JOURNAL OF VIROLOGY, Sept. 1997, p. 7124–7127 Vol. 71, No. 9
0022-538X/97/$04.00+0

Chemokine Receptor CCR5 Genotype Influences the Kinetics of Human Immunodeficiency Virus Type 1 Infection in Human PBL-SCID Mice†

GASTÓN R. PICCHIO, RICHARD J. GULIZIA, AND DONALD E. MOSIER*
Department of Immunology, The Scripps Research Institute, La Jolla, California 92037

Received 4 March 1997/Accepted 30 May 1997

Individuals homozygous for a 32-bp deletion (Δ32) in the CCR5 gene encoding the coreceptor for macrophage-tropic human immunodeficiency virus type 1 (HIV-1) are resistant to virus infection, and heterozygous individuals show some slowing of disease progression. The impact of the CCR5 genotype on HIV-1 infection was assessed in vitro and in the human PBL-SCID (hu-PBL-SCID) model. Cells and hu-PBL-SCID mice from CCR5 Δ32/Δ32 donors were resistant to infection with macrophage-tropic HIV-1 and showed slower replication of dual-tropic HIV-1. hu-PBL-SCID mice derived from CCR5 Δ32/+ heterozygotes showed delayed replication of macrophage-tropic HIV-1 despite a small and variable effect of heterozygosity on viral replication in vitro. The level of CCR5 expression appears to limit replication of macrophage-tropic and dual-tropic HIV-1 strains in vivo.

Macrophage-tropic (M-tropic) human immunodeficiency virus type 1 (HIV-1) uses both CD4 and the CCR5 chemokine receptor (9, 28) for fusion with target cells (1, 6, 13–15), whereas T-cell-line-tropic (T-tropic) HIV-1 uses CD4 and CXCR-4 (fusin) for viral entry (5, 17). Most individuals homozygous for a 32-bp deletion in CCR5 (CCR5 Δ32/Δ32) are resistant to HIV-1 infection, and their cells resist infection with M-tropic but not T-tropic HIV-1 in vitro (11, 22, 26, 29), although at least one infected CCR5 Δ32/Δ32 patient has recently been identified (4). Heterozygous individuals (CCR5 Δ32/+) are susceptible to HIV-1 infection but show some protective effects since they are slightly overrepresented in long-term nonprogressors and have slower increases in viral RNA levels after seroconversion (12, 19). Despite the fact that some dual-tropic (M/T-tropic) viruses can use alternative chemokine receptors for entry, e.g., CCR2b, CCR3, and CXCR4 (14, 27), the CCR5 coreceptor seems to be the most important for viral entry into the primary target cell involved in all routes of HIV-1 transmission. The observation that M/T-tropic HIV-1 isolates are more common than previously assumed (5, 31) makes it even more paradoxical that the CCR5 deletion has such a clear protective effect and that heterozygosity at the CCR5 locus has any protective effect, since M/T-tropic isolates should be able to use chemokine coreceptors other than CCR5 for primary infection. Perhaps the primary targets for infection are macrophages or dendritic cells (18) and CCR5 expression is critical for infection of these targets. In an attempt to understand these issues, the impact of the homozygous or heterozygous CCR5 Δ32 genotype on in vitro and in vivo infection with HIV-1 was examined. The impact of CCR5 coreceptor expression on the kinetics of HIV-1 infection in vivo was examined in the human PBL-SCID (hu-PBL-SCID) model (23–25), and the results were compared to those from a study on the kinetics of in vitro HIV-1 replication in cultures of peripheral blood mononuclear cells (PBMC) from the same donors.

* Corresponding author. Mailing address: Department of Immunology—IMM7, The Scripps Research Institute, 10550 N. Torrey Pines Rd., La Jolla, CA 92037. Phone: (619) 784-9121. Fax: (619) 784-9190. E-mail: dmosier@scripps.edu.

† Publication 10630-IMM of The Scripps Research Institute.

