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Altered Cytokine Expression in T Lymphocytes from Human Immunodeficiency Virus Tat Transgenic Mice

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Examination of the interaction between human immunodeficiency virus (HIV) regulatory gene products and the host immune system is fundamental to understanding the pathogenesis of HIV and could reveal possible targets for therapeutic intervention in the treatment of AIDS. The HIV Tat gene is a potential candidate for this type of strategy. Transgenic mice can be used to investigate the in vivo effects of Tat on the developing and dynamic immune system and on cellular gene expression. Thus, we have generated transgenic mice that harbor the HIV type 1 Tat gene under the transcriptional control of the human CD2 gene regulatory elements. This expression cassette results in high-level, tissue-specific transcription of the transgene within the T-cell compartment. In this report, we demonstrate the effects of Tat on the in vivo immune system. CD2-Tat transgenic mice show no signs of aberrant thymic development and have normal levels of T-cell subsets in the thymus and peripheral lymphoid organs. However, activated T cells from transgenic mice contain increased levels of tumor necrosis factor beta mRNA as well as biologically active tumor necrosis factor protein and express elevated levels of transforming growth factor β and interleukin-4 receptor mRNA. These increased cytokine levels do not appear to alter mitogen- or antigen-stimulated responses or induce the formation of dermal lesions in ageing mice. Such investigations should provide insight into the combination of host immune factors mediating pathogenesis in HIV infection.

Human immunodeficiency virus type 1 (HIV-1) encodes a small nuclear protein, Tat, which is required for viral replication. When Tat is bound to the transactivation response sequence found at the 5′ end of all HIV-1 mRNAs, it acts as a potent transactivator of transcription from the long terminal repeat (LTR) (reviewed in reference 17). Tat-induced HIV-1 LTR transcription also requires the interaction of cellular proteins and transcription factors such as Sp1 and/or NF-κB (6, 33, 40). The effect of Tat on the HIV-1 LTR is to increase the level of transcription by activation of transcription initiation and elongation (reviewed in reference 18).

Tat has been found to function as an extracellular protein during acute HIV-1 infection or after transfection of the Tat gene. In this extracellular form, it has an effect on cell proliferation and function (22) and has been found to stimulate the growth of spindle-like cells derived from Kaposi's sarcoma (KS) lesions in patients with AIDS. Recombinant Tat protein also has been reported to inhibit the proliferation of human peripheral blood lymphocytes in antigen-specific but not polyclonal mitogen responses (52, 54). This inhibition has been suggested to be due to Tat-mediated induction of immunosuppressive cytokines such as transforming growth factor β1 (TGF-β1), since transfection of the Tat gene or direct addition of Tat protein to human astrocytic glial cells transactivates transcription from the TGF-β1 promoter (19).

Recent evidence indicates that HIV-1 Tat can transactivate several other cellular and cytokine genes, which may explain the observation that numerous cytokines have been found to be elevated in the serum or produced at high levels by cultured mononuclear cells of HIV-1-infected patients (11, 38). A human B-cell line transfected with the Tat gene has demonstrated an increase in tumor necrosis factor beta (TNF-β) mRNA and protein (46). Further studies on mitogen-stimulated T cells infected with HIV-1 or transfected with the Tat gene have also suggested the effect of Tat on TNF-β expression (12). In addition, Tat has been shown to increase interleukin-4 (IL-4) receptor (IL-4R) expression on a human B-cell line (45) and to upregulate IL-2 secretion from activated T cells (58). Hence, a mechanism by which Tat can induce cellular gene expression is through its action as a transcription factor, and such effects of Tat on cellular gene expression may, in part, play a role in the progressive deterioration of the immune system of HIV-infected patients.

Previously, HIV transgenic mice have been successfully used as in vivo models to demonstrate the effects of HIV gene products on the development and function of the lymphoid system (9). For example, the results of HIV Nef transgenic studies have confirmed and extended observations seen in human cell lines and have increased our understanding of the T-cell subsets affected by HIV Nef regulatory protein (10, 49). To study the transactivation effects of Tat on cellular gene and cytokine expression and on the kinetics and development of T lymphocytes in vivo, we have produced transgenic mice which express HIV-1 Tat in all T cells. While Tat-mediated transactivation has been suggested to be ineffective in mouse cells because of the absence of a region of human chromosome 12 (29, 41), others have clearly shown high levels of Tat-mediated transactivation in mouse cell lines (4, 21, 37). In addition, studies using the HIV LTR to drive transcription of Tat in transgenic mice have indicated possible KS-like lesions in ageing animals. In this report, we describe the generation of CD2-Tat transgenic mice and the results of examinations on T-cell subset distribution, and we demonstrate the effects of Tat on cytokine mRNA and protein expression in activated T cells. We show that the cytokine profile of T cells is altered in

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CD2-Tat transgenic mice and present the results of polyclonal mitogen- and antigen-induced T-cell responses in transgenic mice. In addition, we show that ageing mice which express high levels of Tat do not develop skin lesions.

