CD4-Specific Transgenic Expression of Human Cyclin T1 Markedly Increases Human Immunodeficiency Virus Type 1 (HIV-1) Production by CD4+ T Lymphocytes and Myeloid Cells in Mice Transgenic for a Provirus Encoding a Monocyte-Tropic HIV-1 Isolate

Jinglin Sun,† Timothy Soos,2,3† Vineet N. KewalRamani,2,3 Kristin Osiecki,1 Jian Hua Zheng,1 Laurie Falkin,1 Laura Santambrogio,4 Dan R. Littman,2,3 and Harris Goldstein1,5*

Departments of Microbiology & Immunology,1 Pathology,4 and Pediatrics,5 Albert Einstein College of Medicine, Bronx, New York 10461, and Molecular Pathogenesis Program2 and The Howard Hughes Medical Institute,3 Skirball Institute of Biomolecular Medicine, New York University School of Medicine, New York, New York 10016

Received 24 August 2005/Accepted 30 November 2005

Human immunodeficiency virus type 1 (HIV-1)-encoded Tat provides transcriptional activation critical for efficient HIV-1 replication by interacting with cyclin T1 and recruiting P-TEFb to efficiently elongate the nascent HIV transcript. Tat-mediated transcriptional activation in mice is precluded by species-specific structural differences that prevent Tat interaction with mouse cyclin T1 and severely compromise HIV-1 replication in mouse cells. We investigated whether transgenic mice expressing human cyclin T1 under the control of a murine CD4 promoter/enhancer cassette that directs gene expression to CD4+ T lymphocytes and monocytes/macrophages (hu-cycT1 mice) would display Tat responsiveness in their CD4-expressing mouse cells and selectively increase HIV-1 production in this cellular population, which is infected primarily in HIV-1-positive individuals. To this end, we crossed hu-cycT1 mice with JR-CSF transgenic mice carrying the full-length HIV-1JR-CSF provirus under the control of the endogenous HIV-1 long terminal repeat and demonstrated that human cyclin T1 expression is sufficient to support Tat-mediated transactivation in primary mouse CD4+ T lymphocytes and monocytes/macrophages and increases in vitro and in vivo HIV-1 production by these stimulated cells. Increased HIV-1 production by CD4+ T lymphocytes was paralleled with their specific depletion in the peripheral blood of the JR-CSF/hu-cycT1 mice, which increased over time. In addition, increased HIV-1 transgene expression due to human cyclin T1 expression was associated with increased lipopolysaccharide-stimulated monocyte chemoattractant protein 1 production by JR-CSF mouse monocytes/macrophages in vitro. Therefore, the JR-CSF/hu-cycT1 mice should provide an improved mouse system for investigating the pathogenesis of various aspects of HIV-1-mediated disease and the efficacies of therapeutic interventions.

The transcriptional activator Tat, encoded by human immunodeficiency virus (HIV), is essential for efficient viral replication (13, 19, 54). Transcriptional activation by Tat is dependent upon its binding to the transcriptional response element (TAR), an RNA stem-loop structure that is located downstream from the transcription initiation site in the 5′ long terminal repeat (LTR) (5, 18, 45). TAR displays a secondary structure that includes a 5′ bulge from position +23 to position +25 and a central loop at positions +30 to +35 (57). Although the 5′ bulge and central loop are both required for Tat functional activity, the restricted binding of Tat to the 5′ bulge and not to the central loop indicated that host cell factors were required to mediate the interaction between Tat and the central loop and subsequent transcriptional activation (32). The requirement for Tat to interact with a human-host-specific cell factor to mediate transactivation provided a rationale for the functional activity of Tat in human cells but not in mouse cells and for the capacity of transferred human chromosome 12 to confer mouse cells with the ability to support Tat transactivation (29, 46). The cellular factor involved in Tat-mediated transactivation was identified as cellular positive transcription elongation factor b (P-TEFb) (40). After P-TEFb is recruited to the nascent HIV transcript by Tat, it clears RNA polymerase II from the promoter by phosphorylating its carboxy terminal domain and thereby increasing template processivity and transcriptional elongation (42). The kinase associated with the phosphorylation activity of P-TEFb, cyclin-dependent kinase-9 (CDK-9), is required for Tat-mediated transactivation (40, 52, 71). An associated cyclin protein, cyclin T1, coordinates CDK-9 binding to Tat and is required for RNA polymerase II transcriptional elongation (65). The sequences of human cyclin T1 (hu-cycT1) and mouse cyclin T1 are 90% homologous, and the inability of Tat to function as a transcriptional activator in mouse cells is due to a single-amino-acid reciprocal change that prevents the binding of mouse cyclin T1 to Tat (7, 23, 24).
Mouse cells transfected with a construct encoding human cyclin T1 supported Tat-mediated transactivation, confirming that human cyclin T1 was the species-specific HIV type 1 (HIV-1) cofactor and that human cyclin T1 was able to interact with mouse CDK-9 to rescue Tat function in mouse cells (65).

Because the biological behavior of cell lines in vitro differs from that of primary cells in an in vivo environment, further delineation of the interaction of human cyclin T1 with Tat and its role in the activation of HIV-1 replication would be greatly facilitated by the availability of an in vivo model system. Mice are extremely useful tools for investigating the pathogenesis of infectious diseases, the regulation of the immune system, and the generation of protective immunity, but the inability of HIV-1 to infect mouse cells has severely limited the use of mice to investigate HIV-1 pathogenesis (38, 39). In addition to the inefficiency of Tat in mouse cells that is described above, another major block preventing infection of mouse cells is the inability of HIV-1 to enter mouse cells due to the fact that its envelope protein, gp120, does not bind to the murine homologues of human HIV receptors (41). The failure of HIV-1 to penetrate mouse cells can be circumvented by constructing mice transgenic for an infectious provirus derived from HIV-1, such as the X4 laboratory isolate NL4-3 (1, 37). These transgenic mice have been used to investigate in vivo activation of HIV-1 in monocytes by pathogens (10, 14, 21, 25), the efficacy of treatments that block proviral expression (55, 56), and the role of Toll-like receptors in the pathogen-induced activation of the HIV LTR (2, 16). To enable study of the behavior of primary HIV-1 isolates, we expanded upon this approach by developing a different mouse line that is transgenic for HIV-1JR-CSF, a full-length HIV-1 provirus derived from HIV-1, and these mice are populated with T lymphocytes and monocytes that produce infectious HIV-1 (8, 47). The HIV-1 produced by the JR-CSF mouse cells does not infect mouse cells, allowing our transgenic mouse system to be used to investigate factors that modulate the postintegration phase of HIV-1 replication independent of the confounding effects of secondary infection. A limitation of HIV transgenic mouse models is that, in contrast to what is seen for humans, where HIV-1 replication occurs predominantly in CD4-expressing cells, in the HIV transgenic mice the provirus is integrated in every cell and can be expressed by any cell that supports HIV LTR transcription, including cells of many lineages normally not infected by HIV. Consequently, HIV transgenic mice may not recapitulate many aspects of the pathophysiology of HIV infection. We hypothesized that expression of a human cyclin T1 transgene would markedly increase HIV-1 replication in the HIV transgenic mouse cells by enabling Tat-mediated transcriptional activation. By targeting expression of the human cyclin T1 transgene to CD4-expressing cells in the JR-CSF transgenic mice, we would selectively increase HIV-1 replication in the cell population which is the primary target of HIV-1 infection in humans. In this study we demonstrated that expression of human cyclin T1 under the control of a CD4 expression construct in the JR-CSF mice markedly increased in vivo and in vitro HIV-1 production by mouse CD4+ T lymphocytes and myeloid cells, was associated with selective depletion of CD4+ T lymphocytes in the peripheral blood, and was associated with increased monocyte chemoattractant protein 1 (MCP-1) production by stimulated mouse myeloid cells.