Adult C.B-17 SCID mice were repopulated with 20×10^6 PBMC from CCR5 Δ32/Δ32, CCR5 Δ32/+, and normal +/+ donors by previously described techniques (23, 25). Donors were normal volunteers participating in The Scripps Research Institute General Clinical Research Center donor pool. DNA samples from 237 donors were typed for the presence of the 32-bp deletion by PCR amplification of CCR5 sequences with the following flanking primers: 5′-GTCTTCATTACACCTGCAGCTCT-3′ (sense) and 5′-CACAGCCCTGTGCCTCTT-3′ (antisense). The resulting PCR products (184 bp for wild-type CCR5 and 152 bp for the Δ32 allele) were separated on a 6% acrylamide gel and visualized by ethidium bromide staining. Two CCR5 Δ32/Δ32 donors and 38 CCR5 Δ32/+ heterozygotes were identified. Both homozygous donors and 36 of the 38 heterozygotes were Caucasian, in agreement with previous findings (12, 19), making the allele frequencies in our sample of Caucasians 1 and 20% for Δ32/Δ32 and Δ32/+, respectively.

The hu-PBL-SCID mice generated from these donors were challenged 2 weeks later with 10^3 tissue culture infectious doses of the M-, M/T-, and T-tropic HIV-1 isolates listed in Table 1. The V3 sequences of the isolates, which correlate with chemokine coreceptor usage (5, 7), are also presented in Table 1. Low-passage primary isolates were included to preclude in vitro selection of variants with altered cell tropism (20). For in vitro experiments, PBMC were activated with 2 μg of phytohemagglutinin per ml and 20 U of interleukin-2 per ml for 2 to 3 days prior to HIV-1 infection. Viral replication in hu-PBL-SCID mice was assessed in serial measurements of plasma HIV RNA copy number in individual animals by using the Amplicor HIV-1 Monitor assay (Roche Molecular Systems, Branchburg, N.J.). Virus replication in culture was measured by an HIV-1 p24 viral capsid antigen enzyme-linked immunosorbent assay (Dupont Medical Products, Boston, Mass.).

Hu-PBL-SCID mice constructed from CCR5 Δ32/Δ32 donors were resistant to infection with the M-tropic virus isolates CS93 and AB28, showed 10-fold-reduced plasma viremia 1 week following infection with the M/T-tropic virus 89.6, and had near-normal kinetics of infection with the T-tropic virus SF2 compared to hu-PBL-SCID mice constructed from normal donors (Table 2). The decline in plasma viremia 2 weeks after infection with HIV-1 89.6 has been observed in several other

TABLE 1. V3 sequences of patient and laboratory HIV-1 isolates used in these studies[a]

Isolate	V3 sequence	Cell tropism
CS93	CTRPNNNTRK--SIHI--GPGRAFYATGDIIGNIRQAHC	M-tropic
FS3	CTRPSNNTRK--SIHI--GPGRAFYATGTITGDIRQAHC ----------A-A--------	M-tropic
CD65	CTRPNNNTRK--GIHI--GPGRAVYATDRIIGDIRQAHC	T-tropic
AB28	CTRPNNNTRR--SIHI--GPGRAFYATGDIIGDIRQAHC ---------K----------	M-tropic
89.6	CTRPNNNTRRRLSI----GPGRAFYARRNIIGDIRQAHC	M/T-tropic
SF2	CTRPNNNTRR--SIYI--GPGRAFHTTGRIIGDIRKAHC	T-tropic

[a] Virus isolates from patients were expanded in cultured PBMC for 2 weeks to generate the low-passage stock used for further in vitro and in vivo studies. When two sequences are given, the second sequence was a minor variant in the pool. The CP66 T-tropic isolate has not been sequenced. The sequences of isolates 89.6 and SF2 have been previously reported (4, 12). CS93 and FS3 are primary M-tropic, non-syncytium-inducing isolates from long-term nonprogressor (>12 years) hemophiliac patients. AB28 is an M-tropic, non-syncytium-inducing isolate from a patient with AIDS. CD65 is a T-tropic, syncytium-inducing isolate from a patient with AIDS. The SF2 and 89.6 isolates have been described previously (8, 21). SF2 has been typed as T-tropic, although recent evidence suggests that it can use CCR5 for entry into transfected cell lines (5).

