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The Envelope gp120 Gene of Human Immunodeficiency Virus Type 1 Determines the Rate of CD4-Positive T-Cell Depletion in SCID Mice Engrafted with Human Peripheral Blood Leukocytes†

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We have used envelope recombinant viruses generated between two molecular clones of human immunodeficiency virus type 1 (HIV-1), T-cell-tropic HIV-1_{SF2} and macrophage-tropic HIV-1_{SF162}, to assess pathogenic potential in the human peripheral blood leukocyte-reconstituted severe combined immune deficiency mouse model. Recombinant HIV-1_{SF2} viruses expressing the envelope gp120 gene of HIV-1_{SF162} caused as rapid a $CD4^+$ T-cell depletion as did HIV-1_{SF162}. The reciprocal HIV-1_{SF162} recombinant virus with the HIV-1_{SF2} envelope caused slower $CD4^+$ T-cell loss. Although changing the V3 loop sequence of HIV-1_{SF162} to that of HIV-1_{SF2} did not change the rate of $CD4^+$ T-cell depletion, replacing the V3 of HIV-1_{SF2} with the sequence of HIV-1_{SF162} resulted in virus that was poorly infectious in vivo but not in vitro. These studies suggest that the envelope gene determines properties important for pathogenesis in vivo as well as for cell tropism in vitro. HIV-1 infection in vivo may have more stringent requirements for envelope conformation.

Infection with the human immunodeficiency virus type 1 (HIV-1) leads to a characteristic loss of CD4-positive T lymphocytes in patients (11) and in small animal models constructed by grafting human lymphoid tissue (1, 13, 19) or cells (17, 18) into mice with the severe combined immune deficiency (SCID) mutation. Infection with HIV-1 isolates which differ in their cell tropisms, cytopathic effects, sensitivities to neutralization, and replicative capacities caused different rates of $CD4^+$ T-cell depletion as measured in the hu-PBL-SCID model (15, 17). The replication rate and cell tropism appear to be the primary determinants in the hu-PBL-SCID model, in which infection with macrophage-tropic isolates that produce high viral burdens uniformly leads to the most rapid and extensive $CD4^+$ T-cell depletion (15, 16). To determine if the recombinant viruses originally used to map macrophage tropism to the envelope gene in studies of replication in monocytes/macrophages, primary peripheral blood mononuclear cell (PBMC) cultures, and T-cell lines (4, 21) could also map the determinants of $CD4^+$ T-cell depletion in vivo, we have employed a series of recombinant or mutant viruses constructed from the HIV-1_{SF2} and HIV-1_{SF162} molecular clones to infect hu-PBL-SCID mice.

We have previously shown that two macrophage-tropic isolates, HIV-1_{SF162} and HIV-2_{UC1} (3–5, 8), cause an unexpectedly rapid loss of human $CD4^+$ T lymphocytes in infected hu-PBL-SCID mice (15). We infected hu-PBL-SCID mice, which were prepared by injecting C.B-17 *scid/scid* mice intraperitoneally with 2×10^7 human PBMCs prepared from normal HIV- and Epstein-Barr virus-seronegative donors as described previously (15, 18), with two additional macrophage-tropic isolates, HIV-1_{128A} (12) and HIV-$1_{Ba\text{-}L}$ (9), to determine if our earlier observations reflected a general feature of macrophage-tropic viruses. The percentage of surviving $CD4^+$ T cells was determined after 2 weeks of virus infection, a time which distinguishes HIV strains that cause high, intermediate, or low rates of $CD4^+$ T depletion (15, 16). Cells were recovered from the peritoneal cavity and local lymph nodes of hu-PBL-SCID mice and analyzed by flow cytometry after they were stained with the following antibodies: anti-H-$2K^d$ (Pharmingen, San Diego, Calif.) and anti-CD45, anti-CD3, anti-CD4, and anti-CD8 (all Becton Dickinson, Mountain View, Calif.). To standardize for the variable recovery of $CD45^+$ human cells, which ranged from 13.4 to 92% (mean ± standard error, 56% ± 2%; $n = 97$) in peritoneal lavage and from 21 to 96% (mean ± standard error, 67% ± 4%; $n = 31$) in local lymph nodes, $CD4^+$ T-cell recovery was expressed as a percentage of the number of $CD3^+$ human T cells. The results