MATERIALS AND METHODS

Transgene construct. The 623-bp *Sal*I-*Asp* 718 fragment from pTZ19 Tat 1-72 (gift from E. Blair, Wellcome) was blunted and ligated into the unique blunted *Eco*RI site in the first exon of the p2629 CD2 expression plasmid (gift from D. Kioussis, National Institute for Medical Research) to give pCD2Tat. The *Sal*I-*Asp* 718 fragment contained the first exon of HIV Tat (amino acids 1 to 72) derived from HIV Rod 35 and is identical in nucleotide sequence to the same region in HTLV IIIB (HXB2). A 4.5-kb *Bam*HI-*Not*I fragment containing the 3′ CD2 locus control region (LCR) from p2694 (gift from D. Kioussis) was subsequently built into the unique *Bam*HI-*Not*I sites in pCD2Tat, resulting in pCD2TatLCR. The 12-kb *Sal*I-*Not*I fragment containing the chimeric CD2-Tat construct and LCR was prepared from this plasmid for microinjection into CBA × C57BI/10 fertilized mouse oocytes as previously described (30). Transgenic founders were bred with (CBA × C57BI/10)F_1 mice, and the resulting lines were maintained as heterozygotes.

DNA and RNA analysis. Tail DNA (10 μg) was examined from founder animals by Southern blot analysis following digestion with *Eco*RI and *Hin*dIII. DNA was fractionated on 1% agarose gels with Tris-acetate-EDTA as the running buffer. Gels were transferred onto Nytran membranes and probed with a randomly primed, ^{32}P-labeled 623-bp *Sal*I-*Asp* 718 fragment from pTZ19 Tat 1-72. A 1.2-kb *Xba*I-*Nru*I 5′ Thy-1.2 fragment was also used as an internal loading control. Increasing amounts of pCD2TatLCR were also added to negative control genomic tail DNA and analyzed simultaneously as copy number controls. Quantitation was performed by PhosphorImager (Molecular Dynamics) analysis.

Total cellular RNA was prepared by using either the lithium chloride-urea method (25) or Trizol (Gibco, BRL), essentially according to the manufacturer's instructions. For Northern (RNA) blot analysis, 5 to 20 μg of RNA was electrophoresed on 1% denaturing formaldehyde gels as described previously (1) and transferred onto nylon filters (Hybond-N; Amersham). After UV cross-linking, the membranes were hybridized with randomly primed, ^{32}P-labeled DNA probes for the following transcripts: TNF-α (24), TNF-β (26), TGF-β (20), IL-4R (28), and β-actin (1). For S1 analysis, between 10 and 30 μg of total cellular RNA was hybridized for 16 h at 53°C with probes (end labeled with ^{32}P) for Tat as well as internal control transcripts, either β-actin or U6 small nuclear RNA. Following hybridization, the samples were digested at 23°C with 100 U of S1 nuclease and examined on denaturing acrylamide gels.

Flow cytometric analysis. Fluorescence-activated cell sorting (FACS) analysis was performed to examine the surface expression of T-cell subset markers on lymphocytes from transgenic animals (6 to 8 weeks old). The antibodies used were a phycoerythrin-conjugated rat monoclonal antibody against murine CD4 (L3T4) and a fluorescein isothiocyanate-conjugated rat monoclonal antibody against murine CD8 (Ly-2) (clones RM4-5 and 53-6.7, respectively, from Pharmingen, San Diego, Calif.). Briefly, single-cell suspensions were obtained from thymus, spleen, and lymph nodes and incubated in the presence of antibodies on ice for 30 min. After two washings with cold phosphate-buffered saline, the cells were fixed with 1% formaldehyde, and stained cells were analyzed with a Becton Dickinson FACScan and the Lysis II software package.

TNF bioassay. Culture supernatants from transgenic T cells (CT1 and CT2) and nontransgenic controls that had been activated in culture (with phorbol myristate acetate [PMA] plus ionomycin as described below) were examined for the production of TNF, using the cytokine-sensitive cell line L929 (51). The amount of biologically active TNF was determined relative to known amounts of recombinant cytokine and in the presence of TNF-neutralizing antibodies. Cell numbers were assessed by absorbance measurements at 540 nm after dye solubilization following staining with crystal violet.

T-cell proliferative responses. T-cell proliferation assays were performed in 96-well tissue culture plates in RPMI 140 medium containing 10% fetal calf serum and 20 μM 2-mercaptoethanol. For Fig. 7, thymocytes were cultured at 10^6 cells per ml and activated by the following: polyclonal activating mitogen concanavalin A (2 μg/ml). T-cell receptor cross-linking using the anti-CD3ε antibody 145-2C11 previously coated at 10 μg/ml, and phorbol ester in combination with ionophore (10). Cells were pulsed with [^{3}H]thymidine at 48 h of culture for an additional 16 h and harvested, and thymidine incorporation was measured by scintillation counting in order to assess cell proliferation. Each sample was assayed in triplicate, and the individual points shown in Fig. 7 represent the average amount of [^{3}H]thymidine incorporated by thymic T cells.