experiments and is associated with accelerated depletion of $CD4^+$ T cells (24). This may explain the decline in plasma viral RNA levels 2 weeks after infection of hu-PBL-SCID mice derived from the CCR5 Δ32/Δ32 donor. The absence of the CCR5 coreceptor thus precludes infection with M-tropic virus, slows infection with a M/T-tropic isolate, and has little effect on infection with the T-tropic SF2 isolate. The partial protective effect of CCR5 deletion on infection by M/T-tropic HIV-1 may help explain the apparent absence of any HIV-1 infection in all CCR5 Δ32/Δ32-exposed individuals surveyed (12, 19). Exposure to T-tropic HIV-1 may be rarer since such variants generally arise late in the course of the disease (10, 30), but from these results, CCR5 deletion would not be predicted to have any protective effect against infection with such isolates.

In two separate experiments, the kinetics of virus replication were examined in hu-PBL-SCID mice derived from CCR5 Δ32/+ donors. These mice showed significantly delayed kinetics of virus replication after infection with the primary M-tropic virus isolate CS93 compared to hu-PBL-SCID mice derived from homozygous wild-type donors (Fig. 1). The mean plasma viral RNA copy numbers were 20- to 50-fold lower at 1 week after infection of hu-PBL(Δ32/+)-SCID mice compared to mice derived from CCR5 +/+ donors and continued to remain at least 1 log lower at subsequent time points (Fig. 1). This kinetic delay in the plasma virus levels could be explained by a substantially lower efficiency of primary infection followed by an equivalent virus doubling time, since the rates of increase of plasma viremia were similar for the two groups of animals.

PBMC from the same set of donors were used for in vitro studies of HIV-1 infection. As previously reported (11, 19, 22) for

TABLE 2. Plasma HIV RNA copy numbers in hu-PBL-SCID mice at 1 and 2 weeks postinfection

CCR5 genotype	HIV-1 isolate	Cell tropism	RNA copy number (mean ± SE) at week	
			1	2
+/+	89.6	M/T-tropic	70,435 ± 35,025	<400
Δ32/Δ32	89.6	M/T-tropic	6,609 ± 3,492	519 (1/5 +)[b]
+/+	SF2	T-tropic	<800	2,999 ± 1,431
Δ32/Δ32	SF2	T-tropic	1,685 ± 1,157	3,249 ± 1,040
+/+	CS93	M-tropic	1,782 ± 721	4,751 ± 1,298
Δ32/Δ32	CS93	M-tropic	Undetectable	Undetectable
+/+	AB28	M-tropic	80,192 ± 49,010	129,116 ± 111,959
Δ32/Δ32	AB28	M-tropic	Undetectable	Undetectable

[a] SCID mice were reconstituted with 20×10^6 PBMC from donors who were classified as CCR5 Δ32/Δ32 homozygotes or wild type (+/+) by PCR typing. Mice were infected with 10^3 tissue culture infectious doses of the indicated HIV-1 isolate 2 weeks after reconstitution, and plasma samples were obtained 1 and 2 weeks after infection. Plasma concentrations of HIV-1 RNA were determined by the Amplicor HIV-1 Monitor assay (Roche Molecular Systems). The values shown represent the means (± standard error) of RNA copy numbers from three to five mice per HIV-1 isolate, except for CCR5 Δ32/Δ32 hu-PBL-SCID mice at 2 weeks after infection with isolate 89.6, in which case the HIV-1 RNA level of the one positive animal is reported.

[b] Only one of five SCID mice in this group had detectable HIV RNA in plasma at 2 weeks postinfection.