**MATERIALS AND METHODS**

**Construction of mouse transgenic for human cyclin T1 and HIV-1JR-CSF**

The 1.15 kb cDNA fragment encoding human cyclin T1 cloned into an engineered SalI site in a murine CD4 expression cassette targeting expression to CD4 T lymphocytes, macrophages, and dendritic cells (34). The cassette contains the murine CD4 enhancer/promoter and the human CD4 intronic sequence, which encompasses the macrophage enhancer and the CD4 silencer elements. The resulting plasmid was linearized and microinjected into the pronuclei of fertilized embryos derived from FVB × C57Bl6 mouse crosses. Transgenic founders were identified by PCR analysis of genomic DNA extracted from tails using a primer pair specific for the amplification of human cyclin T1 DNA as described below. The JR-CSF mice were constructed and characterized as previously described (8). The animal studies performed in this report were performed under a protocol reviewed and approved by the Albert Einstein College of Medicine Animal Institute Committee.

**Detection of human cyclin T1 DNA and RNA expression**

DNA was extracted from transgenic mouse tails using a DNeasy kit (QIAGEN, Valencia, CA). Human cyclin T1 was detected by PCR amplification (35 cycles at 94°C for 45 s, 55°C for 45 s, and 72°C for 1 min) with Taq polymerase (Promega, Madison, WI) using primers 5'-TCCCAACTTCAGTTGACT-3' and 5'-TACCCACGAC CGGAGGTCA-3', which were designed to be specific for the human cyclin T1 sequence and which yielded an amplimer of ~400 bp. The specificity of these primers for human cyclin T1 was confirmed by demonstrating that no PCR products were generated after PCR amplification of control mouse DNA. After RNA was purified from the indicated cells by use of an RNAeasy kit (QIAGEN) and treated with DNase, human cyclin T1 mRNA was the internal control was detected using a OneStep reverse transcription-PCR (RT-PCR) kit (QIAGEN). Briefly, RNA (1 µg) was mixed with 10 µl of 5x QIAGEN OneStep RT-PCR buffer, 2 µl of dNTP mix (containing 10 µM of each dNTP), 2 µl of QIAGEN OneStep RT-PCR enzyme mix, and 0.6 µl of the human cyclin T1-specific primers described above or 18S rRNA-specific primers 5'-TCT AAGAACGAAAATCGGAGG-3' and 5'-GGCATACTAAAGGCATCAC A-3' (58). The RT-PCR mix was heated at 95°C for 10 min and then amplified for 35 cycles (94°C for 45 s, 55°C for 45 s, and 72°C for 1 min) and electrophoresed through a 1.5% agarose gel containing ethidium bromide; the amplified product was then detected under UV light.

**Western blotting for human cyclin T1 detection**

Whole-cell extracts of thymocytes and reversed-phase column-depleted splenocytes were prepared in NP-40 radiomunonprecipitation assay buffer (150 mM NaCl, 20 mM Tris [pH 7.5], 1% NP-40, 5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 10 mM benzamidine, 2 µg/ml leupeptin), sonicated for three 1-min cycles, and clarified by centrifugation at 18,000 rpm for 15 min at 4°C. Cellular lysate (50 µg) was resolved on 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels, transferred to polyvinylidene difluoride membranes, and incubated with anti-human cyclin T1 rabbit polyclonal antibody (kindly provided by Katherine Jones, The Salk Institute) and anti-mouse actin murine monoclonal antibody (Santa Cruz Biotechnologies, Santa Cruz, CA). Bands were visualized after an incubation of the blot with the appropriate conjugated antibody and subsequently with substrate.

**Flow cytometric analysis**

Mononuclear cells harvested from the peripheral blood of the mice were stained with a phycoerythrin-conjugated rat monoclonal antibody to mouse CD8 and a fluorescein isothiocyanate-conjugated rat monoclonal antibody to CD4 (BD Biosciences, San Jose, CA) as described previously (8). Expression of surface proteins was assessed by flow cytometric analysis using a FACScan cell analyzer with LYSIS-II software (BD Biosciences). Nonviable cells and unlysed red blood cells were gated out based on their forward and side scatter profiles.

**Isolation of myeloid cells from the mouse bone marrow**

Bone marrow cells extracted from mouse femurs by lavage with phosphate-buffered saline (PBS) were dispersed with vigorous pipetting, washed, resuspended in complete medium (RPMI medium containing 10% fetal calf serum and 2-mercaptoethanol [50 µM]), and cultured in 100-mm petri dishes at a density of 2 x 10^6 cells/ml. Following overnight culturing, the nonadherent cells were gently rinsed away, fresh complete medium chilled to 4°C was added, and the adherent cells were harvested by scraping. The adherent cells were washed and were found to be >95% viable by trypan blue exclusion, and >80% of the cells expressed the monocyte marker CD11b, as detected by flow cytometric analysis.
Purification of mouse CD4+ T lymphocytes, CD8+ T lymphocytes, and CD11b+ monocytes. CD4+ T lymphocytes and CD8+ T lymphocytes were purified from mouse splenocytes, and CD11b+ monocytes were isolated from mouse bone marrow cells by use of an AutoMACS system (Miltenyi Biotec, Auburn, CA) in accordance with the manufacturer's protocol. Mononuclear cells isolated from the splenocytes or bone marrow cells by Ficoll-Hypaque density centrifugation were washed twice with PBS; incubated with MACS MicroBeads coupled to anti-CD4, anti-CD8, or anti-CD11b antibody; and passed through a positive selection AutoMACS separation column by use of an AutoMACS automated benchtop magnetic cell sorter. The purity of the sorted cells was determined by flow cytometry and was greater than 80%.