Antigen-induced T-cell proliferation. Transgenic and nontransgenic mice from lines CT1 and CT2 were immunized subcutaneously at the base of the tail with 100 μg of the antigen keyhole limpet hemocyanin (KLH) emulsified in Freund's complete adjuvant (7). After in vivo immunization (5 to 8 days), the peripheral lymph nodes (periaortic and inguinal) were removed and dissaggregated, and the resulting cell suspensions were cultured in triplicate in the presence of increasing concentrations of KLH (0.1 to 100 μg/ml). After 4 days, the cultures were pulsed with [^{3}H]thymidine, and the resultant cell proliferation was assessed by incorporation of radiolabel as described above.

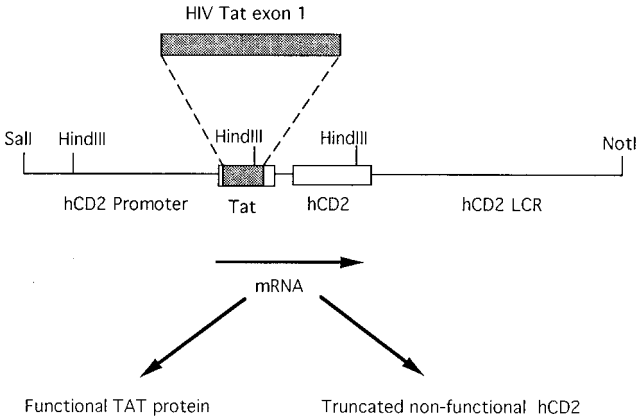

FIG. 1. Tat transgene construction. A *Sal*I-*Asp* 718 fragment containing HIV-1 Tat exon 1 (amino acids 1 to 72) was blunt-end cloned into an *Eco*RI site upstream of the ATG codon in exon 1 of the human CD2 (hCD2) expression cassette with the CD2 promoter, coding region and the 3′ LCR. The unique *Eco*RV site in the coding region of the human CD2 gene was previously removed so as to yield only a truncated nonfunctional human CD2 protein. A 12-kb *Sal*I-*Not*I fragment was used in the generation of transgenic mice.

RESULTS

CD2-Tat transgenic mice express high levels of HIV-1 Tat in T cells. Transgenic mice were produced with a construct containing the transcriptional regulatory units (promoter and LCR) of the human CD2 gene (27) and the exon 1 sequence of the HIV-1 Tat gene encoding amino acids 1 to 72 of the Tat protein (2, 50). The truncated Tat protein (exon 1, 72 amino acids) has been demonstrated to be sufficient for both transactivation response sequence binding and transactivated transcription from the HIV LTR promoter (47). The human CD2 sequences are known to direct expression of inserted genes to the T-cell lineage beginning early in T-cell differentiation (CD4 and CD8 double-negative cells) and to continue expression in double-positive and single-positive cells in the thymus as well as in peripheral T cells (35, 43). To direct expression of Tat to T cells, we used the strategy outlined in Fig. 1 to construct the CD2-Tat transgene. While this construct transcribes both Tat and CD2 sequences, only functional Tat protein will be produced since a downstream frameshift mutation engineered in the unique *Eco*RV site in the coding region of the human CD2 gene results in the nonfunctional translation of CD2. Southern blot analysis (Fig. 2) of DNA from the three Tat transgenic lines demonstrates that lines CT1, CT2, and CT3 carry 14, 29, and 43 copies of the CD2-Tat transgene, respectively, as determined by PhosphorImager analysis.

The levels and tissue-specific pattern of expression of the CD2-Tat transgene in each of the lines were assessed by S1 nuclease protection analysis. Total mRNA from lymphoid and nonlymphoid tissues was examined with a 5′ S1 probe to demonstrate the correct initiation of the Tat transcript within the CD2 promoter. All three transgenic lines showed high levels of expression of the transgene (Fig. 3). Expression of Tat was confined to lymphoid tissues, i.e., thymus, spleen, lymph nodes, and peripheral blood. No transgene expression was detected in the RNA from skin, ovaries, muscle, lung, liver, kidney, heart, or brain of the three transgenic lines (Fig. 3).

Thymus and peripheral T-cell subset percentages are normal. Previously we have shown that overexpression of HIV-1

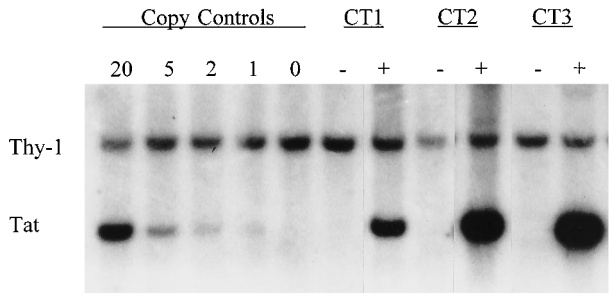

FIG. 2. Transgene copy number determined by Southern blot analysis. Genomic DNAs from the three transgenic lines CT1, CT2, and CT3 and various copy number controls were digested with *Hin*dIII and *Eco*RI and probed with radiolabeled Tat- and Thy-1-specific probes. Thy-1 was used as a DNA loading control. After normalization, transgenic copy numbers were determined by comparing Tat signal with known quantities of plasmid (copy controls) added to genomic DNA. − and + represent transgenic and nontransgenic littermates, respectively.