TABLE 1. Macrophage-tropic HIV isolates uniformly cause rapid CD4 T-cell depletion

Viral strain	Cell tropism[a]	Mean % control CD4 T cells ± SE at 2 weeks after infection[b]	No. of expts
None		100	7
HIV-1_{SF162}	Macrophage/monocyte	12 ± 2.6	7
HIV-1_{SF128A}	Macrophage/monocyte	7	1
HIV-2_{UC1}	Macrophage/monocyte	8.5 ± 0.5	2
HIV-$1_{Ba\text{-}L}$	Macrophage/monocyte	6.5 ± 2.5	2
HIV-1_{SF2}	T-cell line	41 ± 12	5

[a] Cell tropism was determined by the ability to replicate in primary macrophage/monocyte cultures and T-cell lines. The macrophage/monocyte-tropic isolates will also replicate in freshly isolated human $CD4^+$ T cells but not in such established T-cell lines as HUT 78, CEM, and Jurkat.

[b] hu-PBL-SCID mice were infected with 1,000 $TCID_{50}$ of the indicated viral strains at 2 weeks after peripheral blood lymphocyte reconstitution, and recovery of human CD4, CD8, and $CD3^+$ T cells in the peritoneal lavage was determined by flow cytometry 2 weeks later. The percentage of $CD4^+$ T cells was calculated relative to the number of $CD3^+$ T cells for each of three to five animals, and the mean for the group of animals was calculated. The data shown here are the percentages of recovered $CD4^+$ T cells relative to the number for control uninfected animals in one to seven replicate experiments, and the standard error is the experiment-to-experiment variation. At later time points after infection, T-cell-tropic HIV-1 isolates lead to a more complete loss of $CD4^+$ T cells.

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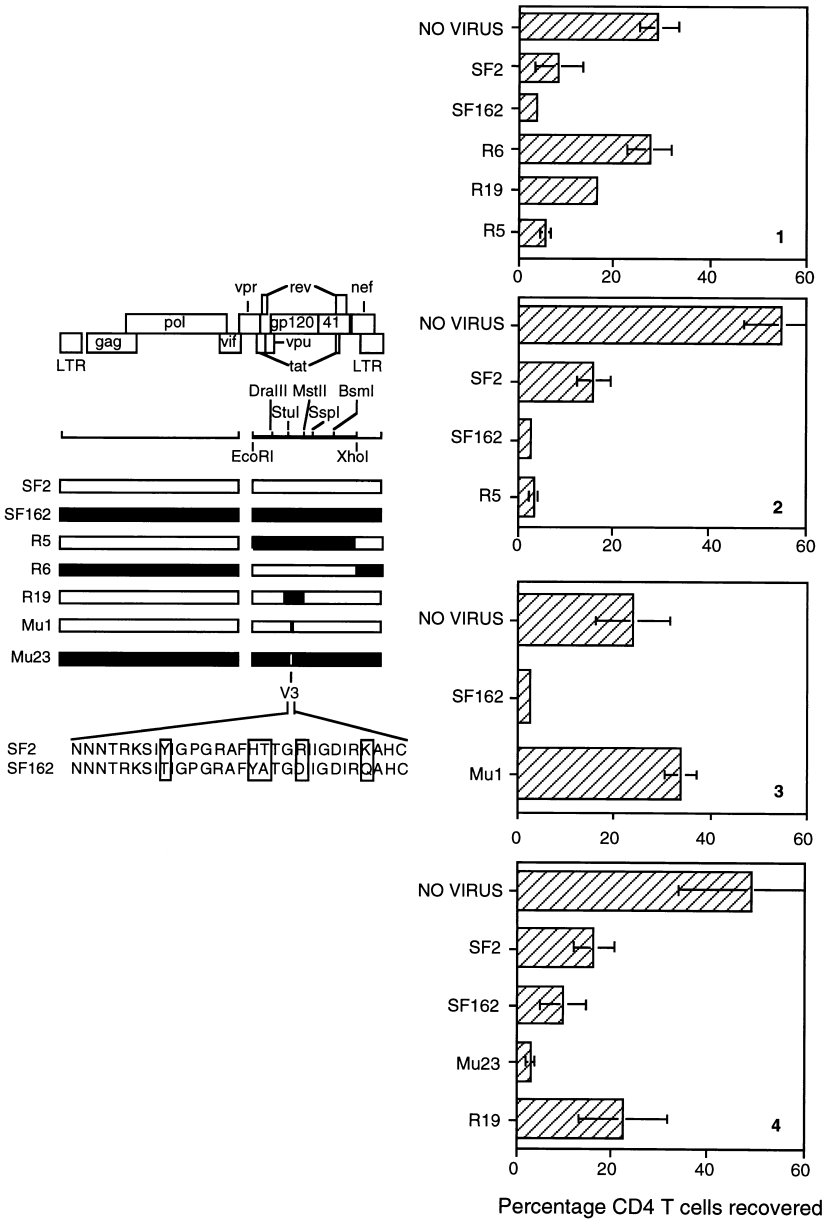

