

Human Kaposi's Sarcoma Cell-Mediated Tumorigenesis in Human Immunodeficiency Type 1 Tat-Expressing Transgenic Mice

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Background: The human immunodeficiency virus type 1 (HIV-1) transactivator (Tat) protein has been linked to the development and course of Kaposi's sarcoma (KS) associated with acquired immunodeficiency disease syndrome (AIDS-KS). Tat is an 86–101 amino-acid protein encoded by two exons. To evaluate the growth-promoting effects of Tat in AIDS-KS *in vivo*, we developed transgenic mice expressing the one-exon-encoded 72 amino-acid protein (Tat₇₂) and the two-exon-encoded 86 amino-acid protein (Tat₈₆). **Methods:** Human KS SLK cells were injected subcutaneously into CD4⁺ T-cell-depleted male mice, and the tumors that formed after 3–4 weeks were recovered and analyzed for the expression of Tat protein(s), different cytokine messenger RNAs (mRNAs), and matrix metalloproteinases (MMPs). All statistical tests were two-sided. **Results:** The average tumor weight was maximum in Tat₈₆ mice (~600 mg) compared with Tat₇₂ (~200 mg) and nontransgenic (~100 mg) mice ($P < .005$). Histologic examination of tumors showed spindle-shaped SLK cells with prominent infiltrates of inflammatory cells. All of the tumors from Tat mice expressed abundant Tat mRNA, suggesting that the infiltrating mouse cells actively expressed Tat. A comparison of the growth-promoting cytokines in the tumors from Tat₈₆-transgenic and nontransgenic mice showed that the expression of the following cytokines was substantially increased in the tumors of the Tat₈₆ mice: tumor necrosis factor- α , interleukin 6, interleukin 8, granulocyte-macrophage colony-stimulating factor, and basic fibroblast growth factor. Furthermore, these tumors showed abundant expression of a 105-kd MMP activity associated with infiltrates of host leukocytes in the lesions. **Conclusion:** Our *in vivo* data clearly suggest that extracellular Tat can contribute to the growth and tumorigenesis of human KS cells. [J Natl Cancer Inst 2000;92:721–8]

The overall risk of Kaposi's sarcoma (KS) among human immunodeficiency virus type 1 (HIV-1)-infected individuals and those with acquired immunodeficiency syndrome (AIDS-KS) is more than 20 000 times higher than in the uninfected population and 300 times higher than that in the immunosuppressed transplant recipients (1). Although the mechanisms of its pathogenesis are not well understood, the epidemiologic and laboratory findings clearly indicate that the human herpesvirus 8 (HHV8, also called KS-associated herpesvirus) and HIV-1 play critical roles in the high incidence of KS in HIV-1-infected individuals [reviewed in (2)]. The earliest evidence to support the role of HIV-1 in AIDS-KS came from transgenic mouse studies showing that HIV-1 transactivator (Tat) can produce KS-like lesions when it is introduced into the germ line of mice (3). Although Tat expression in both male and female mice was comparable, tumors developed only in male mice, mimicking the male pre-

dominance. The expression of the Tat gene was also not observed in tumor cells. However, these findings suggested an important role of Tat in the pathogenesis of AIDS-KS. Numerous studies [reviewed in (4)] also suggest that inflammatory cytokines produced by infected or activated immune cells play an important role in the pathogenesis of AIDS-KS. Inflammatory cytokines present in the conditioned medium of activated T cells have been shown to support the growth of spindle cells derived from AIDS-KS lesions.