Nef driven by CD2 transcriptional elements and the CD2 LCR results in alterations of T-cell numbers and subset distribution in transgenic mice (10). To determine whether Tat also has quantitative and qualitative effects on T-cell subsets in vivo, FACS analysis was performed on cells from the thymus and peripheral lymphoid organs (spleen and lymph nodes) of CD2-Tat transgenic mice. Antibodies specific for CD4 and CD8 were used for analysis of T cells from transgenic and nontransgenic littermates of lines CT1 and CT2. As shown in Fig. 4, there was no apparent impairment in the development of mature T-cell subsets within the thymic compartment in that no differences in distributions or surface expression levels of CD4 or CD8 were observed. No differences in total thymocyte numbers were found (data not shown). Similarly, in peripheral T cells, no differences were observed in CD4/CD8 subset distributions, surface CD4/CD8 expression levels, or total T-cell numbers in transgenic mice (data not shown).

T cells from CD2-Tat transgenic mice show elevated levels of TNF-β mRNA and protein. It has been previously shown that a B-cell line constitutively expressing Tat upregulates TNF-β production (46). TNF-β upregulation has also been found in promonocytic and T-cell lines which express Tat (12). We examined whether this effect could also be demonstrated for primary T cells isolated from CD2-Tat transgenic mice. Thymocytes were isolated from CT1 and CT2 transgenic and nontransgenic littermates and were activated by treatment with the calcium ionophore ionomycin and the phorbol ester PMA. After 24 to 48 h, the culture supernatants were harvested and tested in a biological assay for TNF-β activity. Cells were harvested, and RNA was extracted for Northern blot analysis.

Northern blot analysis of steady-state mRNA levels of TNF-β, TNF-α, and β-actin (normalization control) in mitogen-induced transgenic and nontransgenic thymocytes is shown in Fig. 5. TNF-β levels of mRNA were increased in the transgenic mice of both lines. The levels were increased an average of 2.3-fold when the TNF-β hybridization signal was quantitated by reference to β-actin controls (Table 1). However, the quantitated levels of TNF-α expression were slightly decreased in both transgenic lines.

To establish whether the increase in TNF-β mRNA was mirrored by an increase in biologically active TNF protein, we examined culture supernatants from the activated thymocytes of CT1 and CT2 transgenic and nontransgenic littermates (Fig. 6). The amount of TNF activity measured in the bioassay with cytokine-sensitive cell line L929 was significantly increased in the thymocytes of both transgenic lines. While nontransgenic thymocytes produced 3 to 4 pg of TNF per ml, transgenic thymocytes produced 11 to 12 pg/ml. This 3- to 4-fold increase in TNF protein is slightly higher than the increase in mRNA levels of TNF-β (2.3-fold) and appears not to be limited by decreased levels of TNF-α mRNA.

Tat affects the cytokine profile of activated T cells. Evidence from studies on cultured cell lines suggests that Tat can upregulate other cytokine and cellular genes apart from TNF-β. Transfection of Tat or direct addition of Tat protein to human astrocytic glial cells shows transactivation of the TGF-β promoter in reporter gene constructs (19). Tat also upregulates the IL-4R expression on a human B-cell line (45). Therefore, we examined the expression of TGF-β and IL-4R in activated T cells from transgenic and nontransgenic littermates of the CT1 and CT2 Tat mouse lines. Figure 5 shows the steady-state level of TGF-β and IL-4R mRNA detected by Northern blot analysis. While TGF-β is expressed only at low levels in nontransgenic activated T cells, there is a clear and significant increase (2.8-fold; Table 1) in TGF-β expression in CT1 and CT2 transgenic littermates. Analysis of IL-4R mRNA levels revealed a slightly greater difference between nontransgenic and Tat transgenic littermates. From almost undetectable levels of IL-4R on activated nontransgenic T cells, IL-4R levels are raised by 3.1-fold in CT1 littermates and by nearly 4.0-fold in CT2 littermates (Fig. 5; Table 1). To ensure that the same quantities of T cells were present in each sample and to ensure that all samples was similarly activated, we used mouse CD2 and IL-2 probes for rehybridization of the Northern blots. All samples yielded the same hybridization signal when normalized to β-actin controls (data not shown).

T cells from Tat transgenic mice are not altered in mitogen- or antigen-stimulated responses. Since we found cytokine expression levels altered in Tat transgenic mice, we sought to determine whether this had consequences for T-cell proliferation in response to mitogenic or antigen-specific stimulation. Antigen-specific responses were of particular interest because the work of Viscidi et al. (54) suggests that Tat inhibits antigen-mediated T-cell proliferation. To assess the effect of mitogen,

FIG. 3. Tissue-specific expression of the Tat transgene. S1 nuclease protection analysis was performed with total RNA from CT1, CT2, and CT3 transgenic lines on the following tissues: thymus (T), spleen (S), skin (Sk), ovaries (Ov), muscle (M), lymph node (L), liver (Li), lung (Lu), kidney (K), heart (H), brain (Br), and blood (Bl). Tat, β-actin, and U6 small nuclear RNA (SnRNA) probes were end labeled, and protected fragments were analyzed on denaturing acrylamide gels as described in Materials and Methods. The faint bands appearing in brain and lung samples from CT1 are derived from incompletely digested β-actin probe.