FIG. 1. Recovery of human $CD4^+$ T cells in four experiments in which recombinant HIV-1 viruses R5, R6, and R19, viruses with five reciprocal amino acid substitutions in V3 of gp120 (Mu1 and Mu23), and the parental SF2 and SF162 molecular clones were used to infect hu-PBL-SCID mice. The restriction map of the recombinant viruses (6, 20, 21) and the V3 sequences involved in the Mu1 and Mu23 viruses (21) are shown at the left, and the percentages of recovered human $CD4^+$ T cells in each of the four experiments (1 to 4) are plotted in the bar graphs at the right. Each bar represents the mean ± standard error of the mean of $CD4^+$ T-cell determinations for three to five replicate animals. The percentage of $CD4^+$ T cells was calculated relative to the number of $CD3^+$ T cells, with each type of T cell being measured by flow cytometry analysis of human cells recovered by peritoneal lavage 2 weeks after HIV-1 infection. Infection with HIV-1 was by intraperitoneal injection of cell-free virus, except for experiment 3, in which 10^6 PBMCs (from the same donor used to engraft SCID mice) infected with either HIV-1_{SF162} or Mu1 were injected to permit infection with the poorly infectious Mu1 recombinant. The animals were infected with 10^3 tissue culture infective doses of cell-free virus, except for the R19 virus in experiment 4, in which 10^4 tissue culture infective doses were used for infection.

are summarized in Table 1. Both HIV-1_{128A} and HIV-$1_{Ba\text{-}L}$ caused as rapid a depletion of human $CD4^+$ T cells as did HIV-1_{SF162}, and all macrophage-tropic strains tested caused more extensive depletion than the T-cell-tropic HIV-1_{SF2} isolate.

Since four of four macrophage-tropic isolates had the same phenotype in hu-PBL-SCID mice, we proceeded to determine if the same genetic determinants of cell tropism were required for rapid $CD4^+$ T-cell depletion in this animal model. We infected groups of five hu-PBL-SCID mice with either HIV-1_{SF2}, HIV-1_{SF162}, or recombinant viruses R5, R6, or R19 (4, 20, 21) and with V3 substitution mutants Mu1 and Mu23 in a series of six double-blinded experiments. Virus stocks were generated in short-term cultures in human PBMCs, and their 50% tissue culture infective doses ($TCID_{50}$) were determined by dilution in PBMCs as described previously (14). R5 and R6 recombinant viruses (Fig. 1) were injected at 1,000 $TCID_{50}$ per mouse, and they infected all hu-PBL-SCID mice in four of four

TABLE 2. Replication of HIV-1 in vitro and in hu-PBL-SCID mice

Virus	Replication in PBMC[a]	Proviral copy number[b]	Plasma RNA copy number[c]
SF2	1,578	63	3,838
SF162	1,081	1,422	217,949
Mu1	1,561	<10	435
Mu23	2,127	>1,000	ND

[a] Data are reverse transcriptase activities (10^3) after 10 days of culture. Data are from reference 21.

