

Mice transgenic for human CD4 and CCR5 are susceptible to HIV infection

JESSIE BROWNING*, JAMES W. HORNER*, MASSIMO PETTOELLO-MANTOVANI†, CHRISTINA RAKER†, SERGEY YURASOV†, RONALD A. DEPINHO*, AND HARRIS GOLDSTEIN*†‡

Departments of *Microbiology and Immunology and †Pediatrics, Albert Einstein College of Medicine, Bronx, NY 10461

Communicated by Barry R. Bloom, Albert Einstein College of Medicine, Bronx, NY, October 29, 1997 (received for review August 20, 1997)

ABSTRACT HIV entry into human cells is mediated by CD4 acting in concert with one of several members of the chemokine receptor superfamily. The resistance to HIV infection observed in individuals with defective CCR5 alleles indicated that this particular chemokine receptor plays a crucial role in the initiation of *in vivo* HIV infection. Expression of human CD4 transgene does not render mice susceptible to HIV infection because of structural differences between human and mouse CCR5. To ascertain whether expression of human CD4 and CCR5 is sufficient to make murine T lymphocytes susceptible to HIV infection, the *lck* promoter was used to direct the T cell-specific expression of human CD4 and CCR5 in transgenic mice. Peripheral blood mononuclear cells and splenocytes isolated from these mice expressed human CD4 and CCR5 and were infectible with selected M-tropic HIV isolates. After *in vivo* inoculation, HIV-infected cells were detected by DNA PCR in the spleen and lymph nodes of these transgenic mice, but HIV could not be cultured from these cells. This indicated that although transgenic expression of human CD4 and CCR5 permitted entry of HIV into the mouse cells, significant HIV infection was prevented by other blocks to HIV replication present in mouse cells. In addition to providing *in vivo* verification for the important role of CCR5 in T lymphocyte HIV infection, these transgenic mice represent a new *in vivo* model for understanding HIV pathogenesis by delineating species-specific cellular factors required for productive *in vivo* HIV infection. These mice should also prove useful for the assessment of potential therapeutic and preventative modalities, particularly vaccines.

Although CD4 was identified initially as the cellular receptor for HIV (1), several lines of evidence indicated that expression of CD4 alone was insufficient to confer susceptibility to infection by the virus (2). Specifically, HIV did not infect mouse cells transfected with a human CD4 expression vector (3) or mice transgenic for the expression of human CD4 (4) despite the binding of the HIV envelope protein, gp120, to the human CD4 expressed on the cell surface. Expression of human CD4 alone is insufficient to confer sensitivity to HIV infection because gp120, after interacting with CD4, must subsequently bind to a second receptor, such as a member of the chemokine receptor superfamily, to initiate membrane fusion and viral penetration (5). Since the initial identification of CXCR4 as a coreceptor for HIV (6), it has been shown that several other chemokine receptors, including CCR5 (7–10) and chemokine receptor-like molecules (11, 12), can also function as coreceptors for HIV infection. Functional analysis of CXCR4 and CCR5 has clarified the basis for the divergent cellular tropisms exhibited by different isolates of HIV (13). T-tropic HIV isolates that infect T cell lines and peripheral T

cells but not monocytes use CXCR4 as a coreceptor, whereas M-tropic HIV isolates that infect monocytes/macrophages and peripheral T cells but not T cell lines use CCR5 as a coreceptor (5). Identification of the phenotype of HIV isolated during different stages of disease from infected individuals has indicated that M-tropic HIV isolates play a critical role in establishing infection (14). The importance of M-tropic HIV isolates in the initial infectious process was further demonstrated by the observation that individuals homozygous for a 32-bp deletion in the CCR5 gene were resistant to HIV infection despite multiple exposures to HIV and that mononuclear cells from these individuals were resistant to *in vitro* HIV infection with M-tropic HIV isolates (15, 16). Thus, although HIV binding and internalization can be mediated by CD4 acting together with one of several members of the chemokine receptor superfamily, CCR5 appears to be the critical coreceptor used by HIV in the initial stages of infection. Because mouse CCR5 differs significantly from human CCR5 (17), it cannot function as a coreceptor for HIV, and thus, expression of human CD4 alone was insufficient to permit entry of HIV into mouse cells. Therefore, we investigated whether mice transgenic for human CD4 and CCR5 would be susceptible to HIV infection.