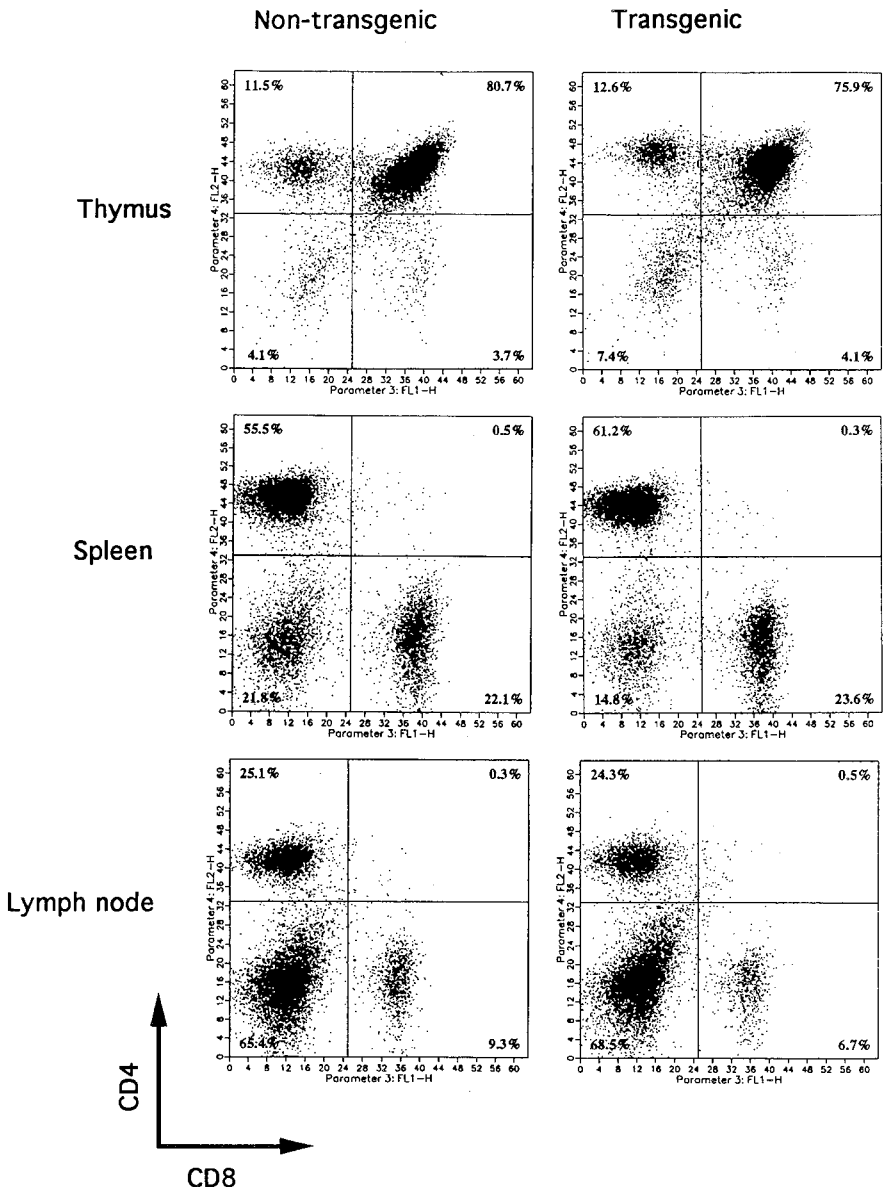

FIG. 4. Distribution of T-cell subsets in lymphoid tissues of CD2-Tat transgenic mice. Thymocytes, splenic cells, and lymph node cells were examined for the expression of CD4 and CD8 by flow cytometry. Cells from transgenic line CT1 and a nontransgenic littermate are shown; the cells were stained for CD4 (phycoerythrin ordinate) and CD8 (fluorescein isothiocyanate abscissa). Relative fluoresence intensities are given on a logarithmic scale, with percentages of double-negative, double-positive, and single-positive CD4/CD8 cells indicated. Analysis of 10,000 cells was performed for each sample with a Becton Dickinson FACScan.

we analyzed the proliferation of Tat transgenic T cells and littermate nontransgenic T cells of the CT1 and CT2 mouse lines to concanavalin A, anti-CD3 antibody, or PMA in combination with ionomycin. As shown in Fig. 7, no significant differences were seen between Tat-containing and Tat-negative T cells.

To examine whether Tat transgenic cells had altered responses to antigen, we primed transgenic and nontransgenic CT1 and CT2 littermates with KLH in complete Freund's adjuvant. Five to eight days later, lymph nodes were isolated, cultured, and restimulated with various concentrations of KLH. As shown in Fig. 8, in two separate experiments, no significant modulation was observed in the in vitro response to recall antigens. No decrease was seen in the ability of peripheral T cells from Tat transgenic mice to respond to KLH compared with their nontransgenic littermates.

