizon.** 1993. Different requirements for membrane fusion mediated by the envelopes of human immunodeficiency virus types 1 and 2. J. Virol. **67:**2355–2359.
21. **Dragic, T., U. Hazan, and M. Alizon.** 1995. Detection of cell fusion mediated by the envelopes of human retroviruses by transactivation of a reporter gene, p. 218–236. *In* K. W. Adolph (ed.), Methods in molecular genetics, vol. 7. Viral gene techniques. Academic Press, Orlando, Fla.
22. **Dragic, T., V. Litwin, G. Allaway, S. R. Martin, Y. Huang, K. A. Nagashima, C. Cayanan, P. J. Maddon, R. A. Koup, J. P. Moore, and W. A. Paxton.** 1996. HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC-CKR5. Nature **381:**667–673.
23. **Fauci, A. S.** 1996. Host factors and the pathogenesis of HIV-induced disease. Nature **384:**529–534.
24. **Feng, Y., C. C. Broder, P. E. Kennedy, and E. A. Berger.** 1996. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane G-protein coupled receptor. Science **272:**872–877.
25. **Folks, T. M., K. A. Clouse, J. Justement, A. Rabson, E. Duh, J. H. Kehrl, and A. S. Fauci.** 1989. Tumor necrosis factor alpha induces expression of human immunodeficiency virus in a chronically infected T-cell clone. Proc. Natl. Acad. Sci. USA **86:**2365–2368.
26. **Frankel, S. S., B. M. Wenig, A. P. Burke, P. Mannan, L. D. Thompson, S. L. Abbondanzo, A. M. Nelson, M. Pope, and R. M. Steinman.** 1996. Replication of HIV-1 in dendritic cell-derived syncytia at the mucosal surface of the adenoid. Science **272:**115–117.
27. **Gao, F., L. Yue, S. Craig, C. L. Thornton, D. L. Robertson, F. E. McCutchan, J. A. Bradac, P. M. Sharp, and B. H. Hahn.** 1994. Genetic variation of HIV type 1 in four World Health Organization-sponsored vaccine evaluation sites: generation of functional envelope (glycoprotein 160) clones representative of sequence subtypes A, B, C, and E. AIDS Res. Hum. Retroviruses **10:**1359–1368.
28. **Gao, F., D. L. Robertson, S. G. Morrison, H. Hui, S. Craig, J. Decker, P. N. Fultz, M. Girard, G. M. Shaw, B. H. Hahn, and P. M. Sharp.** 1996. The heterosexual human immunodeficiency virus type 1 epidemic in Thailand is caused by an intersubtype (A/E) recombinant of African origin. J. Virol. **70:**7013–7029.
29. **Granelli-Piperno, A., B. Moser, M. Pope, D. Chen, Y. Wei, F. Isdell, U. O'Doherty, W. Paxton, R. Koup, S. Mojsov, N. Bhardwaj, I. Clark-Lewis, M. Baggiolini, and R. M. Steinman.** 1996. Efficient interaction of HIV-1 with purified dendritic cells via multiple chemokine coreceptors. J. Exp. Med. **184:**2433–2438.
30. **Harrington, R. D., and A. P. Geballe.** 1993. Cofactor requirement for human immunodeficiency virus type 1 entry into a CD4-expressing human cell line. J. Virol. **67:**5939–5947.
31. **He, J., Y. Chen, M. Farzan, H. Choe, A. Ohagen, S. Gartner, J. Busciglio, X. Yang, W. Hofmann, W. Newman, C. R. Mackay, J. Sodroski, and D. Gabuzda.** 1997. CCR3 and CCR5 are co-receptors for HIV-1 infection of microglia. Nature **385:**645–649.
32. **Koot, M., A. H. Vos, R. P. Keet, R. E. de Goede, M. W. Dercksen, F. G. Terpstra, R. A. Coutinho, F. Miedema, and M. Tersmette.** 1992. HIV-1 biological phenotype in long-term infected individuals evaluated with an MT-2 cocultivation assay. AIDS **6:**49–54.
33. **Koot, M., I. P. Keet, A. H. Vos, R. E. de Goede, M. T. Roos, R. A. Coutinho, F. Miedema, P. T. Schellekens, and M. Tersmette.** 1993. Prognostic value of HIV-1 syncytium-inducing phenotype for rate of CD4+ cell depletion and progression to AIDS. Ann. Intern. Med. **118:**681–688.