METHODS

Construction of Transgenes. A 3.2-kb *NotI/BamHI* fragment containing the proximal promoter for lymphocyte-specific protein tyrosine kinase *p56lck* (obtained from R. Perlmutter, University of Washington, Seattle) was blunted at the 5' *NotI* overhang and subcloned into pBluescript KS(–) at a blunted *KpnI* and a *BamHI* restriction site. The resulting plasmid, pLCK-A+, also contains 847 bp of simian virus 40 poly(A) tail coding sequence, which was inserted into the multicloning site at *BamHI* and *SpeI*. A 1.1-kb DNA fragment containing the CCR5 gene with a 5' *BamHI* site and 3' *XhoI* site was generated by PCR amplification of human genomic DNA. The reaction was run for 30 cycles at 94°C for 30 sec, 60°C for 30 sec, and 68°C for 2 min by using primers 5'-GTCTGAGTCTGAGTCGGATCCAACAAGATGGATTATCAA and 3'-GTCTGAGTCTGAGTCCTCGAGTCCGTGT-CACAAGCCAC that have *BamHI* and *XhoI* sites and overlap the ATG and Stop codons, respectively. The amplified 1.1-kb CCR5 gene was digested with *BamHI* and *XhoI* and subcloned into the pLCK-A+ vector at the *BamHI* and *Sall* site. The resulting plasmid, pLCK-CCR5-A+, was digested with *SfiI* and *NotI* and purified for microinjection into mouse embryos. The plasmid pT4B (obtained from the National Institute of Allergy and Infectious Diseases AIDS Research and Reference Reagent Program, Bethesda, MD) containing

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

© 1997 by The National Academy of Sciences 0027-8424/97/9414637-6\$2.00/0
PNAS is available online at <http://www.pnas.org>.

Abbreviations: PBMCs, peripheral blood mononuclear cells; RT-PCR, reverse transcription-PCR.

‡To whom reprint requests should be addressed at: Albert Einstein College of Medicine, Chanin Building, Room 601, 1300 Morris Park Avenue, Bronx, NY 10461. e-mail: hgoldste@aecom.yu.edu.

the human CD4 cDNA (3) was digested with *EcoRI* and *BamHI* to obtain a 1.8-kb fragment containing the coding sequence. Blunt ends were generated at the 5' and 3' ends, and the CD4 cDNA fragment was inserted at the *EcoRV* site within pLCK-A+. Directionality of the CD4 insert was confirmed by restriction mapping. The construct was liberated by digestion of pLCK-CD4-A+ with *SfiI* and *NotI* and purified for microinjection into mouse embryos.

Detection of CD4 DNA and CCR5 DNA by PCR. DNA was extracted from transgenic mouse tails and purified by phenol extraction and alcohol precipitation. Human CD4 and CCR5 DNA were detected by PCR amplification (45 cycles at 94°C for 30 sec, 65°C for 30 sec, and 72°C for 1 min) with *taq* polymerase (GIBCO/BRL) by using primers 5'-GTGGAGTTCAAATA-GACATCGTG-3' and 5'-CAGCACCCACCCGCTTCC-CGCTT-3', and 5'-CACCTGCAGCTCTCATTTTCC-3' and 5'-TTGTAGGGAGCCAGAAAGAG-3' specific for the human CD4 and CCR5, respectively. No PCR products were detected after PCR amplification of control mouse DNA with these human CCR5 and CD4 primer pairs.

Flow Cytometric Analysis. Mononuclear cells harvested from the peripheral blood of the mice were stained with fluorescein isothiocyanate-conjugated mouse monoclonal antibody to human CD4 (Becton Dickinson) and/or phycoerythrin-conjugated rat monoclonal antibody to mouse CD4, CD8, or B220 (PharMingen), or with fluorescein isothiocyanate-conjugated mouse monoclonal antibody to human CCR5 (PharMingen) and phycoerythrin-conjugated mouse monoclonal antibody to CD4 (Becton Dickinson) as described (18). Expression of the surface proteins was assessed by two-color flow cytometric analysis by using a FACScan cell analyzer with LYSIS-II software (Becton Dickinson), and nonviable cells and unlysed red blood cells were gated out based on their forward- and side-scatter profiles.

Infection with HIV-1. Primary M-tropic HIV-1 strains HIV-1₅₉ (19) and 92RW009A, 92TH022E (20), HIV-1_{JR-CSF}, or HIV-1_{JR-FL} (21) obtained from the National Institute of Allergy and Infectious Diseases AIDS Research and Reference program were used in this study. For *in vitro* infection, peripheral blood mononuclear cells (PBMCs) or splenocytes isolated by density centrifugation were cultured in microplates (10⁵ cells per well) of the indicated isolate. The cultures were washed, and the p24 antigen content of the culture supernatant was measured at the indicated times by using the HIV-1 p24 core profile ELISA assay (DuPont/NEN). For *in vivo* infection, mice were inoculated with the indicated isolate by intrasplenic (300 TCID₅₀) or i.p. (8,000 TCID₅₀) injection. The mice were evaluated for evidence of HIV-1 infection at the indicated time by examining for the presence of HIV-1 *gag* DNA by DNA PCR, *tat/rev* RNA sequences by reverse transcription-PCR (RT-PCR), and infectious virus by coculture with activated human PBMCs as described (18). The positive control for detection of HIV DNA by PCR was HIV DNA (1,000 copies) obtained from 8E5 cells (from the NIH AIDS Research and Reagent Program) and for detection of HIV RNA by RT-PCR was RNA isolated from HIV-infected human T cells, and the negative controls were buffer and enzyme mixtures lacking DNA or cDNA as described (18).