The HIV-1 Tat is an 86–101 amino-acid protein encoded by two exons [reviewed in (5)]. The product of the first exon (72 amino acids) is sufficient for transactivation of the HIV-1 gene expression from the viral promoter. However, the C-terminal (73–86/101 amino acids) encoded by the second exon contains the arginine-glycine-aspartate (RGD) sequence that is believed to function primarily as a cell-attachment domain and binds cell-surface integrin receptors (6,7). Studies (8,9) have shown that Tat protein released into the extracellular medium by HIV-1 infection of T cells stimulates the growth of AIDS-KS-derived spindle cells. Tat also stimulates the growth of and induces spindle morphology in normal endothelial cells that have been pretreated with inflammatory cytokines (10). Tat-induced migration, invasion, proliferation, and collagenase IV expression in KS spindle cells and in cytokine-activated endothelial cells in culture indicated that Tat can support angiogenesis (10,11). The *in vivo* angiogenic response of Tat has been demonstrated by subcutaneous injection of purified Tat in mice (11,12) and in transgenic mice expressing full-length Tat protein (13–15). The angiogenic properties of Tat have been mapped to its RGD sequence (6,7,11) and to the basic amino-acid region that is similar to that of angiogenic growth factors and binds heparin complexes (16,17). Basic fibroblast growth factor (bFGF) and heparin have been shown to significantly enhance the *in vivo* angiogenic effects of Tat (11,16). bFGF and Tat are present in AIDS-KS lesions, and integrin receptors that bind Tat are highly expressed by vessels and spindle cells.

Histologically, KS lesions consist of a heterogeneous population of cells, including characteristic aggregates of spindle-shaped cells, fibroblasts, dendritic cells, and a prominent infiltrate of leukocytes and other inflammatory cells (18). A number

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of studies [(18); reviewed in (19)] suggest that infiltrating leukocytes in AIDS-KS lesions could be a possible source of HIV-1 Tat, inflammatory cytokines, and HHV8, thereby contributing to the aggressive and rapid growth of tumors. HIV-1 infection of leukocytes is also known to induce synthesis and secretion of matrix metalloproteinases (MMPs), the enzymes that degrade components of the extracellular matrix, and aid cells in traversing the tissues (20–22). More recently, HIV-1 Tat protein alone has been shown to increase the production of MMP-9 (92-kd MMP) in monocytes and also to increase their adhesion to endothelial cells (23), causing them to extravasate into lesions.

In this study, we used transgenic mice expressing two-exon-encoded Tat (Tat₈₆) and one-exon-encoded Tat (Tat₇₂) to investigate whether Tat expression will promote the growth and tumorigenic potential of human KS SLK cells subcutaneously injected into these mice. These findings may be relevant for the understanding of the aggressive and rapid course of KS in HIV-1-infected individuals and for the development of targeted anti-AIDS-KS therapies.

MATERIALS AND METHODS

Production of Transgenic Mice

Transgenic mice expressing one-exon-encoded Tat protein (Tat₇₂) and two-exon-encoded Tat protein (Tat₈₆) under the transcriptional control of simian virus 40 promoter were generated as previously described (24). The heterozygous lines of mice established from the transgenic founders expressed Tat protein in several tissues, including bone marrow cells (24). The Tat protein was also biologically active as determined by the transactivation of the HIV-1 long terminal repeat (LTR) in doubly transgenic mice carrying both Tat and chloramphenicol acetyltransferase (CAT) reporter gene under the transcription control of the LTR (LTR-CAT) (24). Tat transgenic mice were routinely identified by the Southern blot analysis of the tail DNA.

Injection of Human KS Cells in Mice

The KS SLK cell line established from a KS lesion of gingiva of an HIV-1-negative immunosuppressed renal transplant recipient (25) was maintained in gelatin-coated flasks as previously described (26). The SLK cell line is of endothelial origin and, when inoculated in nude mice, induces tumors with endothelial characteristics (27). Approximately 1×10^6 KS SLK cells were mixed with liquid Matrigel (Collaborative Biomedical Products, Bedford, MA) in a final volume of 0.5 mL and injected subcutaneously near the abdominal midline of immunosuppressed Tat transgenic and nontransgenic mice. For activation experiments, KS SLK cells were treated with 10 ng/mL of phorbol 12-myristate 13-acetate (PMA) for 1 hour in culture followed by incubation in medium without PMA for 4 hours before injection in mice. PMA (10 ng/mL) was incorporated in the Matrigel before injection, where indicated. Immunosuppression in mice was induced by weekly intraperitoneal injections of 0.3 mg of GK1.5 (American Type Culture Collection, Manassas, VA) antibody, a rat monoclonal antibody targeting the CD4 molecule (28). The antibody was partially purified from ascites fluid by the ammonium sulfate precipitation procedure described previously (29). Flow cytometric analysis of splenic cells with fluorescein isothiocyanate-conjugated anti-CD4 antibody (Pharmingen, San Diego, CA) showed more than a 85% loss of CD4⁺ T cells 5–7 days after anti-CD4 antibody treatment. Mice were killed to recover Matrigel implants, when necessary, for histologic examination or other procedures.