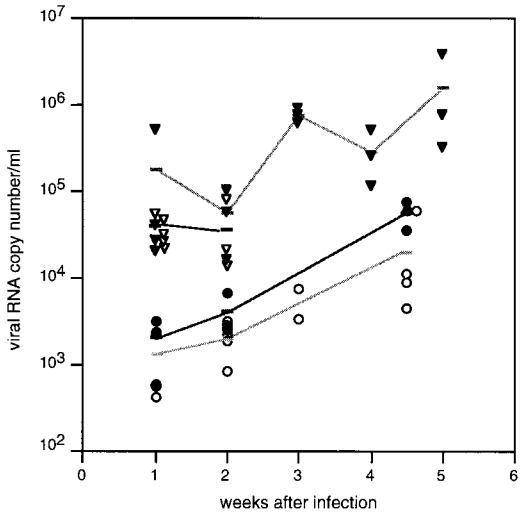

FIG. 1. Kinetics of HIV-1 replication in hu-PBL-SCID mice reconstituted with PBMC from either CCR5 +/+ donors (▼) or CCR5 Δ32/+ donors (○). Two donors of each genotype were used (one indicated by a closed symbol and one designated by an open symbol), and mice derived from the same donor are indicated by the same symbol. Mean values for the individual mice in each group are indicated by the horizontal bars, and lines connect the group means. All mice were infected with 10^3 tissue culture infectious doses of HIV-1 CS93, a primary, M-tropic, non-syncytium-inducing isolate.

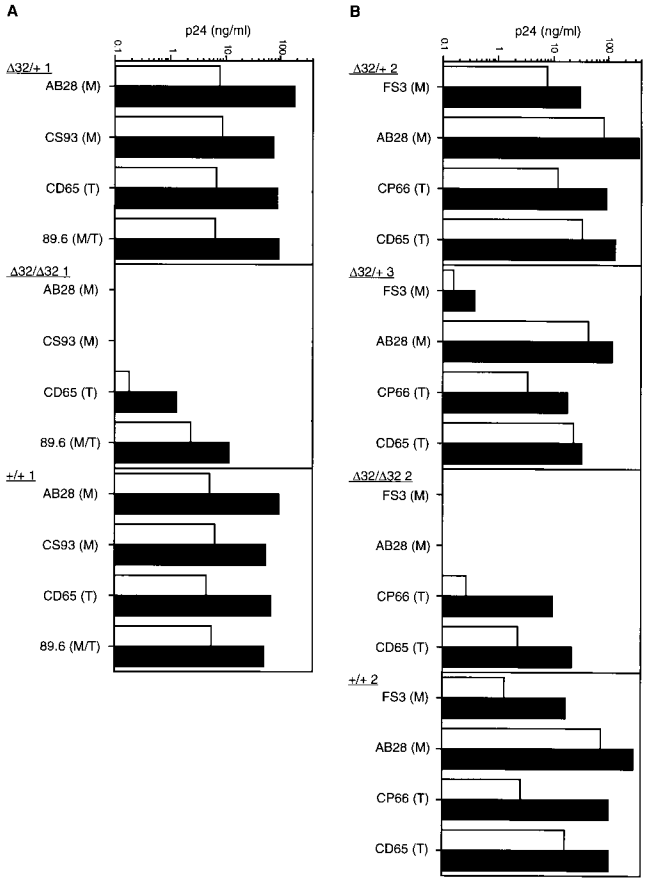

FIG. 2. Infection of PBMC from donors with different CCR5 genotypes in vitro. PBMC were stimulated with phytohemagglutinin (2 μg/ml) and interleukin-2 (20 U/ml) for 2 days and then infected with the indicated HIV-1 isolate. Release of the HIV-1 p24 capsid antigen into the medium was measured at 4 (open columns) and 11 (filled columns) days after infection. Note that results are plotted on a logarithmic scale in nanograms of p24 per milliliter. Panels A and B represent two different experiments, with different donors for each experiment (donor numbers are shown after genotypes). M, M-tropic; T, T-tropic; M/T, M/T-tropic.

other M-tropic isolates, PBMC from CCR5 Δ32/Δ32 donors resisted infection with the primary M-tropic isolates FS3, AB28, and CS93 (Fig. 2). However, PBMC from CCR5 Δ32/+ heterozygous donors were as permissive for HIV-1 replication as cells from homozygous CCR5 wild-type donors in two of three heterozygous donors studied (Fig. 2). One CCR5 Δ32/+ heterozygous donor (donor 3 in Fig. 2B) showed poor replication of the M-tropic FS3 isolate compared to other heterozygous donors and the homozygous wild-type donors. Similar variability in heterozygous donor PBMC has been observed by others (19, 22). Three HIV-1 isolates, the well-characterized M/T-tropic virus 89.6 (14, 27) and the primary CD65 and CP66 isolates, replicated in PBMC from CCR5 Δ32/Δ32 donors, but not as well as in cells from heterozygous or homozygous +/+ donors. These HIV-1 isolates thus can use coreceptors other than CCR5 for entry but at an apparently reduced efficiency. The CD65 and CP66 isolates replicate in MT-2 cells but not in primary macrophages, so they would be classified as T-tropic by traditional criteria. It is unclear whether they can use CCR5 for entry into transfected cell lines but still be unable to infect macrophages, as has recently been reported for the T-tropic HIV-1 isolate SF2 (5).