34. **Korber, B. T., S. Osmanov, J. Esparza, and G. Myers.** 1994. The World Health Organization Global Programme on AIDS proposal for standardization of HIV sequence nomenclature. AIDS Res. Hum. Retroviruses **10:**1355–1358.
35. **Koyanagi, Y., W. A. O'Brien, J. Q. Zhao, D. W. Golde, J. C. Gasson, and I. S. Chen.** 1988. Cytokines alter production of HIV-1 from primary mononuclear phagocytes. Science **241:**1673–1675.
36. **Landor, M., Z. Harish, and A. Rubinstein.** 1989. Human immunodeficiency virus transmission: is the integument a barrier? Am. J. Med. **87:**489.
37. **Langhoff, E., E. F. Terwilliger, H. J. Bos, K. H. Kalland, M. C. Poznansky, O. M. Bacon, and W. A. Haseltine.** 1991. Replication of human immunodeficiency virus type 1 in primary dendritic cell cultures. Proc. Natl. Acad. Sci. USA **88:**7998–8002.
38. **Langhoff, E., K. H. Kalland, and W. A. Haseltine.** 1993. Early molecular replication of human immunodeficiency virus type 1 in cultured-blood-derived T helper dendritic cells. J. Clin. Invest. **91:**2721–2726.
39. **Livingstone, W. J., M. Moore, D. Innes, J. E. Bell, P. Simmonds, and The Edinburgh Heterosexual Transmission Study Group.** 1996. Frequent infection of peripheral blood CD8-positive T-lymphocytes with HIV-1. Lancet **348:**649–654.
40. **Ludewig, B., J. Holzmeister, M. Gentile, H. R. Gelderblom, K. Rokos, Y. Becker, and G. Pauli.** 1995. Replication pattern of human immunodeficiency virus type 1 in mature Langerhans cells. J. Gen. Virol. **76:**1317–1325.
41. **Lui, R., W. A. Paxton, S. Choe, D. Ceradini, S. R. Martin, R. Horuk, M. E.**

McDonald, H. Stuhlmann, R. A. Koup, and N. R. Landau. 1996. Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. Cell **86:**367–377.

42. **McCutchan, F. E., P. Hegerich, T. Brennan, P. Phanuphak, P. Preecha, T. Achasa, P. Berman, A. K. Fowler, and D. S. Burke.** 1992. Genetic variants of HIV-1 in Thailand. AIDS Res. Hum. Retroviruses **8:**1887–1895.
43. **McKnight, A., P. R. Clapham, and T. F. Schulz.** 1995. Detection of HIV entry into cells, p. 129–141. *In* J. Karn (ed.), HIV, a practical approach, vol. 1. IRL Press, Oxford, United Kingdom.
44. **McKnight, Á., R. A. Weiss, C. Shotton, Y. Takeuchi, H. Hoshino, and P. R. Clapham.** 1995. Change in tropism upon immune escape by human immunodeficiency virus. J. Virol. **69:**3167–3170.
45. **McKnight, Á., D. Wilkinson, G. Simmons, S. Talbot, L. Picard, M. Ahuja, M. Marsh, J. A. Hoxie, and P. R. Clapham.** 1997. Inhibition of human immunodeficiency virus fusion by a monoclonal antibody to a coreceptor (CXCR4) is both cell type and virus strain dependent. J. Virol. **71:**1692–1696.
46. **Miller, C. J., N. J. Alexander, P. Vogel, J. Anderson, and P. A. Marx.** 1992. Mechanism of genital transmission of SIV: a hypothesis based on transmission studies and the location of SIV in the genital tract of chronically infected female rhesus macaques. J. Med. Primatol. **21:**64–68.
47. **Miller, C. J., J. R. McGhee, and M. B. Gardner.** 1993. Biology of disease. Mucosal immunity, HIV transmission, and AIDS. Lab. Invest. **68:**129–145.
48. **Morris, J., M. Alaibac, M.-H. Jia, and T. Chu.** 1992. Purification of functional active epidermal Langerhans cells: a simple and efficient new technique. J. Invest. Dermatol. **99:**1595–1601.
49. **Murphy, E., B. Korber, M. C. Georges Courbot, B. You, A. Pinter, D. Cook, M. P. Kieny, A. Georges, C. Mathiot, F. Barre Sinoussi, et al.** 1993. Diversity of V3 region sequences of human immunodeficiency viruses type 1 from the Central African Republic. AIDS Res. Hum. Retroviruses **9:**997–1006.