RESULTS

Construction of Mice Transgenic for Human CD4 and CCR5. *In vitro* studies have demonstrated that mouse fibroblast cell lines such as 3T3 can become susceptible to HIV-1 entry after transient transfection with human CD4 and CCR5 expression vectors (7, 8). To further examine the role of CCR5 as a coreceptor for HIV-1 in a more physiological context, we developed several independent mouse lines transgenic for both human CD4 and CCR5 and investigated whether lymphocytes obtained from these mice could become infected with HIV-1.

Although M-tropic HIV-1 isolates can infect both monocytes and peripheral T cells, T cells are the critical target for HIV-1 infection as evidenced by their production of greater than 90% of the plasma virus present in infected individuals (22, 23). Therefore, the T cell-specific *lck* promoter (24) was used to construct vectors LCK-CD4-A+ and LCK-CCR5-A+ (Fig. 1A) targeting expression of human CD4 and CCR5 to T cells in the transgenic mice. Transgenic founders harboring both transgenes were generated by co-microinjection of these constructs into the pronuclei of F2 hybrid oocytes from FVBxC57/B6 parents as described (25). Of 54 offspring, PCR analysis of tail DNA identified 10 transgenic founder mice that had integrated both the LCK-CD4-A+ and LCK-CCR5-A+ constructs (Fig. 1B). Flow cytometric analysis using a monoclonal antibody specific for human CD4 demonstrated that three of these transgenic founder mice expressed varying levels of human CD4 on the cell surface of their PBMCs (Fig. 2A). In addition, human CCR5 mRNA was detected in PBMCs isolated from these three transgenic founder by RT-PCR (data not shown). Expression of both human CD4 and CCR5 on the surface of PBMCs isolated from progeny of transgene founder mouse 18 was demonstrated by two-color flow cytometric analysis (Fig. 2B). Human CD4 and CCR5 expression were detected in 5 of 10 progeny of transgene founder mouse 18, suggesting that the integrated genes in this transgenic line were in strong linkage disequilibrium and most likely integrated in tandem. To determine whether human CD4 was expressed on T cells, two-color flow cytometry analysis was performed on the mouse PBMCs to evaluate human CD4 expression by the CD4+ T cells, CD8+ T cells, and B cells in the peripheral blood of two F1 mice derived from transgene founder mouse 18. Although human CD4 was expressed on the majority of mouse CD4+ and CD8+ T cells, it was not detected on the mouse B cells (Fig. 3), indicating that the *lck* promoter was directing expression of the transgene to the mouse T cells as expected.

In Vitro Infection of Leukocytes from Human CD4/CCR5 Transgenic Mice. To determine the susceptibility of leukocytes from these transgenic mice to infection by HIV-1, PBMCs isolated from founder transgenic mice and control mice were incubated with a primary patient M-tropic isolate of HIV-1,

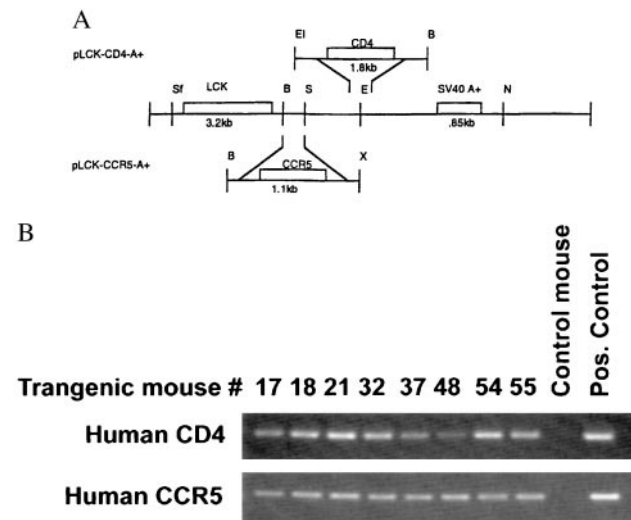


FIG. 1. (A) Constructs used to generate transgenic mice. Human CD4 or CCR5 was cloned into the transgene under the control of the *lck* proximal promoter as shown. Restriction enzyme sites shown are: *SfiI* (Sf), *BamHI* (B), *SalI* (S), *EcoRV* (E), *NotI* (N), *XhoI* (X), and *EcoRI* (EI). (B) Detection of integrated human CD4 and CCR5 in the transgenic mice. DNA was extracted from the tails of the transgenic mice, and integrated human CD4 and CCR5 were detected by PCR amplification with primer pairs specific for human CD4 and CCR5.