These results demonstrate that coreceptor usage influences the efficiency of primary infection in the hu-PBL-SCID model to a greater extent than is observed with infection of activated PBMC in vitro or by the ability of virus to enter cell lines transfected with CD4 and chemokine coreceptors. For example, the M/T-tropic virus 89.6 uses CCR5, CCR3, CCR2b, and CXCR4 for virus entry in transfected cell lines with equal efficiency (6, 14), although the absence of CCR5 expression in hu-PBL-SCID mice infected with 89.6 led to a 1-log reduction of the plasma HIV-1 RNA copy number (Table 2) and replication of virus 89.6 was reduced in cultured PBMC from a CCR5 Δ32/Δ32 donor (Fig. 2A). A more striking contrast between the hu-PBL-SCID model and PBMC cultures was observed when heterozygous CCR5 Δ32/+ donors were studied.

The delayed kinetics of HIV-1 CS93 infection in hu-PBL-SCID mice derived from heterozygous CCR5 Δ32/+ donors (Fig. 1) suggests that primary infection of heterozygous individuals may be slowed, perhaps resulting in a more effective immune response and lower plasma viral RNA levels (19). No effect of CCR5 heterozygosity was observed in PBMC cultures infected with HIV-1 CS93 (Fig. 2A). The advantage of the hu-PBL-SCID model for revealing a phenotype for CCR5 Δ32/+ donors may relate to the rapid clearance of free virus in vivo, which may make the efficiency of virus entry more of a contributory factor to virus replication than in in vitro assays. The level of CCR5 expression in heterozygous individuals is unknown, but it is possible that the truncated protein product of the deleted allele interferes with intracellular trafficking of the intact protein, resulting in a lower level of CCR5 expression than the anticipated 50% reduction. It appears from the limited number of heterozygous individuals examined that there may be variation in CCR5 expression between different heterozygous donors (Fig. 2) and that the effect of the proposed low-level CCR5 expression may be more apparent with certain virus isolates (e.g., FS3). Different HIV-1 isolates may interact with the same CCR5 receptor in different ways (3) and be more or less dependent on expression levels. Heterozygosity for the CCR5 Δ32 allele thus could be more protective against disease progression with some HIV-1 isolates than expected, and more extensive studies with the hu-PBL-SCID model may be useful for determining the extent and mechanism of protection. These issues could be important in the context of the proposed use of chemokine receptor antagonists to block HIV-1 infection (2, 16).

Nucleotide sequence accession numbers. The sequence data for AB28, CD65, CS93, and FS3 are available from GenBank under accession no. AF001428 to AF001431.

This work was supported by NIH grant AI29182.

We thank Rebecca Sabbe, Andrew Beernink, and Matthew Kohls for skilled technical assistance. The General Clinical Research Center of The Scripps Research Institute (supported by NIH grant M01 RR00833) provided DNA samples for CCR5 genotyping as well as blood collection from volunteer donors.