[b] Data are proviral copy numbers per 10^5 recovered cells and were determined by limiting dilution of input DNA from hu-PBL-SCID spleen cells. Data are from reference 15.

[c] RNA copy numbers were determined in plasma samples from hu-PBL-SCID mice used in experiment 3 (Fig. 1). Competitive RNA PCR was performed with the Amplicor HIV Monitor assay kit provided by Roche Molecular Systems, Somerville, N.J. ND, not done.

experiments (see below), as did the same $TCID_{50}$ of parental SF2 and SF162 viruses. Infection of hu-PBL-SCID mice was documented by the recovery of infectious virus by coculture of peritoneal lavage and spleen cells with human PBMCs that had been activated with phytohemagglutinin (2 μg/ml) and interleukin 2 (5 U/ml) and by amplification of proviral *gag* sequences with the SK38 and SK39 primers as previously described (15, 18). In one experiment (Table 2), plasma HIV-1 RNA levels were determined by the Amplicor HIV Monitor assay (Roche Molecular Systems, Somerville, N.J.). The R19 recombinant containing the 0.49-kb *Stu*I-*Mst*II fragment from SF162 infected only 40% of hu-PBL-SCID mice at 1,000 $TCID_{50}$ per mouse in experiment 1 (Fig. 1; only data from infected mice are presented) and an additional preliminary experiment, but increasing the infecting dose to 10,000 $TCID_{50}$ resulted in the infection of five of five mice in experiment 4 (Fig. 1). We also used the Mu1 and Mu 23 (Mu 10) mutant viruses (21). Mu1 has five amino acid substitutions in the V3 loop of SF2 to generate the SF162 V3 sequence (Fig. 1). Cell-free virus stocks of Mu1 were not infectious for hu-PBL-SCID mice at 1,000 $TCID_{50}$ per animal (data not shown), so we infected hu-PBL-SCID mice with 10^6 cultured, autologous PBMCs infected 3 days earlier with Mu1 or SF162 (Fig. 1, experiment 3). This procedure resulted in the infection of all mice but poor virus replication (Table 2). Mu23 has the five amino acid substitutions to generate the SF2 V3 sequence in the context of the SF162 genome (21), and this virus readily infected all mice. These recombinant and mutant viruses show similar levels of replication in primary PBMC cultures (Table 2).

Figure 1 shows the mean percentages of human $CD4^+$ T cells (relative to the total number of CD3 T cells) recovered in infected mice in four representative experiments and also shows the restriction map of the recombinant viruses studied. Infection with the R5 recombinant virus caused nearly as much $CD4^+$ T-cell depletion as did infection with HIV-1_{SF162} (Fig. 1, experiments 1 and 2). The reciprocal R6 recombinant virus caused little loss of $CD4^+$ T cells in two experiments (e.g., Fig. 1, experiment 1) and a $CD4^+$ T-cell loss similar to that caused by infection with SF2 in a third experiment (data not shown). The R19 recombinant virus caused less $CD4^+$ T-cell depletion than HIV-1_{SF162} in two experiments (Fig. 1, experiments 1 and 4) although viral burdens were comparable to infection with HIV-1_{SF2}. Introduction of cells infected with Mu1 led to no loss of $CD4^+$ T cells compared with levels in uninfected mice (Fig. 1, experiment 3). However, Mu1 was not only poorly infectious after being introduced as free virus, it replicated to very low levels even after infection of mice was achieved with peripheral blood lymphocytes infected in vitro (Table 2). In contrast, infection with Mu23, which was highly infectious both in vivo and in vitro, led to more extensive $CD4^+$ T-cell depletion than infection with wild-type HIV-1_{SF162}. The V3 sequence of SF162 thus can be altered to that of SF2 without changing the ability of the virus to induce rapid $CD4^+$ T-cell loss.