Pseudotype HIV production. Pseudotype HIV production and luciferase assays were performed in Dulbecco's modified Eagle's medium with 10% fetal calf serum and cotransfected with 20 μg of an expression plasmid for vesicular stomatitis virus G protein (VSV-G) and 50 μg each of a proviral vector construct, pNL-4.3-Luc-E-R, which contained the firefly luciferase reporter gene under the transcriptional control of the HIV LTR (12, 30) (obtained from the NIAID AIDS Repository, Rockville, MD), or of pHIV-SVREN, which contained the Renilla luciferase reporter gene under the transcriptional control of the SV40 early promoter gene in the nef open reading frame (4), by use of Lipofectamine Plus (Invitrogen). After 48 h, culture supernatants were harvested and passed through 0.45-μm cellulose acetate syringe filters (Gelman Sciences, Inc., Ann Arbor, MI), and virus production was quantified using an HIV-1 p24 enzyme-linked immunosorbent assay (ELISA) (Perkin-Elmer Life Sciences, Boston, MA). Purified CD4+ splenic T lymphocytes (5 × 10^6 T lymphocytes/well) were activated in a 48-well plate with anti-CD3 (3 μg/ml) and anti-CD28 (1 μg/ml) plate-bound antibodies for 48 h and then coinfected with VSV-G-pseudotyped HIV-1-A3-Luc-E-R HIV-1 proviral constructs. Bone marrow-derived myeloid cells were cultured for 1 week in complete medium with added murine macrophage colony-stimulating factor (10 ng/ml; R&D Systems, Minneapolis, MN) and then coinfected with the HIV reporter-pseudotyped viruses described above at a cell density of 1 × 10^6 macrophages per well in a 24-well plate. Whole-cell lysates were prepared 48 h after infection using a dual luciferase reporter assay kit (Promega), and firefly and Renilla luciferase activity was measured using a Luminometer (Anthos Instruments, Carlsbad, CA).

Quantification of HIV production by stimulated T lymphocytes and myeloid cells. The CD4+ T lymphocytes and bone marrow-derived myeloid cells (10^5 cells) isolated from mouse bone marrow described above were cultured in duplicate in 96-well plates in complete medium alone in a total volume of 200 μl. CD4+ T lymphocytes were stimulated with prostratin, a potent activator of HIV LTR expression in T lymphocytes (36), and monocytes were stimulated with granulocyte-macrophage colony-stimulating factor (GM-CSF) (20 ng/ml). At the indicated time, HIV-1 production by the mouse cells was quantified by measuring the p24 antigen content of the culture supernatant with an HIV-1 p24 core antigen quantification ELISA (Perkin-Elmer Life Sciences Inc., Boston, MA) done as described previously (8).

Immunohistological analysis of mouse spleens by immunofluorescence. After mice were sacrificed, they were embedded in Tissue-Tek OCT compound (Sakura Finetek USA, Torrance, CA), frozen in liquid nitrogen, and cut by a cryostat into 10-μm-thick sections. The sections were fixed for 10 min at 4°C in PBS containing 4% paraformaldehyde. After the nonspecific reactivity was blocked by incubation with goat serum and bovine serum albumin blocking solution, the sections were incubated with rat anti-mouse CD4 or rat anti-mouse CD19 (PharMingen) at optimized concentrations, washed, incubated with Alexa Fluor 594-conjugated goat anti-rat immunoglobulin G (Invitrogen Corporation, Carlsbad, CA), washed again, and mounted on slides. The samples were analyzed with a Leica AOBAS laser scanning confocal microscope system.

Evaluation of the infectivities of JR-CSF and JR-CSF/hu-cycT1 murine mouse CD4+ T lymphocytes and monocytes by limiting-dilution coculture. The infectivities of CD4+ T lymphocytes and CD11b+ monocytes isolated from the mice as described above were determined by culturing fourfold dilutions of cells ranging from 1 × 10^3 to 1 × 10^6 cells in quadruplicate in 24-well plates with a fixed number of phytohemagglutinin-activated human donor peripheral blood mononuclear cells (1 × 10^5 cells/well) in complete medium containing interleukin 2 (25 units/ml) as described previously (8). One week later, the p24 antigen content of the culture supernatant was measured as described above. The infectivities of the cells are reported as tissue culture infective dose/100 cells, which was calculated by determining the lowest number of added cells that initiated productive infection of at least half of the quadruplicate cocultures with HIV-1.

RNA extraction and RNAse protection assay. The level of mRNA encoding various chemokines was measured using an RNAse protection assay described previously (43). Briefly, total RNA was extracted from cultured myeloid cells by use of Trizol reagent according to the manufacturer’s protocol (Invitrogen), radiolabeled probes specific for the C-C chemokines Ltn, RANTES, eotaxin, MIP-1α, MIP-1β, IP-10, MCP-1, and TCA-3 and the control housekeeping genes encoding L32 and GADPH (panel m6K5; Pharmingen, San Diego, CA) were generated with a MaxiScript kit (Ambion, Austin, TX) according to the manufacturer’s instructions. An RNAse Protection Assay III kit (Ambion) was used to perform an RNAse protection assay after the radiolabeled probes were hybridized to cellular RNA, and complexes containing mRNA bound to the probes were separated by electrophoresis on 5% denaturing acrylamide gels, which was followed by autoradiography. Bands representing chemokine probe bound to specific chemokine mRNA were quantified by densitometry on multiple film exposures by use of AMBUS Quant-Probe radioanalytic imaging system software (AMBUS, Inc., San Diego, CA), and relative chemokine mRNA expression was determined by calculating the ratio of the density of the indicated chemokine mRNA to that of L32 mRNA or GADPH.

Measurement of MCP-1 and RANTES protein. MCP-1 and RANTES secreted by myeloid cells into culture supernatant were quantified by ELISA with a Quantikine immunoassay (R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions. Briefly, aliquots of culture supernatant were mixed with assay diluent, added in duplicate to wells coated with a polyclonal antibody specific for MCP-1 or RANTES, and incubated for 2 h at room temperature. After plates were aspirated and rinsed with wash buffer, horseradish peroxidase-conjugated polyclonal antibody specific for MCP-1 or RANTES was added to the wells and incubated for 2 h at room temperature. The plates were washed, substrate was added for 30 min, the reaction was stopped with 2 N H2SO4, and the absorbance of each microwell was read at the 450-nm wavelength on a microplate reader (Bio-Rad, Hercules, CA) within 30 min of stopping the reaction. The level of MCP-1 or RANTES in the culture supernatants was then calculated using a standard curve generated using MCP-1 or RANTES standards measured in parallel with the samples. The limit of detection of the assay was 2 pg/ml.

In vivo treatment with GM-CSF. B16-F10 melanoma cells stably transduced with a retroviral vector expressing murine GM-CSF (15) were expanded in Dulbecco minimal essential medium with 10% fetal calf serum added. After the cells were harvested and washed extensively in PBS, approximately 2 × 10^7 cells were subcutaneously injected into each mouse. Two to 3 weeks later, when a subcutaneous tumor with diameter of 2.5 to 3.5 cm had appeared, the indicated mouse tissues were harvested and analyzed. Systemic GM-CSF production by the tumor cells was measured in vivo by quantization of mouse plasma GM-CSF levels by use of a GM-CSF ELISA kit (R&D Systems).