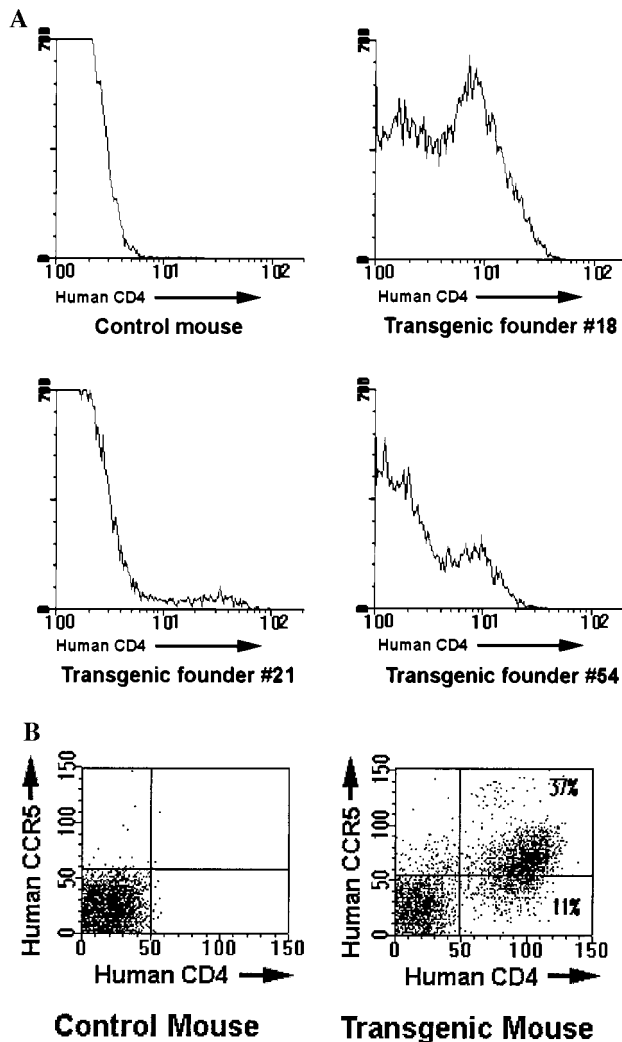


FIG. 2. (A) Expression of human CD4 by PBMCs of transgenic mice. PBMCs were harvested from the transgenic founder mice, and surface expression of human CD4 was detected by flow cytometry by using a fluorescein isothiocyanate-conjugated mouse monoclonal antibody specifically directed against human CD4. (B) Expression of human CCR5 by PBMCs of transgenic mice. PBMCs harvested from a transgenic mouse derived from transgenic founder mouse 18 and a control mouse were evaluated for surface expression of human CD4 and CCR5 by two-color flow cytometry by using mouse monoclonal antibodies specifically directed against human CD4 and CCR5. The percentage of positive cells in each quadrant is indicated.

HIV-1₅₉. Three days later, the cultures were washed, and 6 days later the culture supernatants were evaluated for the presence of HIV p24 antigen. Productive HIV-1 infection indicated by the presence of this antigen in the culture supernatant was detected in cultures of PBMCs from the transgenic mice and not in cultures of PBMCs from the control mice (Fig. 4). The extent of HIV-1 infection in the PBMCs from the transgenic mice correlated with cellular expression of human CD4. Cultures containing cells from transgenic mouse 18, which had the highest level of human CD4 expression, produced the most p24 antigen, and cultures containing cells from transgenic mouse 21, which had the lowest level of human CD4 expression, produced the least p24 antigen.

To further evaluate the *in vitro* sensitivity of leukocytes from the human CD4/CCR5 transgenic mice to infection with HIV-1, splenocytes from transgenic mouse 18 were harvested and infected with several isolates of HIV-1. After 6 days of culture, the cells were washed, and then the p24 antigen concentration in the culture supernatant was measured on day

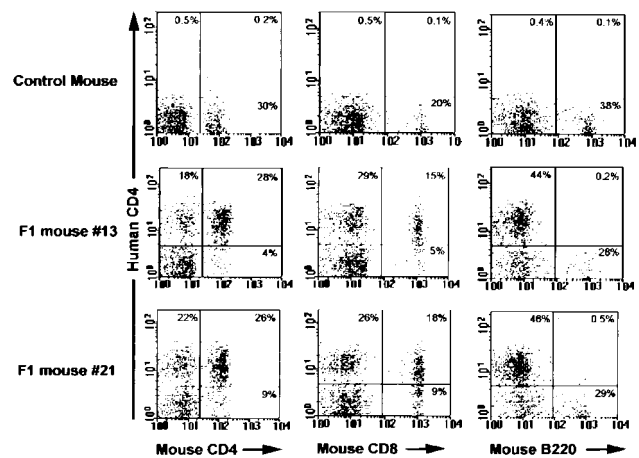


FIG. 3. Selective expression of human CD4 by mouse T cells. PBMCs were isolated from a control mouse and two F1 progeny of founder mouse 18. Expression of human CD4 by the mouse CD4⁺ T cells, CD8⁺ T cells, and B cells was analyzed by two-color flow cytometry. The percentage of positive cells in each quadrant is indicated.