Gelatin Zymography

For zymography, tumor and tissue extracts were prepared by homogenization and sonication in 0.01 M Tris buffer (pH 7.0) containing 0.01% Triton X-100 followed by centrifugation at 10000 rpm for 10 minutes at 4 °C. The protein content of cell-free supernatants was determined by the Bio-Rad protein assay method (Bio-Rad Laboratories, Hercules, CA) (30). Gelatinase activity was assayed by electrophoresis of tissue or cell extracts containing 20 µg protein on 10% polyacrylamide gels with 0.1% gelatin (NOVEX, San Diego, CA), accord-

ing to the supplier's instructions. After electrophoresis, the gels were incubated overnight in developing buffer and stained with Coomassie Blue. Proteolytic activity was detected as unstained bands against a blue background. To characterize these bands as MMPs, gels were incubated with developing buffer containing 20 mM EDTA, where indicated.

Isolation of Mouse Mononuclear Cells and Polymorphonuclear Leukocytes

Mononuclear cells (MNCs) and polymorphonuclear leukocytes (PMNs) from mouse blood and bone marrow were isolated by use of a two-component step gradient procedure (Cardinal Associates, Inc., Santa Fe, NM). A light microscopic examination revealed that the isolated PMN and MNC fractions from blood contained 70% and 4% segmented neutrophils, respectively. On the other hand, the PMN and MNC fractions from bone marrow contained 54% and 80% neutrophils, respectively.

RNA Extraction and Reverse Transcription-Polymerase Chain Reaction for Quantitation of Tat and Cytokine Messenger RNAs From Tumor Tissues

Total cellular RNA from liquid nitrogen-frozen tissues was extracted by use of the Rneasy Mini Kit (Qiagen, Valencia, CA), according to the supplier's instructions. To eliminate DNA contamination, extracted RNA was treated with ribonuclease-free deoxyribonuclease. The final preparation was quantitated spectrophotometrically, examined for the presence of 18S and 28S ribosomal RNA bands, and stored at -70 °C until use.

Reverse transcription (RT) of the RNA (500 ng) was performed in a final volume of 20 µL containing 50 mM Tris-HCl, 75 mM KCl, 3 mM MgCl₂, 10 mM dithiothreitol, 0.5 µM each of the deoxynucleotide triphosphates, 200 ng random primers (Promega Corp., Madison, WI), and 200 U of reverse transcriptase (Life Technologies, Inc. [GIBCO BRL], Gaithersburg, MD). The reaction mixture was incubated at 42 °C for 1 hour and then heated at 94 °C for 10 minutes to inactivate the enzyme. One microliter of this reverse transcribed mixture was used for polymerase chain reaction (PCR) amplification of the complementary DNA in a 50-µL reaction volume.

The primers for the PCR amplification of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH), human nuclear factor-kappa B (p50) (NF-κB [p50]) and all of the human cytokines, except bFGF, and their positive-control PCR products were obtained from Clontech Laboratories, Inc. (Palo Alto, CA). The primer and oligoprobe sequences for bFGF, Tat₈₆, and Tat₇₂ were obtained from the published sequence and were as follows: bFGF (GenBank, Accession No. E02544), 5'-GGCTTCTTCTCCTCGCATCCAC-3', 5'-CTGCCAGTTCGTTTCAGTGC-3', and 5'-TGGCTTCTAAATGTGTACGGATGAGT-GTT-3'; Tat₈₆ (31), 5'-CCTACCTGCCATGGAACCAGTCGACCCTA-3', 5'-GATCCTTACTATTCCTTAGGACCTGTGCG-3', and 5'-GGCTTTGGGTATCTCCTACGG-3'; and Tat₇₂ (32), 5'-TCGACCCGCGTCTGGAAC-CATTGGAAAC-3', 5'-AGAGAAACCTGGTGGGTCTGGGA-3', and 5'-CGGCCGTAAAAACGTCGTC-3'. The PCR amplification was carried out for 30 cycles by use of Perkin-Elmer (The Perkin-Elmer Corp., Foster City, CA) PCR reagents in a 50-µL volume and a DNA Thermal Cycler Model 9700 according to the PCR protocol provided by Clontech Laboratories, Inc. To ascertain that equal amounts of tumor RNAs were used in each RT-PCR reaction, the same RT mixture was used for concurrent amplification of GAPDH.