Statistical analysis of the data. The Student t test for unpaired data was used for all statistical comparisons made. Significance was assigned to P values of <0.05.

RESULTS

Myeloid cells from hu-cycT1 transgenic mice express human cyclinT1. hu-cycT1 transgenic mice were generated using a murine CD4 expression cassette encoding human cyclin T1 predicted to target transgene expression to CD4+ T lymphocytes, macrophages, and dendritic cells (Fig. 1A). Transgenic founders were identified by PCR analysis of genomic DNA extracted from their tails using a primer pair specific for the amplification of human cyclin T1 DNA. Immunoblot analysis using an antibody specific for human cyclin T1 demonstrated expression of the human cyclinT1 transgene in the spleens and thymuses of mice from several independent transgenic lines (Fig. 1B). Expression of the human cyclin T1 transgene in bone marrow-derived myeloid cells from hu-cycT1 mice derived from the T7 line and not in those cultured in the presence or absence of GM-CSF from wild-type littermates was demonstrated by RT-PCR using primers specific for human cyclin T1 (Fig. 1C). Taken together, these results indicated that the hu-cycT1 transgene was expressed in hu-cycT1 mouse T lymphocytes and myeloid cells.

HIV LTR-driven expression is increased in myeloid cells from hu-cycT1 transgenic mice. Expression of human cyclin T1 by the mouse cells should rescue Tat function and permit...
Tat-mediated transactivation of the HIV-1 LTR. To examine whether T lymphocytes and myeloid cells from the hu-cycT1 transgenic mice supported Tat-mediated transactivation, purified CD4⁺/H11001 T lymphocytes and bone marrow-derived myeloid cells from four different hu-cycT1 transgenic mice was evaluated by RT-PCR analysis of RNA extracted from cells cultured with or without GM-CSF for 2 days. Amplified products after PCR amplification using primers specific for human cyclin T1 mRNA or mouse 18S mRNA were resolved and visualized on an agarose gel containing ethidium bromide.

Transgenic expression of human cyclin T1 increases HIV-1 production by stimulated CD4⁺ T lymphocytes and myeloid cells. JR-CSF mice are transgenic for a full-length HIV-1 provirus that is expressed in the spleens, lymph nodes, thymuses, and brains of JR-CSF mouse T lymphocytes, monocytes, and microglia produce infectious HIV-1 (8, 47, 64). JR-CSF mouse splenocytes express Tat mRNA (data not shown), as determined by analysis using RT-PCR as described previously (31). We crossed the hu-cycT1 transgenic mice derived from the T7 line with JR-CSF mice to obtain JR-CSF/hu-cycT1 mice that were transgenic for human cyclin T1 and the HIV-1-JR-CSF provirus regulated by the endogenous HIV-1 LTR. The JR-CSF/hu-cycT1 mice were used to examine the in vivo effect of human cyclin T1 expression on HIV-1 production by primary mouse CD4⁺ T lymphocytes and myeloid cells.

**FIG. 1.** Construction of human cyclin T1 mice and evaluation of transgene expression. (A) Schematic representation of the human cyclin T1 transgene construct. E4/P4, murine CD4 enhancer/promoter; N, Xb, Xh, Cl, Sc, B, and S, restriction enzyme sites for NotI, XbaI, XhoI, ClaI, SacI, BamHI and Sall, respectively; SVpA, SV40 polyadenylation signal. (B) Expression of the human cyclin T1 transgene in the spleens and thymuses of mice from seven transgenic lines. Expression was detected by Western blotting with a polyclonal antibody specific for human cyclin T1. Specificity is indicated by reactivity with 3T3 cells expressing human cyclin T1 and no reactivity with thymocytes from wild-type mice. (C) Human cyclin T1 expression in bone marrow-derived myeloid cells from hu-cycT1 transgenic mice was evaluated by RT-PCR analysis of RNA extracted from cells cultured with or without GM-CSF for 2 days. Amplicons after PCR amplification using primers specific for human cyclin T1 mRNA or mouse 18S mRNA were resolved and visualized on an agarose gel containing ethidium bromide.
JR-CSF mice and JR-CSF/hu-cycT1 mice by use of AutoMACS were stimulated with prostratin, a unique phorbol ester that potently increases HIV-1 production by T lymphocytes carrying integrated proviral templates (36), and cellular HIV-1 production was determined by measuring the level of p24 antigen secreted into the culture supernatant. Prostratin induced levels of HIV-1 production from JR-CSF/hu-cycT1 mouse CD4+ T lymphocytes that were over 50-fold higher than those from CD4+ T lymphocytes from JR-CSF mouse littermates (Fig. 3A). To determine whether the murine CD4 enhancer/promoter-regulated hu-cycT1 transgene specifically increased HIV-1 production by CD4+ T lymphocytes, in another experiment performed in duplicate, purified CD4+ T lymphocytes and CD8+ T lymphocytes were obtained from the spleens of JR-CSF/hu-cycT1 mice, and prostratin-stimulated HIV-1 production by these cellular populations was determined by measuring the level of p24 antigen secreted into the culture supernatant. The mean level of p24 antigen produced by prostratin-stimulated JR-CSF/hu-cycT1 mouse CD4+ T lymphocytes (541 ± 173 pg/ml) was about 10-fold higher than the level produced by JR-CSF/hu-cycT1 mouse CD8+ T lymphocytes (55 ± 30 pg/ml).

We next examined the effect of human cyclin T1 expression on HIV-1 production by bone marrow-derived myeloid cells. Because GM-CSF increases HIV-1 production by myeloid cells (20) and bone marrow-derived myeloid lineage cells from JR-CSF mice (47), we compared the levels of HIV-1 production by GM-CSF-stimulated myeloid cells from JR-CSF mice and from JR-CSF/hu-cycT1 mice. The level of HIV-1 produced by GM-CSF-stimulated JR-CSF/hu-cycT1 mouse myeloid cells was almost 50-fold higher than the level of HIV-1 produced by GM-CSF-stimulated JR-CSF mouse myeloid cells (Fig. 3B). Furthermore, the infectivities of purified CD4+ T lymphocytes and CD11b+ myeloid cells from JR-CSF/hu-cycT1 mice were 5-fold and 100-fold higher, respectively, than that of cells from JR-CSF littermates, as measured by determining the lowest number of cells capable of generating a productive infection of activated human peripheral blood mononuclear cells (Fig. 4). Thus, expression of the hu-cycT1 transgene markedly enhanced the HIV-1 production and infectivity of primary mouse myeloid cells and CD4+ T lymphocytes from JR-CSF mice.