6, day 8, and day 11 of culture. After 11 days of culture, the splenocytes were further evaluated for integrated HIV-1 by *gag*-specific DNA PCR and for active viral replication by RT-PCR detection of spliced *tat/rev* RNA sequences. Infection by HIV-1 isolates HIV-1_{JR-CSF}, HIV-1_{JR-FL}, and HIV-1₅₉ was indicated by the production of increasing amounts of p24 antigen (Fig. 5A) and the detection of HIV-1 *gag* DNA (Fig. 5B) and *tat/rev* RNA (Fig. 5C) sequences in the transgenic mouse splenocytes. In comparison, 1–2 weeks after infection of human PBMCs with the same inoculum of HIV-1_{JR-CSF}, HIV-1_{JR-FL}, HIV-1₅₉, 92RW009a, and 92TH022E isolates, p24 antigen production of 34,655, 29,935, 43,945, 37,045, and 33,848 pg/ml, respectively, was obtained.

In Vivo Infection of Human CD4/CCR5 Transgenic Mice. The susceptibility of the human CD4/CCR5 transgenic mice to *in vivo* HIV-1 infection was evaluated by inoculating them with different HIV-1 strains either by intrasplenic injection or by i.p. injection. As shown in Table 1, although HIV-1 *gag* DNA sequences were present in mononuclear cells isolated from the spleen and lymph nodes of the human CD4/CCR5 transgenic mice 4 weeks after inoculation, HIV-1 *tat/rev* RNA sequences were not detectable by RT-PCR and HIV-1 could

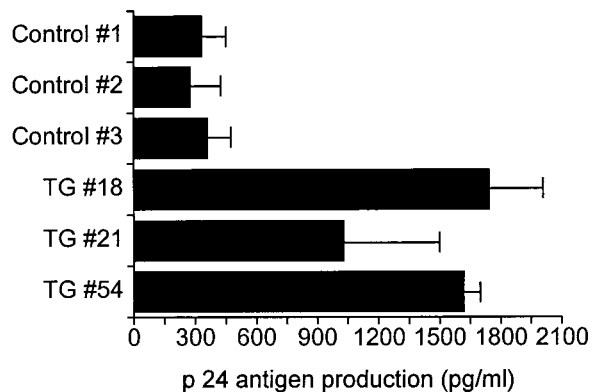


FIG. 4. HIV infection of PBMCs of the founder mice. Peripheral blood mononuclear cells from three founder mice and three control mice were infected with HIV-1₅₉ at a multiplicity of infection of 0.025 (4,394 pg of p24). Three days after infection the cells were extensively washed, and 6 days later the level of p24 antigen in the culture supernatant was determined. The data shown are the mean \pm SEM of two separate experiments.