REFERENCES

1. **Alkhatib, G., C. Combadiere, C. Broder, Y. Feng, P. Kennedy, P. Murphy, and E. Berger.** 1996. CC CKR5: a RANTES, MIP-1α, MIP-1β receptor as a fusion cofactor for macrophage-tropic HIV-1. Science **272:**1955–1958.
2. **Arenzana-Seisdedos, F., J.-L. Virelizier, D. Rousset, I. Clark-Lewis, P. Loetscher, B. Moser, and M. Baggiolini.** 1996. HIV blocked by chemokine antagonist. Nature **383:**400.
3. **Atchison, R., J. Gosling, F. Monteclaro, C. Franci, L. Digilio, I. Charo, and M. Goldsmith.** 1996. Multiple extracellular elements of CCR5 and HIV-1 entry: dissociation from response to chemokines. Science **274:**1924–1926.
4. **Biti, R., R. French, J. Young, B. Bennetts, G. Stewart, and T. Liang.** 1997. HIV-1 infection in an individual homozygous for the CCR5 deletion allele. Nature Med. **3:**252–253.
5. **Cheng-Mayer, C., R. Liu, N. R. Landau, and L. Stamatatos.** 1997. Macrophage tropism of human immunodeficiency virus type 1 and utilization of CC-CKR5 coreceptor. J. Virol. **71:**1657–1661.
6. **Choe, H., M. Farzan, Y. Sun, N. Sullivan, B. Rollins, P. Ponath, L. Wu, C. Mackay, G. LaRosa, W. Newman, N. Gerard, C. Gerard, and J. Sodroski.**