Expression of human cyclin T1 by JR-CSF mice is associated with decreased levels of CD4+ lymphocytes in the peripheral blood and lymphoid tissues. A hallmark of HIV-1 infection is the selective depletion of CD4+ T lymphocytes in the peripheral blood of infected individuals. To determine the effect of expression of the JR-CSF transgene combined with CD4-targeted expression of the human cyclin T1 gene on T-cell subpopulations, the percentages of CD4+ and CD8+ T lymphocytes in the peripheral blood of control mice, JR-CSF mice, hu-cycT1 mice and JR-CSF/hu-cycT1 mice which ranged in age from 2 to 4 months were evaluated by flow cytometry (Fig. 5). Comparable levels of CD8+ lymphocytes were detected in the peripheral blood of control mice (14% ± 2%), hu-cycT1 mice (14% ± 1%), JR-CSF mice (12% ± 2%), and JR-CSF/hu-cycT1 mice (10% ± 1%). Similar levels of CD4+ lymphocytes were also detected in peripheral blood samples...
from control mice (47% ± 2%), hu-cycT1 mice (45% ± 4%), and JR-CSF mice (42% ± 2%), indicating that expression of the hu-cycT1 gene or of the JR-CSF transgene alone did not affect the CD4⁺ lymphocyte population in the peripheral blood. In contrast, the level of CD4⁺ lymphocytes in the peripheral blood of JR-CSF/hu-cycT1 mice (27% ± 5%) was significantly lower than the level of CD4⁺ lymphocytes in the peripheral blood of JR-CSF mice (P = 0.005) or of hu-cycT1 mice, with JR-CSF/hu-cycT1 mice older than 1 year having significantly (P = 0.006) lower numbers of CD4⁺ lymphocytes (11.5% ± 3.4%) than JR-CSF mice older than 1 year (30% ± 11%), with no significant difference (P = 0.18) between the numbers of CD8⁺ lymphocytes in these older JR-CSF/hu-cycT1 mice (14.9% ± 3.5%) and in the older JR-CSF mice (12.2% ± 1%). As a result of this depletion, an inverted CD4/CD8 ratio (mean, 0.57 ± 0.09) was present in the peripheral blood of all four JR-CSF/hu-cycT1 mice over 1 year of age, in contrast to the normal CD4/CD8 ratio in the four JR-CSF mice (mean, 3.07 ± 0.21) examined.

The effect of combined expression of the JR-CSF transgene with CD4-targeted expression of the human cyclin T1 gene on the CD4 population in lymphoid tissues was examined by immunohistochemistry and flow cytometry. Immunohistochemical analysis of the spleens of 5-month-old JR-CSF/hu-cycT1 mice demonstrated preserved architecture of the lymphoid regions, with B cells present in the lymphoid follicles and CD4⁺ T lymphocytes present in the periarteriolar lymphoid sheath (Fig. 6). There was a moderate decrease in the numbers of CD4⁺ T lymphocytes in the marginal zones of the JR-CSF/hu-cycT1 mice from those for hu-cycT1 mouse littersmates. The CD4⁺ lymphocyte populations in the spleens, lymph nodes, and thymuses of JR-CSF/hu-cycT1 mouse (n = 3 mice), JR-CSF mouse (n = 3 mice), and hu-cycT1 mouse (n = 3 mice) littersmates were measured by flow cytometry. The fraction of CD4⁺ lymphocytes (17.8% ± 0.3%) in the spleens of the JR-CSF/hu-cycT1 mice was significantly less (P < 0.03) than those for JR-CSF mice (26.9% ± 3.5%) and hu-cycT1 mice (21.7% ± 1.4%). Similarly, the fraction of CD4⁺ lymphocytes (41.9% ± 0.3%) in the lymph nodes of the JR-CSF/hu-cycT1 mice was significantly less (P < 0.037) than those for JR-CSF mice (59.3% ± 3.5%) and hu-cycT1 mice (56.5% ± 6.4%). The percentages of doubly positive and singly positive thymocytes in the thymuses from JR-CSF/hu-cycT1 mice were not significantly different from those found in thymuses from hu-cycT1 mice and JR-CSF mice. Taken together, these findings indicate that expression of the hu-cycT1 gene coupled with carriage of the JR-CSF provirus results in the selective depletion of the CD4⁺ lymphocyte population in the peripheral blood and secondary lymphoid tissues of mice.

Expression of the hu-cycT1 transgene increases in vivo HIV-1 production in JR-CSF mice treated with GM-CSF. We demonstrated above that hu-cycT1 transgene expression increased in vitro HIV-1 production by GM-CSF-treated JR-CSF mouse myeloid cells. We next examined the in vivo effect of rescuing Tat function by transgenic expression of human cyclin T1 by comparing in vivo HIV-1 production by JR-CSF mice to that of JR-CSF/hu-cycT1 mice after chronic treatment with GM-CSF. To provide the mice with a continuous source of GM-CSF, the mice were subcutaneously injected with B16-F10-GM-CSF, a melanoma cell line stably transfected with a GM-CSF expression vector that forms a subcutaneous tumor that continuously secretes GM-CSF. Two weeks after injection with the B16-F10-GM-CSF cells, there was no significant difference (P < 0.31) between the GM-CSF serum level of injected JR-CSF mice (mean, 245 ± 116 pg/ml) and that of JR-CSF/hu-cycT1 mice (mean, 306 ± 10 pg/ml). In vivo HIV-1 production induced by GM-CSF treatment was determined by harvesting bone marrow cells and spleens from these treated mice and measuring their p24 antigen content levels. After in vivo stimulation with GM-CSF, JR-CSF/hu-cycT1 mouse bone marrow cells contained levels of p24 antigen 7-fold higher than those from JR-CSF littermate bone marrow cells (P = 0.004) (Fig. 7A), and JR-CSF/hu-cycT1 splenocytes contained levels of p24 antigen 18-fold higher than those from JR-CSF littermate splenocytes (P = 0.047) (Fig. 7B). Thus, the rescue of Tat function in the JR-CSF mice by transgenic expression of human cyclin T1 was associated with a significant increase in the in vivo HIV-1 production induced by GM-CSF.