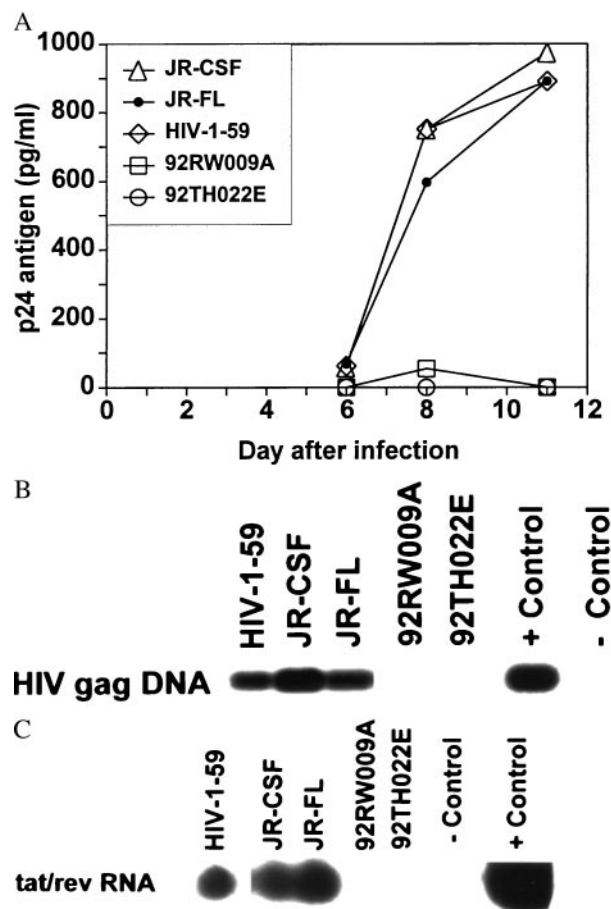


FIG. 5. HIV infection of splenocytes from a human CD4/CCR5 transgenic mouse. Splenocytes from founder mouse 18 were isolated, cultured in 24-well plates (1×10^6 per well) in the presence of IL-2 (50 units/ml) and infected with the indicated isolates at a multiplicity of infection of about 0.004 (p24 antigen concentration of 3,465 pg, 2,993 pg, 4,394 pg, 3,704 pg, and 3,384 pg for the JR-CSF, JR-FL, HIV-159, 92RW009A, and 92TH022E isolates, respectively). After 6 days of culture, all of the medium was removed and the cells were washed three times and fed with fresh medium containing IL-2. (A) At the designated time points, cleared supernatant was harvested and analyzed for the presence of p24 antigen. After 2 weeks of culture, the splenocytes were evaluated for the presence of *gag* DNA (B) and *tat/rev* RNA (C) by PCR.

not be isolated by coculture (data not shown). Thus, although expression of human CD4 and CCR5 in the transgenic mice overcame the block that prevented entry of HIV-1 into mouse cells, sustained *in vivo* replication of HIV-1 still did not occur.

DISCUSSION

The pathogenesis of HIV-1 infection is a result of the complex interaction between many different factors that can best be studied by using an *in vivo* model. Because humans and primates appear to be the sole hosts for HIV-1 infection, studies investigating the effectiveness of novel therapies and vaccines in preventing or modifying the course of HIV-1 infection have been restricted. The present report provides verification that *in vivo* expression of human CD4 and CCR5 in primary mouse lymphocytes permits these cells to be infected with primary HIV-1 isolates. Low levels of productive *in vitro* infection of the transgenic mouse splenocytes was indicated by the rise in p24 antigen levels produced during the course of culture after infection with certain M-tropic isolates, HIV-1_{JR-CSF}, and HIV-1_{JR-FL}, but not with others, such as 92RW009A and 92TH022E. Cellular infection was indi-

cated further by the detection of HIV-1 *gag* DNA and spliced *tat/rev* RNA in the cells. The observation that the transgenic mouse splenocytes were infected by some M-tropic isolates and not by others suggested that different HIV-1 isolates may have varying capacity to productively infect mouse cells. It is possible that many rounds of *in vitro* passage of these isolates in the transgenic mouse T cells would generate HIV-1 isolates that are better adapted for replicating in mouse cells.

The major barrier preventing HIV-1 infection of mouse cells, a block in cellular penetration because of structural differences between human and mouse CD4 and CCR5, was overcome by the expression of human CD4 and CCR5 by T cells in these transgenic mice. Although leukocytes from the human CD4/CCR5 transgenic mice were infectible with HIV-1 *in vitro*, the degree of p24 antigen production was 1–2 logs less than that observed after infection of human leukocytes, and infectious virus was not isolated from the culture supernatant by secondary coculture with human PBMCs (data not shown). The decreased replication of HIV-1 in mouse cells has been attributed to the reduced function of certain HIV-1 regulatory genes in mouse cells (26). The activity of HIV-1 *tat*, the major positive regulator of HIV-1 gene expression, is markedly decreased in mouse cell lines (27). The resistance of mouse cells to *tat*-mediated trans-activation is a result of the absence of a species-specific cofactor encoded by human chromosome 12 that is required for the binding of Tat to its cis-responsive binding element, TAR (28). The function of another HIV-1 regulatory gene product, Rev, which facilitates transport of incompletely spliced HIV-1 mRNAs from the nucleus into the cytoplasm, is also markedly diminished in mouse fibroblast cell lines (27, 29). However, although the activity of Tat and Rev may be restricted in some mouse tissues such as fibroblasts, they may be functional in other mouse tissues such as lymphoid cells. For example, substantial Tat-mediated trans-activation was detected in a mouse macrophage line, RAW264, that was mediated by interaction between Tat and TAR (30). Furthermore, although Rev function was severely blocked in some mouse cell lines, functional Rev activity was observed in a murine T cell hybridoma (28). Thus, sufficient activity of these regulatory genes may occur in the T cells from the human CD4/CCR5 transgenic mice, permitting them to be infected with HIV-1 albeit at a much lower level than human T cells. Nevertheless, our results indicated that these levels were insufficient to permit sustained productive *in vivo* infection in mice transgenic for human CD4 and CCR5. It is also possible that the absence of *in vivo* HIV-1 replication in these mice was a result of the defective function of other HIV-1 regulatory genes in mouse cells, such as *vif*, which is responsible for the production of infectious virions (31) and the lack of other factors required for virion assembly and budding.