50. **Neote, K., D. DiGregorio, J. Y. Mak, R. Horuk, and T. J. Schall.** 1993. Molecular cloning, functional expression, and signalling characteristics of a C-C chemokine receptor. Cell **72:**415–425.
51. **Nkengasong, J. N., W. Janssens, L. Heyndrickx, K. Fransen, P. M. Ndumbe, J. Motte, A. Leonaers, M. Ngolle, J. Ayuk, P. Piot, and G. van der Groen.** 1994. Genotypic subtypes of HIV-1 in Cameroon. AIDS **8:**1405–1412.
52. **Ou, C.-Y., Y. Takebe, B. G. Weniger, C.-C. Luo, M. L. Kalish, W. Auwanit, S. Yamazaki, H. D. Gayle, N. L. Young, and G. Schochetman.** 1993. Independent introduction of two major HIV-1 genotypes into distinct high-risk populations in Thailand. Lancet **341:**1171–1174.
53. **Patterson, S., and S. C. Knight.** 1987. Susceptibility of human peripheral blood dendritic cells to infection by human immunodeficiency virus. J. Gen. Virol. **68:**1177–1181.
54. **Patterson, S., J. Gross, P. Bedford, and S. C. Knight.** 1991. Morphology and phenotype of dendritic cells from peripheral blood and their productive and nonproductive infection with human immunodeficiency virus type 1. Immunology **72:**361–367.
55. **Patterson, S., M. S. Roberts, N. R. English, S. E. Macatonia, M. N. Gompels, A. J. Pinching, and S. C. Knight.** 1994. Detection of HIV DNA in peripheral blood dendritic cells of HIV-infected individuals. Res. Virol. **145:**171–176.
56. **Pau, C. P., M. Kai, D. L. Holloman Candal, C. C. Luo, M. L. Kalish, G. Schochetman, B. Byers, and J. R. George.** 1994. Antigenic variation and serotyping of HIV type 1 from four World Health Organization-sponsored HIV vaccine sites. AIDS Res. Hum. Retroviruses **10:**1369–1377.
57. **Ponath, P., S. Oln, T. Post, J. Wang, L. Wu, N. Gerard, W. Newman, C. Gerard, and C. Mackay.** 1996. Molecular cloning and characterization of a human eotaxin receptor expressed selectively on eosinophils. J. Exp. Med. **183:**1–12.
58. **Pope, M., M. G. Betjes, N. Romani, H. Hirmand, P. U. Cameron, L. Hoffman, S. Gezelter, G. Schuler, and R. M. Steinman.** 1994. Conjugates of dendritic cells and memory T lymphocytes from skin facilitate productive infection with HIV-1. Cell **78:**389–398.
59. **Pope, M., S. Gezelter, N. Gallo, L. Hoffman, and R. M. Steinman.** 1995. Low levels of HIV-1 infection in cutaneous dendritic cells promote extensive viral replication upon binding to memory CD4+ T cells. J. Exp. Med. **182:**2045–2056.
60. **Raport, C. J., J. Gosling, V. L. Schweickart, P. W. Gray, and I. F. Charo.** 1996. Molecular cloning and functional characterization of a novel human CC chemokine receptor (CCR5) for RANTES, MIP-1β, and MIP-1α. J. Biol. Chem. **271:**17161–17166.
61. **Romani, N., S. Gruner, D. Brang, E. Kämpgen, A. Lenz, B. Trockenbacher, G. Konwalinka, P. O. Fritsch, R. M. Steinman, and G. Schuler.** 1994. Proliferating dendritic cell progenitors in human blood. J. Exp. Med. **180:**83–93.
62. **Roos, M. T., J. M. Lange, R. E. de Goede, R. A. Coutinho, P. T. Schellekens, F. Miedema, and M. Tersmette.** 1992. Viral phenotype and immune response in primary human immunodeficiency virus type 1 infection. J. Infect. Dis. **165:**427–432.
63. **Rübsamen Waigmann, H., H. von Briesen, H. Holmes, A. Bjorndal, B. Korber, R. Esser, S. Ranjbar, P. Tomlinson, B. Galvao Castro, E. Karita, et al.** 1994. Standard conditions of virus isolation reveal biological variability of HIV type 1 in different regions of the world. AIDS Res. Hum. Retroviruses **10:**1401–1408.