Ageing Tat transgenic mice show no development of KS-like lesions. Previously it has been shown that transgenic mice expressing Tat under the transcriptional control of the HIV-1 LTR develop skin lesions (56) and show an increased incidence of liver cancer by 12 to 18 months of age (55). The skin lesions were described as having characteristics similar to those seen in KS. We have observed over 150 F_1 transgenic CT1 and CT2 mice for up to 2 years for such Tat-induced abnormalities. In contrast to previous results, the CD2-Tat transgenic mice appear normal, have shown no incidence of dermal lesions, KS-like or otherwise, and have not developed liver or any other tumors. Upon sacrifice of some of these mice, we isolated RNA and performed S1 nuclease protection analysis to confirm the continued expression of the Tat transgene. As shown in Fig. 9, the CD2-Tat transgene continues to be expressed at 18 months of age at high levels and in a lymphoid cell-specific manner.

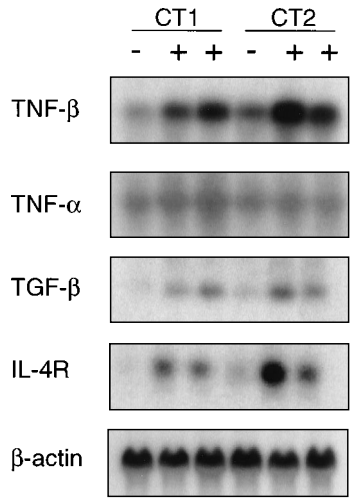

FIG. 5. Northern blot analysis of cytokine expression. Northern analysis was performed on RNA from T cells of CD2-Tat transgenic mice to quantitatively examine the levels of cytokine gene expression. Thymocytes were isolated from transgenic and nontransgenic CT1 and CT2 littermates and activated in the presence of PMA and ionomycin as described in Materials and Methods. After activation, the cells were harvested, total RNA was extracted, and 10 μg of RNA was fractionated on formaldehyde-agarose gels. Following transfer onto nylon membranes, the RNA was hybridized with probes for TNF-α, TNF-β, TGF-β, IL-4R, and β-actin (the latter used as an internal mRNA control). Relative signal intensities were quantitated with a PhosphorImager.

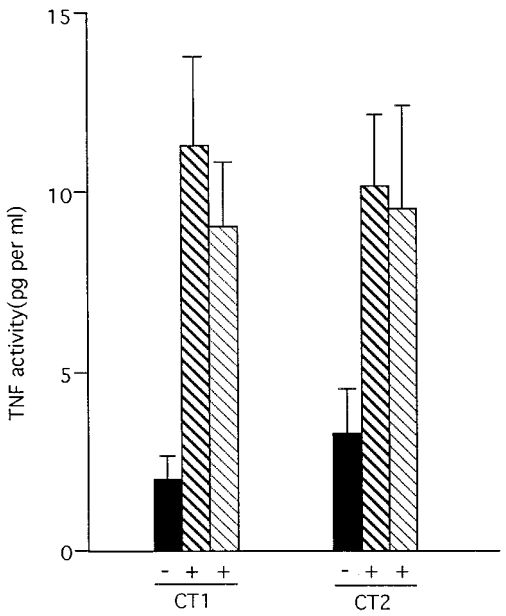

FIG. 6. Bioassay for TNF production. Culture supernatants from activated T cells of transgenic line CT1 (+), transgenic line CT2 (+), and nontransgenic littermate controls (−) were examined for the production of TNF, using the cytokine-sensitive cell line L929. The amount of TNF was determined relative to known amounts of recombinant cytokine titrated in this assay. Three separate experiments were performed with triplicate supernatant samples from transgenic and nontransgenic littermates. Inhibition by TNF-neutralizing antibodies demonstrated that the activity was specifically due to TNF (data not shown). L929 cell numbers were assessed by absorbance measurements at 540 nm after dye solubilization following staining with crystal violet.

DISCUSSION

We have generated a transgenic mouse model to study the biological effects of HIV-1 Tat on the developing and activated immune system in vivo. High-level expression of Tat was directed to T cells, the primary affected cell type in HIV-1 infection, using the transcriptional control elements of the human CD2 gene and the LCR (10, 27). Unlike the results obtained with our HIV Nef transgenic mouse model using the CD2 expression cassette, we saw no change in the CD4/CD8 T-cell populations or T-cell numbers of the Tat transgenic mice in either the thymus or peripheral lymphoid organs, nor did we see the downregulation of cell surface CD4, CD8, or CD3 (data not shown). Thus, the effects of Tat are unique and specific for this transgene, and the previously reported cytotoxic effects of HIV-1 Tat protein on lymphocytes (5) are not apparent in our system.

The observation that several cytokines are present at elevated levels in the serum or in cultured lymphocytes of HIV-1-infected patients (11, 38) has led to investigations probing the role of individual HIV regulatory proteins in the induction of cytokine gene expression. Previously, the mechanism of HIV-1-induced cytokine gene expression has been addressed by transfection of individual HIV genes into cultured cell lines. Tat gene transfection into a human B-cell line induced an eight-fold increase in TNF-β mRNA. While no increase was found in a Tat-transfected T-cell line (46) before activation, increases in TNF-β expression have been demonstrated in mitogen-stimulated Tat-transfected T-cell lines and in HIV-1-infected primary T cells (12). Results from our Tat transgenic mice showing induction of TNF-β mRNA and protein in primary activated T cells support these findings and suggest the efficacy of this in vivo animal model. Furthermore, the Tat-mediated effect in transgenic mice is specific since TNF-α mRNA expression was not similarly upregulated.