Taken together, these results indicated that although expression of human CD4 and a chemokine receptor such as CCR5 may be sufficient to permit entry of HIV-1 into mouse cells, the combined effect of impairment in other stages of HIV-1 replication in mouse cells may prevent the development of sustained *in vivo* infection in these human CD4/CCR5 transgenic mice. Therefore, the presence of additional blocks that prevent efficient HIV-1 replication in mouse cells complicates the use of transgenic mice to investigate the immunopathology of HIV-1 infection. It is possible that modification of HIV-1 proviruses so that they contain alternate regulatory genes that are active in mouse cells would permit productive infection to occur in these mice. For example, when the murine leukemia virus (MLV) core enhancer was inserted into the HIV-1 LTR, increased transcriptional activity of the HIV-1 LTR was observed in mouse cells, and mice transgenic for an HIV-1 provirus containing this MLV/HIV chimeric long terminal repeat produced infectious virus particles (32). If productive infection would occur in these human CD4/

Table 1. *In vivo* infection of human CD4/CCR5 transgenic mice

Mouse	HIV isolate	Time after last inoculation	Spleen			Lymph node		
			<i>gag</i> DNA	<i>tat/rev</i> RNA	Coculture	<i>tat/rev</i> RNA	<i>gag</i> DNA	Coculture
Control mice								
#1	HIV-1 ₅₉	2 weeks	–	–	–	–	–	–
#2	HIV-1 ₅₉	4 weeks	–	–	–	–	–	–
#3	HIV-1 ₅₉	4 weeks	–	–	–	–	–	–
#4	HIV-1 ₁₇₇₉	4 weeks	–	–	–	–	–	–
CD4/CCR TG mice								
TG#1	HIV-1 ₅₉	2 weeks	–	–	–	–	–	–
TG#2	HIV-1 ₅₉	2 weeks	–	–	–	–	–	–
TG#3	HIV-1 ₅₉	4 weeks	+	–	–	–	ND	–
TG#4	HIV-1 ₁₇₇₉	4 weeks	–	–	–	–	+	–
TG#5	HIV-1 ₅₉	4 weeks	+	–	–	–	–	–
TG#6	HIV-1 _{JR-CSF}	4 weeks	+	–	–	–	+	–
TG#7	HIV-1 _{JRFL}	4 weeks	+	–	–	–	–	–

Mice were infected by intrasplenic inoculation of 300 TCID₅₀ (control #1, #2, #3, and TG #1, #2, and #3) or i.p. injection of 8,000 TCID₅₀ (control #4 and TG #4, #5, and #6) of the indicated isolate. TG #4, #5, and #6 were inoculated weekly four times. The mice were sacrificed, and the splenocytes and lymph node cells were evaluated for HIV *gag* DNA and *tat/rev* RNA by PCR and for the presence of infectious virus by coculture. ND, not done.

CCR5 transgenic mice after infection with modified HIV-1 proviruses, then they could be used to examine whether a vaccine-stimulated immune response could prevent infection. Thus, the human CD4/CCR5 transgenic mice described in this report represent a productive first step in the development of a new *in vivo* system to evaluate the effectiveness of candidate HIV-1 vaccines and to study the pathobiology of HIV-1 infection.

This work was supported by the National Institutes of Health (National Institute of Allergy and Infectious Diseases Grant AI-36664 and Centers for AIDS Research Grant AI-27741). M.P.M. was supported by a National Institutes of Health training grant (CA09173). Flow cytometry was performed in the Flow Cytometry Core Facility, and oligonucleotides were synthesized in the Oligonucleotide Synthesis Core Facility supported by the Cancer Center Grant (5P30CA13330).

- Klatzmann, D., Champagne, E., Chamaret, S., Gruet, J., Guetard, D., Herceud, T., Gluckman, J. C. & Montagnier, L. (1984) *Nature (London)* **312**, 20–27.
- Fauci, A. S. (1988) *Science* **239**, 617–622.
- Maddon, P. J., Dagleish, A. G., McDougal, J. S., Clapham, P. R., Weiss, R. A. & Axel, R. (1986) *Cell* **47**, 333–358.
- Lores, P., Boucher, V., Mackay, C., Pla, M., Von Boehmer, H., Jami, J., Barre-Sinoussi, F. & Weill, J. C. (1992) *AIDS Res. Hum. Retro.* **8**, 2063–2071.
- Berger, E. A. (1997) *AIDS* **11**, S3–S16.
- Feng, Y., Broder, C. C., Kennedy, P. E. & Berger, E. A. (1996) *Science* **272**, 872–877.
- Deng, H., Liu, R., Ellmeier, W., Choe, S., Unutmaz, D., Burkhart, M., Di Marzio, P., Marmon, S., Sutton, R. E., Hill, C. M., Davis, C. B., Peiper, S. C., Schall, T. J., Littman, D. R. & Landau, N. R. (1996) *Nature (London)* **381**, 661–667.
- Dragic, T., Litwin, V., Allaway, G. P., Martin, S. R., Huang, Y., Nagashima, K. A., Cayanan, C., Maddon, P. J., Koup, R. A., Moore, J. P. & Paxton, W. A. (1996) *Nature (London)* **381**, 667–673.
- Choe, H., Farzan, M., Sun, Y., Sullivan, N., Rollins, B., Ponath, P. D., Wu, L., Mackay, C. R., LaRosa, G., Newman, W., Gerard, N., Gerard, C. & Sodroski, J. (1996) *Cell* **85**, 1135–1148.
- Doranz, B. J., Rucker, J., Yi, Y., Smyth, R. J., Samson, M., Peiper, S. C., Parmentier, M., Collman, R. G. & Doms, R. W. (1996) *Cell* **85**, 1149–1158.
- Deng, H. K., Unutmaz, D., Kewal Ramani, V. N. & Littman, D. R. (1997) *Nature (London)* **388**, 296–300.
- Liao, F., Alkhatib, G., Peden, K. W., Sharma, G., Berger, E. A. & Farber, J. M. (1997) *J. Exp. Med.* **185**, 2015–2023.