1996. The β-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. Cell **85:**1135–1148.
7. **Cocchi, F., A. DeVico, A. Garzino-Demo, A. Cara, R. Gallo, and P. Lusso.** 1996. The V3 domain of the HIV-1 gp120 envelope glycoprotein is critical for chemokine-mediated blockade of infection. Nature Med. **2:**1244–1247.
8. **Collman, R., J. W. Balliet, S. A. Gregory, H. Friedman, D. L. Kolson, N. Nathanson, and A. Srinivasan.** 1992. An infectious molecular clone of an unusual macrophage-tropic and highly cytopathic strain of human immunodeficiency virus type 1. J. Virol. **66:**7517–7521.
9. **Combadiere, C., S. Ahuja, H. Tiffany, and P. Murphy.** 1996. Cloning and expression of CC CKR5, a human monocyte CC chemokine receptor selective for MIP-1 (alpha), MIP-1 (beta), and RANTES. J. Leukocyte Biol. **60:**147–152.
10. **Connor, R. I., H. Mohri, Y. Cao, and D. D. Ho.** 1993. Increased viral burden and cytopathicity correlate temporally with $CD4^+$ T-lymphocyte decline and clinical progression in human immunodeficiency virus type 1-infected individuals. J. Virol. **67:**1772–1777.
11. **Connor, R. I., W. A. Paxton, K. E. Sheridan, and R. A. Koup.** 1996. Macrophages and $CD4^+$ T cells from two multiply exposed, uninfected individuals resist infection with primary non-syncytium-inducing isolates of human immunodeficiency virus type 1. J. Virol. **70:**8758–8764.
12. **Dean, M., M. Carrington, C. Winkler, G. Huttley, M. Smith, R. Allikmets, J. Goedert, S. Buchbinder, E. Vittinghoff, E. Gomperts, S. Donfield, D. Vlahov, R. Kaslow, A. Saah, C. Rinaldo, R. Detels, and S. O'Brien.** 1996. Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CKR5 structural gene. Science **273:**1856–1861.
13. **Deng, H., R. Liu, W. Ellmeier, S. Choe, D. Unutmaz, M. Burkhart, P. Di Marzio, S. Marmon, R. Sutton, C. Hill, C. Davis, S. Peiper, T. Schall, D. Littman, and N. Landau.** 1996. Identification of a major co-receptor for primary isolates of HIV-1. Nature **381:**661–666.
14. **Doranz, B., J. Rucker, Y. Yi, R. Smyth, M. Samson, S. Peiper, M. Parmentier, R. Collman, and R. Doms.** 1996. A dual-tropic primary HIV-1 isolate that uses fusin and the β-chemokine receptors CKR-5, CKR-3 and CKR-2b as fusion cofactors. Cell **85:**1149–1158.
15. **Dragic, T., V. Litwin, G. Allaway, S. Martin, Y. Huang, K. Nagashima, C. Cayanan, P. Maddon, R. Koup, J. Moore, and W. Paxton.** 1996. HIV-1 entry into $CD4^+$ cells is mediated by the chemokine receptor CC-CKR-5. Nature **381:**667–673.
16. **D'Souza, M., and V. Harden.** 1996. Chemokines and HIV-1 second receptors. Nature Med. **2:**1293–1300.
17. **Feng, Y., C. Broder, P. Kennedy, and E. Berger.** 1996. HIV-1 entry co-factor: functional cDNA cloning of a seven-transmembrane, G-protein coupled receptor. Science **272:**873–877.
18. **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. Steinman.** 1996. Efficient interaction of HIV-1 with purified dendritic cells via multiple chemokine receptors. J. Exp. Med. **184:**2433–2438.
19. **Huang, Y., W. Paxton, S. Wolinsky, A. Neumann, L. Zhang, T. He, S. Kang, D. Ceradini, Z. Jin, K. Yazdandakhsh, K. Kunstman, D. Erickson, E. Dragon, N. Landau, J. Phair, D. Ho, and R. Koup.** 1996. The role of a mutant CCR5 allele in HIV-1 transmission and disease progression. Nature Med. **2:**1240–1243.
20. **Koito, A., L. Stamatatos, and C. Cheng-Mayer.** 1995. Small amino acid sequence changes within the V3 domain can affect the function of a T-cell line-tropic human immunodeficiency virus type 1 envelope gp120. Virology **206:**878–884.
21. **Levy, J., A. Hoffman, S. Kramer, J. Landis, J. Shimabukuro, and L. Oshiro.** 1984. Isolation of lymphocytotropic retroviruses from San Francisco patients with AIDS. Science **225:**840–842.
22. **Liu, R., W. Paxton, S. Choe, D. Ceradini, S. Martin, R. Horuk, M. MacDonald, H. Stuhlmann, R. Koup, and N. Landau.** 1996. Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. Cell **86:**367–377.
23. **Mosier, D., R. Gulizia, P. MacIsaac, B. Torbett, and J. Levy.** 1993. Rapid loss of CD4+ T cells in human-PBL-SCID mice by noncytopathic HIV isolates. Science **260:**689–692.
24. **Mosier, D. E.** 1996. Human immunodeficiency virus infection of human cells transplanted to severe combined immunodeficient mice. Adv. Immunol. **63:**79–125.
25. **Mosier, D. E., R. J. Gulizia, S. M. Baird, D. B. Wilson, D. H. Spector, and S. A. Spector.** 1991. Human immunodeficiency virus infection of human-PBL-SCID mice. Science **251:**791–794.
26. **Paxton, W. A., S. R. Martin, D. Tse, T. R. O'Brien, J. Skurnick, N. L. VanDevanter, N. Padian, J. F. Braun, D. P. Kotler, S. M. Wolinsky, and R. A. Koup.** 1996. Relative resistance to HIV-1 infection of CD4 lymphocytes from persons who remain uninfected despite multiple high-risk sexual exposure. Nature Med. **2:**412–417.
27. **Rucker, J., M. Samson, B. Doranz, F. Libert, J. Berson, Y. Yi, R. Smyth, R. Collman, C. Broder, G. Vassart, R. Doms, and M. Parmentier.** 1996. Regions in β-chemokine receptors CCR5 and CCR2b that determine HIV-1 cofactor specificity. Cell **87:**437–446.
28. **Samson, M., O. Labbe, C. Mollereau, G. Vassart, and M. Parmentier.** 1996. Molecular cloning and functional expression of a new CC-chemokine receptor gene. Biochemistry **35:**3362–3367.
29. **Samson, M., F. Libert, B. Doranz, J. Rucker, C. Liesnard, C.-M. Farber, S. Saragosti, C. Lapoumeroulie, J. Cognaux, C. Forceille, G. Muyldermans, C. Verhofstede, G. Burtonboy, M. Georges, T. Imai, S. Rana, Y. Yi, R. Smyth, R. Collman, R. Doms, G. Vassart, and M. Parmentier.** 1996. Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. Nature **382:**722–725.
30. **Tersmette, M., R. E. Y. de Goede, B. J. M. Al, I. N. Winkel, R. A. Gruters, H. T. Cuypers, H. G. Huisman, and F. Miedema.** 1988. Differential syncytium-inducing capacity of human immunodeficiency virus isolates: frequent detection of syncytium-inducing isolates in patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. J. Virol. **62:**2026–2032.
31. **Zhang, L., Y. Huang, T. He, Y. Cao, and D. Ho.** 1996. HIV-1 subtype and second-receptor usage. Nature **383:**768.