Myeloid cells from JR-CSF/hu-cycT1 mice display increased chemokine production after lipopolysaccharide (LPS) stimulation. The C-C chemokine family members, including MIP-1α, MIP-1β, MCP-1, and RANTES, induce migration of activated T lymphocytes and monocytes and stimulate the mobilization of inflammatory cells to injured or infected locations (3, 66). HIV-1 infection of monocytes may induce chemokine production that facilitates the spread of infection by inappropriate induction of migration of T lymphocytes and inflammatory cells to the infected cells (62). We had previously demonstrated that MCP-1 gene expression in JR-CSF mouse microglia and brains was more responsive to in vitro and in vivo stimulation with LPS than that in microglia and brains from control mice (64). Therefore, we examined whether expression of the integrated HIV-1 provirus also affects chemokine production by stimulated JR-CSF bone marrow-derived mouse myeloid cells and whether Tat-mediated transactivation contributes to dysregulation of the chemokine response. After bone marrow-derived myeloid cells from JR-CSF mice and
control littermates were treated with GM-CSF alone or with LPS, chemokine gene expression was evaluated by an RNase protection assay (Fig. 8A). GM-CSF- and LPS-stimulated JR-CSF myeloid cells produced levels of MCP-1 mRNA (Fig. 8B) over 2-fold higher and levels of MCP-1 protein (Fig. 8C) over 2.4-fold higher than those of GM-CSF- and LPS-stimulated wild-type mouse myeloid cells; they also produced 1.5-fold-higher levels of RANTES protein (Fig. 8D). We next examined whether the chemokine production by stimulated JR-CSF mouse myeloid cells would be further dysregulated by conferring support for Tat-mediated trans-activation by expression of the human cyclin T1 transgene (Fig. 9A). GM-CSF- and LPS-stimulated JR-CSF/hu-cycT1 myeloid cells produced levels of MCP-1 mRNA (Fig. 9B) and of MCP-1 protein (Fig. 9C) over 2-fold higher than those produced by GM-CSF and LPS-stimulated JR-CSF littermate myeloid cells and also produced minimally higher levels of RANTES production (Fig. 9D). LPS-induced chemokine gene expression by monocytes from mice transgenic for human cyclin T1 alone was similar to that observed for wild-type mice (data not shown). Thus, HIV-1 proviral expression in myeloid cells is associated with increased production of MCP-1 in response to LPS stimulation that is further increased by the rescue of Tat function through expression of the hu-cycT1 transgene.

DISCUSSION

Although the cyclin T1 subunit of the human P-TEFb complex is the critical factor limiting Tat-mediated transactivation in vitro in mouse cells (7, 23, 65), rodent cells engineered to support HIV-1 replication by stable expression of human CD4, CCR5, and cyclin T1 produced levels of HIV-1 much lower than those produced by human cells (6, 41). The continued severely compromised HIV-1 replication in these rodent cells despite expression of human cyclin T1 and the rescue of Tat function was attributed to the presence of other postintegration blocks in HIV-1 replication, including compromise of the Rev-mediated transport of unspliced mRNA encoding HIV-1...
produced HIV-1 after infection with 20 and 60 copies of the provirus encoding an X4 isolate, NL4.3, and contained from two to four copies of the provirus. We recently reported that the combination of GM-CSF and LPS stimulated JR-CSF mouse bone marrow-derived monocytes to produce levels of p24 antigen (1.595 ± 3 pg/ml) markedly higher than those produced by stimulation with either GM-CSF (89 ± 10 pg/ml) or LPS (44 ± 1 pg/ml) alone (47). We attributed the high level of HIV-1 production by monocytes from these mice carrying low numbers of integrated provirus and lacking human cyclin T1 to the synergistic activation by GM-CSF and LPS of Sp1, a transcription factor reported to robustly activate the HIV-1 LTR in the absence of Tat or TAR (69). These and other mechanisms may permit the production of HIV-1 by primary mouse monocyte/macrophage cells that are unable to support Tat function. The data in this paper extend those findings by demonstrating that stimulated JR-CSF mouse CD4+ T lymphocytes and myeloid cells able to support Tat-mediated transactivation by the expression of human cyclin T1 produce levels of HIV-1 about 50-fold higher than those produced by JR-CSF/hu-cycT1 mice was about eightfold higher than the level of HIV-1 produced by stimulated T lymphocytes. In our JR-CSF transgenic mice, production of HIV-1 by stimulated T lymphocytes decreased in subsequent generations from the higher levels first measured in the F1 generation (8) to the lower levels described here, possibly due to a stabilization of the JR-CSF transgene during breeding. Doherty et al. also reported for their HIV transgenic mice that HIV production by the T lymphocytes was markedly lower than HIV production from the monocyte/macrophage lineage cells (14).

Rats exhibit a similar phenotype. After rats transgenic for the expression of human CD4/CCR5 were infected with HIV-1, they displayed infection of T lymphocytes that was extremely limited compared to infection of monocytes; this extremely limited level of infection was ascribed to a T-lymphotropic-specific posttranscriptional block to HIV-1 replication (34, 35). Because the HIV-1 provirus is integrated in the JR-CSF mouse cells, the decreased HIV-1 production by JR-CSF mouse T lymphocytes is not attributable to the postentry, preintegration block recently described for murine T lymphocytes that is related to the reduced efficiency of reverse transcription and the transfer of the preintegration complex into the nucleus (4). These results, combined with those of other groups, indicate that in addition to having restricted Tat function, mouse and rat T lymphocytes, like mouse 3T3 fibroblasts, harbor another block compromising the postintegration replication of HIV-1 that can be bypassed by mouse myeloid lineage cells. This rodent-specific block to replication may be due to altered RNA processing in mouse 3T3 cells, because the murine homologue of the human splicing inhibitor, p32, insufficiently suppresses mRNA splicing, resulting in extensive splicing of the 9-kb HIV mRNA transcript required for synthesis of structural and enzymatic proteins (70). This posttranscriptional

structural proteins (6) and the inability of mouse cells to support virion assembly (6, 41). The data presented in this paper extend the findings of previous in vitro studies with rodent cell lines by examining the in vivo effect of transgenic expression of human cyclin T1 on the rescue of Tat function and its effect on HIV-1 replication in primary murine CD4+ T lymphocytes and myeloid cells. Expression of the human cyclin T1 transgene conferred to primary hu-cycT1 mouse monocytes/macrophages and CD4+ lymphocytes the capacity to support Tat-mediated transactivation of the HIV LTR. This was demonstrated by the fact that the levels of luciferase produced by hu-cycT1 primary mouse monocytes/macrophages and CD4+ lymphocytes after infection with a VSV-G-pseudotyped HIV carrying a luciferase reporter were severalfold higher than those produced by equivalent cells from wild-type littersmotes. Furthermore, the rescue of Tat function by the hu-cycT1 transgene significantly increased HIV-1 production by stimulated JR-CSF mouse cells, particularly by GM-CSF-stimulated myeloid cells, indicating that the other HIV-1 replication blocks reported in mouse fibroblast cells lines (6, 41) are less pronounced in primary mouse leukocytes. We and other investigators have reported that a lack of human cyclin T1 does not preclude HIV transgenic mouse cells from producing HIV-1. For example, Doherty et al. reported that transgenic mice carrying between 20 and 60 copies of the provirus encoding an X4 isolate, NL4.3, produced HIV-1 after infection with Mycobacterium avium predominately from monocyte/macrophage lineage cells (14). Production of HIV-1 in a Tat-independent manner by cells from these transgenic mice may result from the additive effect of transcription of the many copies of the HIV provirus carried by each transgenic mouse monocyte/macrophage. The JR-CSF mice used in the current study are transgenic for a provirus encoding the R5 isolate, HIV-1JR-CSF, and contained from two to four copies of the provirus (8). We recently reported that the combination of GM-CSF and LPS stimulated JR-CSF mouse bone marrow-derived monocytes to produce levels of p24 antigen (1.595 ± 3 pg/ml) markedly higher than those produced by stimulation with either GM-CSF (89 ± 10 pg/ml) or LPS (44 ± 1 pg/ml) alone (47). We attributed the high level of HIV-1 production by monocytes from these mice carrying low numbers of integrated provirus and lacking human cyclin T1 to the synergistic activation by GM-CSF and LPS of Sp1, a transcription factor reported to robustly activate the HIV-1 LTR in the absence of Tat or TAR (69). These and other mechanisms may permit the production of HIV-1 by primary mouse monocyte/macrophage cells that are unable to support Tat function. The data in this paper extend those findings by demonstrating that stimulated JR-CSF mouse CD4+ T lymphocytes and myeloid cells able to support Tat-mediated transactivation by the expression of human cyclin T1 produce levels of HIV-1 about 50-fold higher than those produced by JR-CSF/hu-cycT1 mice was about eightfold higher than the level of HIV-1 produced by stimulated T lymphocytes. In our JR-CSF transgenic mice, production of HIV-1 by stimulated T lymphocytes decreased in subsequent generations from the higher levels first measured in the F1 generation (8) to the lower levels described here, possibly due to a stabilization of the JR-CSF transgene during breeding. Doherty et al. also reported for their HIV transgenic mice that HIV production by the T lymphocytes was markedly lower than HIV production from the monocyte/macrophage lineage cells (14).