- Schuitemaker, H., Kootstra, N. A., de Goede, R. E., de Wolf, F., Miedema, F. & Tersmette, M. (1991) *J. Virol.* **66**, 1354–1360.
- Schuitemaker, H., Koot, M., Kootstra, N. A., Dercksen, M. W., de Goede, R. E., van Steenwijk, R. P., Lange, J. M., Schattenkerk, J. K., Miedema, F. & Tersmette, M. (1992) *J. Virol.* **66**, 1354–1360.
- Liu, R., Paxton, W. A., Choe, S., Ceradini, D., Martin, S. R., Horuk, R., MacDonald, M. E., Stuhlmann, H., Koup, R. A. & Landau, N. R. (1996) *Cell* **86**, 367–377.
- Samson, M., Libert, F., Doranz, B. J., Rucker, J., Liesnard, C., *et al.* (1996) *Nature (London)* **382**, 722–725.
- Atchison, R. E., Gosling, J., Monteclaro, F. S., Franci, C., Digilio, L., Charo, I. F. & Goldsmith, M. A. (1996) *Nature (London)* **274**, 1924–1926.
- Kollmann, T. R., Pettoello-Mantovani, M., Zhuang, X., Kim, A., Hachamovitch, M., Smarnworawong, P., Rubinstein, A. & Goldstein, H. (1994) *J. Exp. Med.* **179**, 513–522.
- Kollmann T. R., Kim, A., Pettoello-Mantovani, M., Hachamovitch, M., Rubinstein, A., Goldstein, M. M. & Goldstein, H. (1995) *J. Immunol.* **154**, 907–921.
- Koyanagi, Y., Miles, S., Mitsuyasu, R. T., Meril, J. E., Vinters, H. V. & Chen I. Y. (1987) *Science* **236**, 819–826.
- WHO Network for isolation and characterization (1994) *AIDS Res. Hum. Retroviruses* **10**, 1327–1343.
- Perelson A. S., Neumann A. U., Markowitz, M., Leonard, J. M. & Ho, D. D. (1996) *Science* **271**, 1582–1586.
- Chun, T. W., Carruth, L., Finzi, D., Shen, X., DiGiuseppe, J. A., Taylor, H., Hermankova, M., Chadwick, K., Margolick, J., Quinn, T. C., Kuo, Y. H., Brookmeyer, R., Zeiger, M. A., Barditch-Crovo, P. & Siliciano, R. F. (1997) *Nature (London)* **387**, 183–188.
- Wilden, R. S., Garvin, A. M., Pawar, S., Lewis, D. B., Abraham, K. M., Forbush, K. A., Ziegler, S. F., Allen, J. M. & Perlmutter, R. M. (1991) *J. Exp. Med.* **173**, 383–393.
- Hogan, B., Constantini, F. & Lacey, E. (1986) *Manipulating the Mouse Embryo: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
- Chesbro, B., Wehrly, K. & Maury, W. (1990) *J. Virol.* **64**, 4553–4557.
- Winslow, B. J. & Trono, D. (1993) *J. Virol.* **67**, 2349–2354.
- Newstein, M., Stanbridge, E. J., Casey, G. & Shank, P. R. (1990) *J. Virol.* **64**, 4565–4567.
- Trono, D. & Baltimore, D. (1990) *EMBO J.* **9**, 4155–4160.
- Murphy, K. M., Sweet, M. J., Ross, I. L. & Hume, D. A. (1993) *J. Virol.* **67**, 6956–6964.
- Von Schwedler, U., Song, J., Aiken, C. & Trono, D. (1993) *J. Virol.* **67**, 4945–4955.
- Dickie, P., Gazzinelli, R. & Chang, L.-J. (1996) *AIDS Res. Hum. Retroviruses* **12**, 1103–1117.