These studies with recombinant viruses demonstrate that the envelope sequences previously shown to determine macrophage tropism also are important in determining the rate of human $CD4^+$ T-cell depletion observed following infection of hu-PBL-SCID mice. Nonetheless, the in vitro phenotypes of the chimeric viruses containing changes within the envelope gene did not always predict the in vivo biologic properties of these viruses. Several experiments showed that four independent macrophage-tropic isolates, including HIV-1_{SF162}, produced rapid loss of $CD4^+$ T cells (Table 1). Similarly rapid depletion was caused by infection with the R5 recombinant virus containing nucleotides 5720 to 8914 of the SF162 molecular clone (Fig. 1). The R6 recombinant virus containing only the 3.2-kb *Eco*RI-*Xho*I fragment of HIV-1_{SF2} caused $CD4^+$ T-cell depletion that was less than or equivalent to that caused by SF2 in three experiments (Fig. 1 and data not shown). The R19 recombinant virus containing only the 0.48-kb *Stu*I-*Mst*II fragment of SF162 gp120 was much less effective than R5 in causing CD4 T-cell loss in three of three experiments. Thus, expression of amino acids 174 to 332 of gp120, while sufficient to confer macrophage tropism in vitro (20), is insufficient to cause the rapid loss of $CD4^+$ T cells in vivo. The experiments with Mu1 and Mu23 (21) indicate that changes in only the V3 loop alter the in vivo pathogenic potentials of the viruses in ways that can't be predicted on the basis of their in vitro phenotype. Mu1 is severely attenuated for both infectivity and CD4 T-cell depletion compared with SF2 in the hu-PBL-SCID model (Fig. 1 and Table 2), whereas it shows low-level replication in macrophages and high-level replication in PBMCs in vitro (21). Mu23 also shows poor replication in primary macrophages compared with that of SF162 but replicates well in PBMC cultures (21). Despite this modest difference in vitro, Mu23 was able to induce as much $CD4^+$ T-cell depletion as SF162 in vivo. Changes in the envelope conferred by the V3 sequence thus can be important for pathogenesis in the hu-PBL-SCID model and can lead to poorly infectious virus in vivo. Minor changes in V3 may have a major impact on infectivity in animal models, as has been noted with simian immunodeficiency virus type 1 envelope recombinants (10). The viral determinants of macrophage tropism may therefore influence other biologic properties of the virus in vivo, including replication or initial infectivity, and these properties may have different sensitivities to envelope conformation.

One explanation for our data thus is that macrophage-tropic viruses are most effective than most T-cell-line tropic HIV-1 isolates in inducing $CD4^+$ T-cell depletion because the different envelope sequences (7) alter the conformation of gp120. However, other biologic variables related to cell tropism may contribute to our results. Virus replicating in macrophages may acquire membrane components expressed uniquely or at higher levels on macrophages (2). Alternatively, macrophage-tropic viruses may replicate to higher levels in the hu-PBL-SCID model than T-cell-tropic viruses. Although the data in Table 2 support this possibility, data from other studies do not. No difference in proviral copy number was observed for HIV-1_{SF33} replication and HIV-1_{SF162} replication (21). Other T-cell-tropic isolates (e.g., NL4-3 and LAI) show levels of replication as high as those of HIV-1_{SF162} and yet cause rates of $CD4^+$ T-cell depletion lower than those of HIV-1_{SF162} (data

not shown). Although replication to some threshold level of viral burden may be required to cause the loss of $CD4^+$ T cells, replication beyond that threshold does not appear to have a predictable effect in the hu-PBL-SCID model. These data thus indicate that the envelope sequence is a determinant of in vivo pathogenic potential in ways not always predicted by in vitro studies and that envelope determinants associated with macrophage tropism often, but not always, influence the rate of $CD4^+$ T-cell depletion in the hu-PBL-SCID model.

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