FIG. 7. JR-CSF mouse expressing human cyclin T1 display increased HIV-1 production after continuous in vivo stimulation with GM-CSF.

- **A**. JR-CSF mice (n = 4) or JR-CSF/hu-cycT1 mice (n = 4) were implanted with B16-F10-GM-CSF melanoma cells. Two weeks later, GM-CSF levels in the serum were measured by ELISA, and the spleens and bone marrow cells were harvested. HIV-1 production was determined by measuring the HIV p24 antigen content of the lysates from (A) bone marrow cells and (B) spleens and results are presented as the mean ± SEM of p24 antigen content/10⁷ cells.
block can be overcome by providing mouse 3T3 cells with human p32, a more potent inhibitor of mRNA splicing than mouse p32, which increases levels of the unspliced 9-kb HIV mRNA transcript and thereby markedly increases HIV-1 production by the 3T3 cells (70). Monocytes may not be affected by this posttranscriptional block because their splicing machinery may differ from that of T lymphocytes and 3T3 cells due to expression of lower levels of splicing factors or higher levels of alternative splicing inhibitors. These differences may cause less-extensive splicing of the 9-kb HIV mRNA transcript in monocytes than in T lymphocytes and consequently result in the production of higher levels of HIV-1 by monocytes. The degree of posttranscriptional blocking displayed by T lymphocytes, 3T3 cells, and monocytes may also be related to the utilization of different mechanisms for HIV-1 assembly by these different cell lineages (60). The primary location for HIV-1 assembly in T lymphocytes and 3T3 cells is the plasma membrane, where a block to the assembly of HIV-1 may exist.

FIG. 8. Effect of JR-CSF provirus expression on chemokine gene expression and production by stimulated mouse myeloid cells. Bone marrow-derived myeloid cells from JR-CSF mice and control mice were cultured in 24-well plates (2 × 10^6 cells/well) with GM-CSF and were either stimulated with LPS or not stimulated. After 24 h, the cells were harvested, and RNA was extracted from the myeloid cells and analyzed for chemokine gene expression by use of an RNase protection assay. (A) An autoradiograph of an RNase protection assay of RNA extracted from myeloid cells from a wild-type or a JR-CSF mouse and (B) densitometric analysis of the gel. After 72 h of stimulation, the levels of (C) MCP-1 and (D) RANTES in an aliquot of culture supernatant were measured by ELISA. The ELISA data shown represent the mean levels of MCP-1 and RANTES production in four independent experiments using myeloid cells isolated from different mice, with statistical significance indicated.
in mouse cells (41). In contrast, the major location of HIV-1 assembly in monocytes is in the late endosomal compartment, particularly the major histocompatibility class II compartment (50, 51, 53). Assembly in this compartment may utilize a mechanism different from that occurring at the plasma membrane that prevents the efficient assembly on the plasma membrane of mouse cells. We are currently examining these possibilities.

Nevertheless, stimulated CD4+ lymphocytes from the JR-CSF/hu-cycT1 mice produced moderate levels of HIV-1 and levels of HIV-1 significantly higher than those produced by CD4+ lymphocytes from the JR-CSF mice. A limitation of HIV transgenic mouse models is that in contrast to humans, where HIV-1 replication occurs predominantly in CD4-expressing cells, in the HIV transgenic mice the provirus is integrated in every cell and can be expressed by any