64. **Samson, M., F. Libert, B. J. Doranz, J. Rucker, C. Liesnard, C. M. Farber, S. Saragosti, C. Lapoumeroulie, J. Cogniaux, C. Forceille, G. Muyldermans, C. Verhofstede, G. Bortonboy, M. Georges, T. Imai, S. Rana, Y. Yi, R. J. Smyth, R. G. Collman, R. W. Doms, G. Vassart, and M. Parmentier.** 1996. Resistance to HIV-1 infection of Caucasian individuals bearing mutant alleles of the CCR5 chemokine receptor gene. Nature **382:**722–725.
65. **Simmons, G., D. Wilkinson, J. D. Reeves, M. T. Dittmar, S. Beddows, J. Weber, G. Carnegie, U. Desselberger, P. W. Gray, R. A. Weiss, and P. R. Clapham.** 1996. Primary, syncytium-inducing human immunodeficiency virus type 1 isolates are dual-tropic and most can use either Lestr or CCR5 as coreceptors for virus entry. J. Virol. **70:**8355–8360.
66. **Simmons, G., P. R. Clapham, L. Picard, R. E. Offord, M. M. Rosenkilde, T. W. Schwartz, R. Buser, T. N. C. Wells, and A. E. I. Proudfoot.** 1997. Potent inhibition of HIV-1 infectivity in macrophages and lymphocytes by a novel CCR5 antagonist. Science **276:**276–279.
67. **Soto-Ramirez, L. E., B. Renjifo, M. F. McLane, R. Marlink, C. O'Hara, R. Sutthent, C. Wasi, P. Vithayasai, V. Vithayasai, C. Apichartpiyakul, P. Auewarakul, V. Pena Cruz, D.-S. Chui, R. Osathanondh, K. Mayer, T.-H. Lee, and M. Essex.** 1996. HIV-1 Langerhans cell tropism associated with heterosexual transmission of HIV. Science **271:**1291–1293.
68. **Spira, A. I., P. A. Marx, B. K. Patterson, J. Mahoney, R. A. Koup, S. M. Wolinsky, and D. D. Ho.** 1996. Cellular targets of infection and route of viral dissemination after an intravaginal inoculation of simian immunodeficiency virus into rhesus macaques. J. Exp. Med. **183:**215–225.
69. **Tschachler, E., V. Groh, M. Popovic, D. L. Mann, K. Konrad, B. Safai, L. Eron, F. diMarzo Veronese, K. Wolff, and G. Stingl.** 1987. Epidermal Langerhans cells—a target for HTLV-III/LAV infection. J. Invest. Dermatol. **88:**233–237.
70. **Tsunetsugu-Yokota, Y., K. Akagawa, H. Kimoto, K. Suzuki, M. Iwasaki, S. Yasuda, G. Häusser, C. Hultgren, A. Meyerhans, and T. Takemori.** 1995. Monocyte-derived cultured dendritic cells are susceptible to human immunodeficiency virus infection and transmit virus to resting T cells in the process of nominal antigen presentation. J. Virol. **69:**4544–4547.
71. **Weniger, B. G., K. Limpakarnjanarat, K. Ungchusak, S. Thanprasertsuk, K. Choopanya, S. Vanichseni, T. Uneklabh, P. Thongcharoen, and C. Wasi.** 1991. The epidemiology of HIV infection and AIDS in Thailand. AIDS **5**(Suppl. 2)**:**S71–S85.
72. **Weniger, B. G.** 1994. Experience from HIV incidence cohorts in Thailand: implications for HIV vaccine efficacy trials. AIDS **8:**1007–1010.
73. **Weniger, B. G., and T. Brown.** 1996. The march of AIDS through Asia. N. Engl. J. Med. **335:**343–345.
74. **WHO Network for HIV Isolation and Characterization.** 1994. HIV-1 variation in WHO-sponsored vaccine evaluation sites: genetic screening, sequence analysis and preliminary biological characterization of selected viral strains. AIDS Res. Hum. Retroviruses **10:**1327–1344.
75. **Zhang, L., Y. Huang, T. He, Y. Cao, and D. D. Ho.** 1996. HIV-1 subtype and second-receptor use. Nature **383:**768.
76. **Zhou, L. J., R. Schwarting, H. M. Smith, and T. F. Tedder.** 1992. A novel cell-surface molecule expressed by human interdigitating reticulum cells, Langerhans cells, and activated lymphocytes is a new member of the Ig superfamily. J. Immunol. **149:**735–742.
77. **Zhu, T., H. Mo, N. Wang, D. S. Nam, Y. Cao, R. A. Koup, and D. D. Ho.** 1993. Genotypic and phenotypic characterization of HIV-1 patients with primary infection. Science **261:**1179–1181.