The amplified RT-PCR products were detected by Southern blot analysis of 25 µL (5 µL for Tat₈₆ and GAPDH) of the final reaction mixture by use of the ³²P-labeled positive internal PCR products or oligoprobes. The intensity of the resultant bands on the autoradiograms was quantitated by use of the Molecular Dynamics PhosphorImager Model 840 (Molecular Dynamics Corp., Sunnyvale, CA).

Statistical Analysis

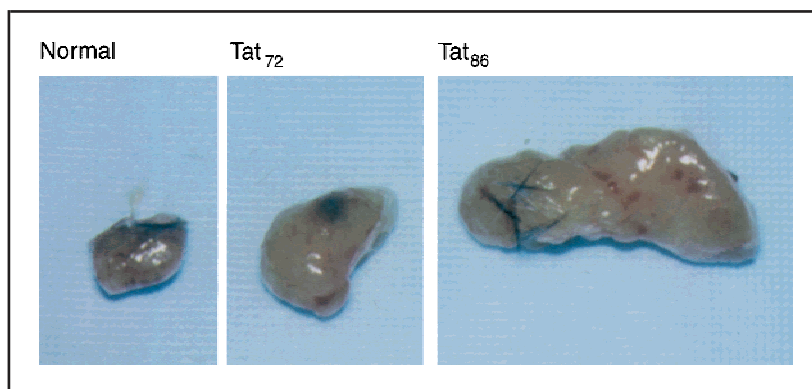
Student's *t* test was applied to estimate the statistical significance of difference (33) in the tumor weights from nontransgenic mice, Tat₇₂, and Tat₈₆-transgenic mice. All *P* values were two-sided.

RESULTS

Human KS SLK Cell-Derived Tumorigenic Response in Tat-Transgenic Mice

Earlier studies (24) have shown that the transgenic mice carrying the one-exon Tat gene (Tat₇₂) and the two-exon Tat gene

Fig. 1. A typical representation of human Kaposi's sarcoma SLK cell-derived tumor-growth in human immunodeficiency virus 1 transactivator (Tat)-transgenic and normal nontransgenic mice. The SLK cells were grown and subcutaneously injected in mice as described in the "Materials and Methods" section. Four to 5 weeks after injection, the animals were killed and the Matrigel implants were recovered and weighed.



(Tat₈₆) express detectable levels of Tat protein in several tissues. Tat is also biologically active in these mice, as seen by the transactivation of the HIV-1 LTR in doubly transgenic mice carrying both Tat and LTR-CAT transgenes (24). To determine whether HIV-1 Tat expression influences the tumorigenic potential of human KS cells, we compared the tumorigenicity of KS SLK cells in nontransgenic and Tat-transgenic mice. All nontransgenic and Tat-transgenic mice developed tumors in 3–4 weeks after inoculation with KS SLK cells. However, the tumor growth response in the three lines of mice was different. In general, the tumor growth in Tat₈₆ mice was considerably higher than that in nontransgenic animals, whereas an intermediate growth response was seen in Tat₇₂ mice (Fig. 1). A statistical analysis performed on the weight differences in tumors obtained from each of the lines of mice revealed a sixfold to sevenfold higher tumor growth in Tat₈₆ mice (~600 mg) compared with nontransgenic mice (~100 mg) and a twofold to threefold higher tumor growth in Tat₇₂ mice (~200 mg) (Fig. 2). The average tumor weight in Tat₈₆ mice was statistically significantly greater than that in the other two groups ($P < .005$). The observed difference between the Tat₇₂ and the nontransgenic groups was not statistically significant ($P = .1$). These observations clearly indicated that Tat₈₆ and, to a lesser extent, Tat₇₂ promote the growth of KS SLK cell-derived tumors in Tat transgenic mice.