The action of the truncated Tat protein (encoded by only the first exon) to upregulate TNF-β transcription (12) is similar to transcriptional transactivation data obtained for the HIV-1 LTR (40, 48) in cell transfection studies. It has been suggested that sequence similarities between the TNF-β gene promoter and the promoter in the HIV-1 LTR may explain the effect of Tat on TNF-β expression (13). In common with these studies, our Tat transgenic mice, which were made with a construct containing only the first exon of the Tat gene, show similar increases in TNF-β transcription.

We have also demonstrated the upregulation of TGF-β mRNA in activated T cells from Tat transgenic mice. Previously, elevated levels of TGF-β have been found in peripheral blood mononuclear cells (34) and the brains of HIV-1-infected individuals (57). Interestingly, not all brain cells producing TGF-β were HIV-1 infected. Direct addition of Tat to human astrocytic glial cells (19) has been demonstrated to transactivate transcription from the TGF-β promoter. In combination, these results suggest that HIV-1-infected cells can influence

TABLE 1. Quantitation of cytokine RNAs in CD2-Tat transgenic mice

Cytokine	Fold RNA change compared with nontransgenic littermates[a]			
	CT1		CT2	
	1	2	1	2
TNF-β	2.2	2.3	2.2	2.2
TNF-α	0.8	0.7	0.7	0.9
TGF-β	2.2	3.3	2.7	2.9
IL-4R	3.4	2.8	4.0	3.8

[a] Quantitated by PhosphorImager and normalized to β-actin levels.

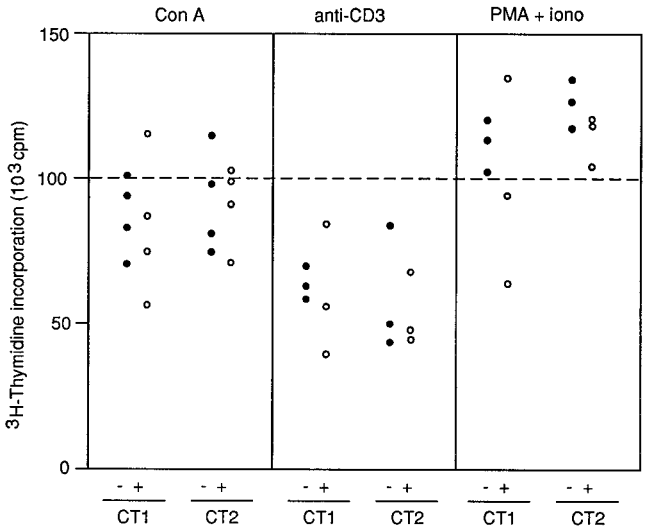

FIG. 7. T-cell proliferative responses of Tat transgenic cells induced via mitogen, T-cell receptor cross-linking, and PMA plus ionophore. Thymocytes from transgenic (closed circles) and nontransgenic (open circles) CT1 and CT2 littermates were cultured at 10^6 cells per ml on 96-well tissue culture plates in RPMI 140 medium containing 10% fetal calf serum and 20 mM 2-mercaptoethanol. T cells were induced to proliferate in the presence of concanavalin A (Con A; 2 μg/ml), anti-CD3ε (145-2C11 coated at 10 μg/ml), or PMA in combination with ionomycin. Forty-eight hours after establishment of culture, cells were pulsed with [^{3}H]thymidine for an additional 16 h. Cells were harvested, and thymidine incorporation was measured by scintillation counting to assess proliferation.

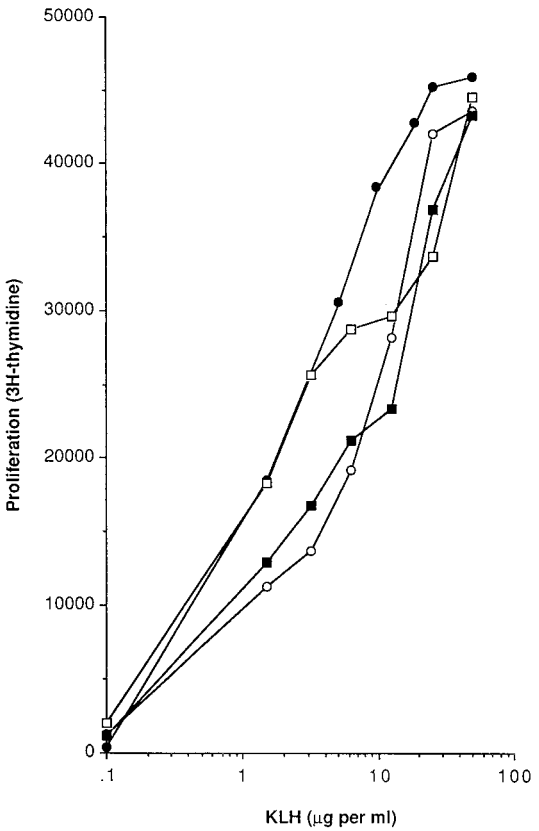

FIG. 8. Antigen receptor-induced proliferation to recall antigens. Peripheral lymph nodes (periaortic and inguinal) from CT1 (●, transgenic; ○, nontransgenic) and CT2 (■, transgenic; □, nontransgenic) animals that had been previously immunized with KLH (50 μg per mouse for 5 to 8 days) were isolated and cultured in vitro in the presence of increasing concentrations of KLH. After 4 days, cells were pulsed with [^{3}H]thymidine as for Fig. 7, and cell proliferation was assessed.