FIG. 9. Effect of human cyclin T1 expression on chemokine gene expression in stimulated JR-CSF mouse myeloid cells. Bone marrow-derived myeloid cells from JR-CSF mice and JR-CSF/hu-cycT1 mice were cultured in 24-well plates (2 x 10^6 cells/well) with GM-CSF and either stimulated with LPS or not stimulated. After 24 h, the cells were harvested, and RNA was extracted from the myeloid cells and analyzed for chemokine gene expression by an RNase protection assay. (A) A representative autoradiograph of an RNase protection assay of RNA extracted from myeloid cells from wild-type or JR-CSF mice from three independent experiments and (B) the mean values of densitometric analysis of autoradiographs from three separate experiments. After 72 h of stimulation, the levels of (C) MCP-1 and (D) RANTES in an aliquot of culture supernatant were measured by ELISA. The ELISA data shown represent the mean levels of MCP-1 and RANTES production in four independent experiments using myeloid cells isolated from different mice, with statistical significance indicated.
cell that supports HIV LTR transcription, including many cell lineages normally not infected by HIV. Consequently, HIV transgenic mice may not recapitulate many aspects of the pathophysiology of HIV infection. We hypothesized that expression of a human cyclin T1 transgene would markedly increase HIV-1 replication in the HIV transgenic mouse cells by enabling Tat-mediated transcriptional activation. To selectively increase HIV-1 production by CD4-expressing cells in the JR-CSF mice and increase the relevance of the JR-CSF mouse model to human disease, we focused expression of the human cyclin T1 transgene to CD4-expressing cells by using a hu-cycT1 transgene under the control of a CD4 promoter that targets expression to CD4+ T lymphocytes, monocytes, and dendritic cells (34). Of great interest was the observation that CD4+ T lymphocytes and not CD8+ T lymphocytes were depleted in the peripheral blood of the JR-CSF/hu-cycT1 mice, while normal levels of CD4+ and CD8+ T lymphocytes were present in the peripheral blood of JR-CSF mice and hu-cycT1 mice. The extent of CD4+ T lymphocyte depletion correlated with mouse age, with an inverted CD4/CD8 ratio (mean, 0.57 ± 0.09) present in the peripheral blood of all four JR-CSF/hu-cycT1 mice over 1 year of age that was in contrast to the normal CD4/CD8 ratio seen for the four JR-CSF mice (mean, 3.07 ± 0.21) examined. While the JR-CSF provirus in the transgenic mice is equally expressed by the CD4+ lymphocytes and the CD8+ lymphocytes, the hu-cycT1 transgene regulated by the CD4 promoter is expressed in CD4+ lymphocytes and not in CD8+ lymphocytes. It is likely that the selective depletion of the CD4+ lymphocytes is a consequence of the increased production of HIV-1 by the CD4+ T cells that express human cyclin T1 and resembles the natural course of HIV-1 infection in humans. An alternative approach that targeted HIV expression to CD4 cells in transgenic mice and that utilized an HIV transgene regulated by a CD4-specific promoter was described previously. Transgenic mice were generated by use of a construct consisting of the CD4C promoter fused to an 8.8-kbp fragment of the HIV-1 pNL4-3 clone, their CD4 cells expressed HIV proteins, and the mice developed an AIDS-like disease with symptoms including muscle wasting, severe atrophy of and fibrosis in lymphoid organs, and nephritis and pneumonia mediated by Nef expression (27, 28). This dramatic phenotype was not seen in our transgenic mice. This may be due to the activity of the CD4C promoter, which was much stronger than that of the HIV LTR regulating the HIV transgene in our transgenic mice, resulting in the production of higher levels of HIV proteins deleterious to tissues in their transgenic mice. However, although the phenotype of the mice is dramatic, regulation of the HIV proviral transgene by the CD4C promoter is different from control by the endogenous LTR and precludes the use of these mice to investigate the in vivo effect of factors that activate the HIV LTR on HIV-1 transcription and viral production. In contrast, because HIV production in the JR-CSF/hu-cycT1 mice is regulated by the endogenous HIV LTR transactivated by Tat in a manner comparable to that observed in HIV-1-infected individuals, the JR-CSF/hu-cycT1 mice should be a useful model to study in vivo regulation of HIV-1 transcription by infected cells.

Chemokines produced by monocytes play an important role in influencing HIV-1 replication, spread of infection, and pathogenesis of disease (17). Localized production of MCP-1 is sufficient to induce the directed migration of monocytes into organs in vivo, as evidenced by the organ-specific influx of monocytes in mice that carry an MCP-1 transgene controlled by an organ-specific promoter (22, 26). In the current study, we demonstrated that after GM-CSF/LPS stimulation, myeloid cells from JR-CSF mice produced levels of MCP-1 that were 2.4-fold higher than those produced by myeloid cells from wild-type mice, and GM-CSF/LPS-stimulated myeloid cells from JR-CSF/hu-cycT1 mice produced levels of MCP-1 that were 2.1-fold higher than those produced by stimulated JR-CSF mouse myeloid cells. These results indicated that carriage of the JR-CSF provirus by mouse myeloid cells is associated with increased MCP-1 production that is further augmented when associated with rescued Tat function mediated by transgenic expression of human cyclin T1. The 4.7-fold enhancement of MCP-1 production by stimulated JR-CSF/hu-cycT1 mouse myeloid cells over the levels produced by stimulated wild-type mouse myeloid cells is comparable to the two- to sevenfold enhancement of MCP-1 production reported after infection of human monocyte-derived macrophages with HIV-1 (44). Because Tat expression has been reported to induce MCP-1 production by endothelial cells (48), astrocytes (67), and dendritic cells (31), it is possible that expression of hu-cycT1 facilitates this effect of Tat. MCP-1 is a chemokine that plays a critical role in initiating inflammation by inducing the migration of monocytes, memory T lymphocytes, and NK cells (61, 62). Because MCP-1 increases the production of HIV-1 by infected cells (63), increased production of MCP-1 by HIV-1-infected monocytes may amplify HIV-1 dissemination by directly increasing the production of HIV-1 by infected cells. Furthermore, increased MCP-1 production may also augment the spread of HIV-1 infection by recruiting susceptible cells, including CD4+ T lymphocytes, to sites of active viral replication, where they can become activated or rendered permissive to infection and infected with HIV-1 as described previously for MIP-1α and MIP-1β (61, 62). The increased production of chemokines by HIV-1-infected macrophages and microglia residing in the central nervous system may contribute to the immunopathogenesis of HIV-mediated encephalitis by inducing the transmigration of HIV-1-infected and/or uninfected monocytes across the blood-brain barrier from the systemic circulation into the brain and inducing the development of inflammatory foci that lead to neuronal damage (33, 68). The physiological relevance of this proposed mechanism is supported by the detection of MCP-1 in the brains and cerebrospinal fluid of patients with HIV-1-associated dementia (11) and the association between elevated levels of MCP-1 in cerebrospinal fluid and the development of encephalitis in simian immunodeficiency virus-infected macaques (72).

Taken together, our results demonstrate that expression of human cyclin T1 markedly increases in vivo HIV-1 production in mice and that blocks preventing efficient HIV-1 replication in mice can be overcome by using transgenic technology to provide human factors. This approach will be used to confirm the role of other factors shown to compromise HIV-1 replication in mouse cells identified by in vitro studies, such as hp32 (70), and ultimately used to develop a mouse model displaying efficient HIV-1 replication. Differences between the biological behaviors of murine and human lymphoid and myeloid cells may compromise the applicability of studies using murine
models for understanding the mechanism by which HIV-1 causes disease in infected patients. Nevertheless, because the lymphocytes and myeloid cells present in this transgenic mouse carry the provirus of a primary R5 HIV-1 isolate under the control of the endogenous HIV-1 LTR and HIV-1 replication is specifically increased in CD4-expressing cells by targeted expression of the hu-cycT1 transgene, it is likely that the in vivo behavior of these cells may recapitulate many aspects of HIV-1 replication and cellular behavior occurring in HIV-1-infected individuals. In particular, we are currently investigating whether the selected depletion of CD4 T lymphocytes in the JR-CSF/hu-cycT1 mice is associated with the development of an immunodeficiency that resembles that seen in AIDS. Therefore, the JR-CSF/hu-cycT1 mice should provide a new system for investigating the pathogenesis of various aspects of HIV-1-mediated disease and the efficacy of therapeutic interventions designed to prevent manifestations of HIV-1-induced disease.

ACKNOWLEDGMENTS

This work was supported by the National Institutes of Health (National Institute of Neurological Disorders and Stroke NS39201, National Institute of Allergy and Infectious Diseases AI48468, and the Center for AIDS Research AI51519). T.S. was supported by NIAID AIDS Training Grant T32AI0732.