Histologic examination of tumors by light microscopy showed spindle-shaped SLK cells (Fig. 3, large arrow) with a prominent infiltrate of inflammatory cells (small arrow). The tumor cell growth in culture showed morphologic features of the parental SLK cells (not shown).

NF- κ B p50 and Cytokine mRNA Expression in KS SLK Cell-Derived Tumors in Tat-Transgenic Mice

Numerous studies [reviewed in (4)] have suggested that inflammatory cytokines that are elevated in HIV-1-infected individuals and cytokines produced by AIDS-KS cells cooperate with HIV-1 Tat in the development of AIDS-KS. To test the possibility that Tat and certain inflammatory cytokines might play a role in the enhanced tumor growth in Tat-transgenic mice, we measured the expression of a number of cytokines, such as interleukin 2 (IL-2), interleukin 4 (IL-4), interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin 1 α (IL-1 α), interleukin 1 β (IL-1 β), tumor necrosis factor- α (TNF- α), interferon gamma (IFN γ), granulocyte-macrophage colony-stimulating factor (GM-CSF), and bFGF. Since the two-exon-encoded Tat is the predominant form of Tat produced during HIV-1 infection and the observed difference between the tumors in one-exon-encoded Tat (Tat₇₂) and the nontransgenic group was not statis-

tically significant, we randomly selected four tumors from nontransgenic and Tat₈₆-transgenic mice for analysis by the semiquantitative RT-PCR method as described in the "Materials and Methods" section. As shown in Fig. 4, all of the tumors from Tat-transgenic mice showed the expression of Tat mRNA, suggesting that the infiltrating mouse cells actively expressed Tat. The comparative profile of cytokine expression in tumors from the Tat-transgenic and nontransgenic mice showed substantial

Fig. 2. Comparison of growth response of Kaposi's sarcoma SLK cell-derived tumors in transactivator (Tat)-transgenic and nontransgenic mice. Data are expressed as the mean \pm standard deviation for nontransgenic and Tat₇₂ groups ($n = 10$) and for Tat₈₆ group ($n = 15$). * = statistically significant difference in tumor weights in the Tat₈₆-transgenic mouse group versus the Tat₇₂-transgenic and nontransgenic mouse groups, with $P < .005$.

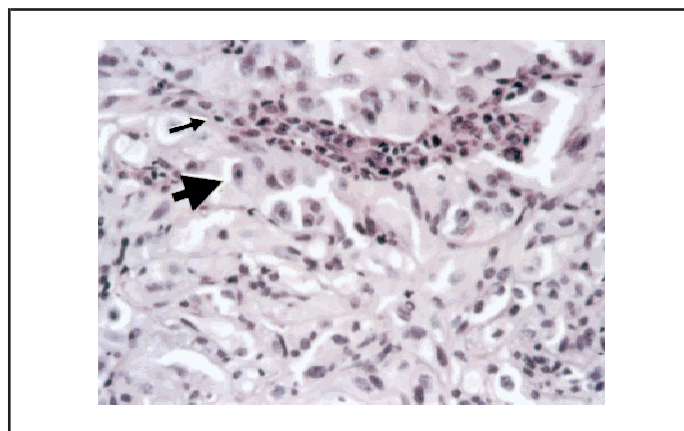
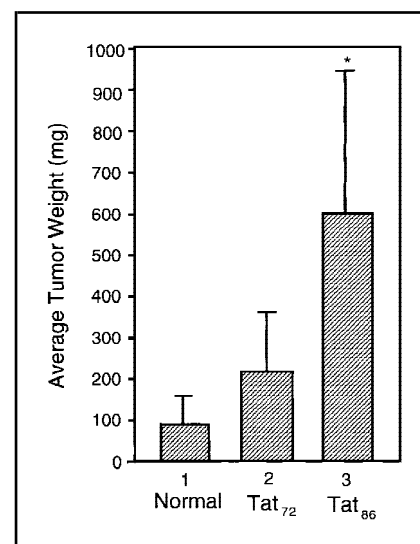
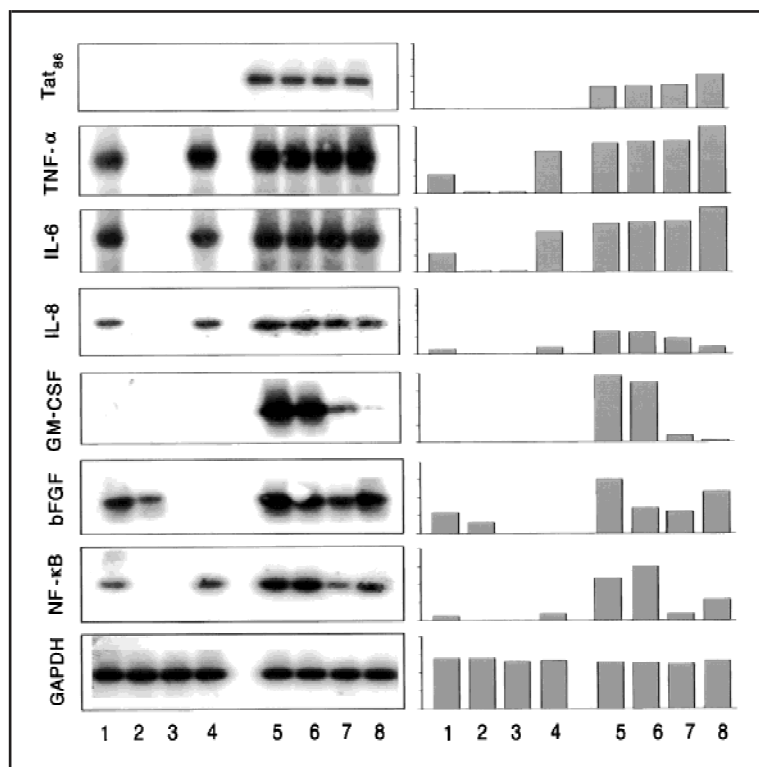