TGF-β production in surrounding cells, perhaps through the action of extracellular Tat. We have found that Tat affects TGF-β expression in activated transgenic T cells in culture, but it is undetermined whether this effect is cell autonomous or mediated extracellularly. The biological consequences of increased TGF-β expression in the Tat transgenic mice are unclear. Although TGF-β is known to have immunosuppressive functions including an antiproliferative effect on human T and B cells (reviewed in reference 36), we have found no differences in the mitogen- and antigen-induced proliferation of T cells in these mice.

A further difference found in the Tat transgenic mice is that the level of IL-4R mRNA is higher in T cells than in nontransgenic T cells, and the increase is similar to those seen previously in a transfected human B-cell line (45). This is particularly interesting since IL-4 (like TGF-β) under certain conditions can mediate immunosuppressive effects by inhibiting IL-2-induced proliferation of human B cells (31), antigen-specific immunoglobulin secretion (14), and proliferation of natural killer cells (59). IL-4 can also enhance HIV-1 replication in macrophages (42). Preliminary data on the levels of IL-4 in the Tat transgenic T cells suggest that the levels are not higher than in controls. Experiments are currently under way to assess the biological effects of Tat-induced IL-4R levels.

Our finding that Tat expression does not profoundly modulate T-cell activation by polyclonal mitogens or PMA is in agreement with previous studies (52, 54). However, these reports also ascribe to Tat the ability to inhibit antigen-induced lymphocyte proliferation. In our experiments, priming Tat transgenic mice with protein antigen KLH and subsequent KLH stimulation of T cells in culture yielded no such inhibition in antigen-induced proliferation. This difference may be explained by studies of Meyaard et al. (39) which show that only very high concentrations (5 μg/ml) of Tat inhibited CD3-mediated activation of purified T cells and that, in the presence of accessory cells, general T-cell activation and responses to recall antigens were inhibited. In our transgenic model, the concentration of Tat is at least 50-fold lower. Furthermore, we used lymph nodes containing both T cells and accessory cells as the source of cells for the proliferation assays. Under these conditions, no influences occur on either mitogen- or antigen-stimulated T-cell proliferation. We have also examined the in vivo response to superantigens SEA and SEB and to lipopolysaccharide and have found no significant differences.

Finally, we have shown that long-term expression (1 to 2

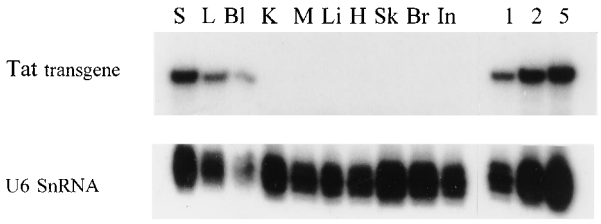

FIG. 9. S1 nuclease protection analysis of long-term transgene expression. The long-term expression of the CD2-Tat transgene was examined in a CT1 transgenic mouse at the age of 18 months. The tissue designations, probes, and protected fragments are as for Fig. 3. Lanes 1, 2, and 5 represent increasing amounts of control total RNA (5, 10, and 25 μg, respectively) from a stably transfected T-cell line expressing the pCD2TatLCR construct.

years) of Tat in the T cells of transgenic mice does not lead to the development of KS-like lesions. Evidence exists clearly showing that Tat (22), TNF, and other cytokines (3) promote the growth and proliferation of AIDS KS-derived cells (22) and more recently that basic fibroblast growth factor and Tat synergize to induce KS-like lesions in nude mice (23). It is interesting that these data indicate that Tat alone in nude mice has no effect, which may explain why our transgenic mice show no skin lesions. Alternatively, the absence of lesions may be due to the restricted expression of the CD2-Tat transgene to the T-cell lineage. Previously, transgenic mice which developed KS-like lesions and/or tumors and endothelial cell proliferation after 15 to 22 months were generated by using either the HIV LTR (56) or the BK polyomavirus promoter (16) to direct transcription of Tat. In these mice, cell proliferation and dermal lesions may be accounted for by the expression of Tat within nonlymphoid cell types since no expression of Tat was detected in $CD4^+$ T cells. In addition, the presence of essential cofactors such as another virus (15) or other immunosuppressive agents suggests that Tat alone is not sufficient for KS-like lesions to develop. Further studies using the T-cell-restricted expression in our CD2-Tat transgenic mice should be advantageous in the examination of the in vivo effects of extracellular Tat and the factors involved in dermal lesion development. They should also serve as an in vivo animal model to test molecular strategies for the inhibition of Tat-mediated HIV replication (32), particularly retrovirus-based systems for delivery of transactivation response sequence RNA decoys (53), Tat-mediated conditional cell ablative constructs (8), and transdominant mutant Tat decoys (44).

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H.J.M.B. and D.J.A. should be considered co-first authors since they made equal contributions to this work.

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