Fig. 3. Hematoxylin-eosin-stained section of a representative tumor from Tat₈₆-transgenic mouse showing abundance of human Kaposi's sarcoma SLK cells (large arrow) and a prominent infiltrate of inflammatory cells of host origin (small arrow).

Fig. 4. Human immunodeficiency virus (HIV) type 1 transactivator (Tat)₈₆, nuclear factor- κ B (p50) (NF- κ B [p50]), and cytokine gene expression in the human Kaposi's sarcoma SLK cell-derived tumors in nontransgenic (lanes 1–4) and Tat₈₆-transgenic mice (lanes 5–8). The semiquantitative reverse transcription–polymerase chain reaction analysis was carried out as described in the “Materials and Methods” section. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene expression was used as an internal control. Since there was no statistically significant difference between the tumors in Tat₇₂-transgenic and the nontransgenic groups, the comparison was made only between the Tat₈₆-transgenic and the nontransgenic groups. bFGF = basic fibroblast growth factor; GM-CSF = granulocyte–macrophage colony-stimulating factor; IL-8 = interleukin 8; IL-6 = interleukin 6; TNF- α = tumor necrosis factor- α .



differences, although GAPDH expression in all of the tumors was comparable. Four of four tumors from Tat-transgenic mice showed expression of TNF- α , IL-6, IL-8, and bFGF compared with only two tumors from the nontransgenic group. The level of mRNA expression was also higher in the tumors from Tat mice than in the tumors from nontransgenic mice. Of interest, three of the four tumors from Tat mice showed expression of GM-CSF, but no expression was detectable in the tumors from nontransgenic animals. The expression of IL-2, IL-4, IL-1 β , and IFN γ mRNAs was undetectable in all tumors. The transcription factor NF- κ B is known to play a key role in the expression of a number of genes involved in inflammatory responses and cellular growth regulation (34,35). The most abundant form of NF- κ B is a complex composed of p50–p65 heterodimer belonging to the Rel family. In most cells, NF- κ B/Rel proteins are sequestered in the cytoplasm by any of several inhibitor proteins I κ B. Activation of NF- κ B involves I κ B degradation and subsequent translocation of NF- κ B/Rel proteins to the cell nucleus. Earlier reports (36–38) have indicated that extracellular Tat can induce the activation of NF- κ B. In this study, we found that NF- κ B p50-related transcripts in three of the four tumors from Tat mice were substantially elevated, whereas negligible transcription was observed in the tumors from nontransgenic animals. The results of cytokine mRNA analysis suggested that exposure of KS SLK cells to extracellular Tat and to certain inflammatory cytokines might play a role in the enhanced growth of KS SLK cell-derived tumors in Tat mice.

Secretion of Leukocyte-Specific MMP in Tumors From Tat Mice

The KS-derived cells in culture have been shown to constitutively release MMPs into the culture supernatants, suggesting that these cells constitutively have an invasive and metastasizing potential (39). To determine whether tumors from Tat-transgenic

mice produced increased levels of MMPs that contributed to more aggressive tumor growth, we performed gelatin zymography. As shown in Fig. 5, A–D, only one of 10 tumors in nontransgenic mice showed a 105-kd MMP band (panel A), whereas all of the tumors from Tat₈₆ mice showed an intense band of 105-kd (panel B). A similar band was observed in four of 10 tumors from Tat₇₂ mice (panel C). All members of the MMP family contain a zinc ion at the catalytic site, require Ca²⁺ for full activity, and are inhibited by chelating agents [reviewed in (40)]. Inhibition with 20 mM EDTA (panel D) showed that the 105-kd band was associated with MMP activity. Blankart et al. (39) have reported that human KS cells produce a 92-kd MMP activity. We also found a similar activity produced by the KS SLK cells (not shown), suggesting that the 105-kd specific activity in our study originated from infiltrating mouse cells in the tumors. We next screened mouse tissues to determine the source of the 105-kd MMP activity. As shown in Fig. 6, A, of all the tissues examined, abundant activity was detectable only in the bone marrow. For the determination of the cell type specificity of the 105-kd MMP, bone marrow cells were fractionated into MNCs and PMNs by gradient centrifugation, and the fractions were analyzed by gelatin zymography. We also determined if a similar activity was present in MNC and PMN fractions collected from mouse blood. As shown in Fig. 6, B (lanes 1–4), the 105-kd MMP activity was detectable almost entirely in the blood-derived PMN fraction (lane 2); only a negligible activity was seen in the MNC fraction (lane 1). In contrast, in the bone marrow, substantial activity was observed in both fractions (lanes 3 and 4). A light microscopic examination (Fig. 7, A–D) of the MNC and PMN fractions from blood and bone marrow from tumor-bearing and control mice revealed that PMN fractions (Fig. 7, A and C) were mainly (>75%) composed of segmented neutrophils, suggesting that these neutrophils might be the source of 105-kd MMP activity. The abundance of MMP

Fig. 5. Metalloproteinase (MMP) activities in representative tumors from nontransgenic (A), transactivator (Tat)₈₆ (B), and Tat₇₂ transgenic (C) mice. The inhibitory effect of EDTA on 105-kd MMP is shown in panel D. Gelatin zymography was carried out as described in the "Materials and Methods" section. kDa = kilodalton.

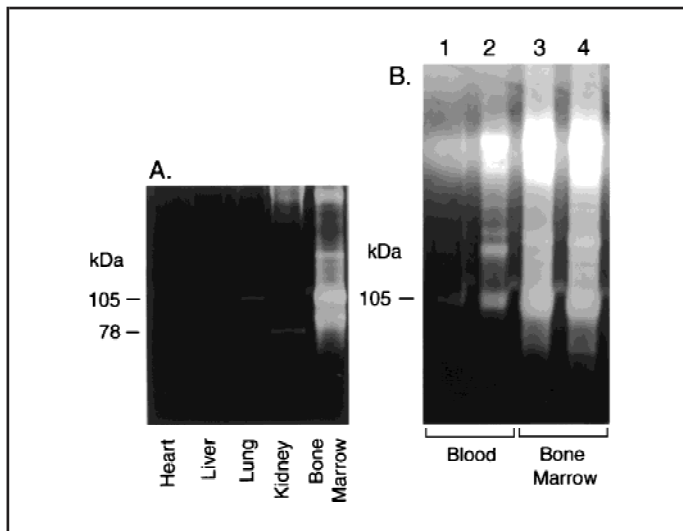
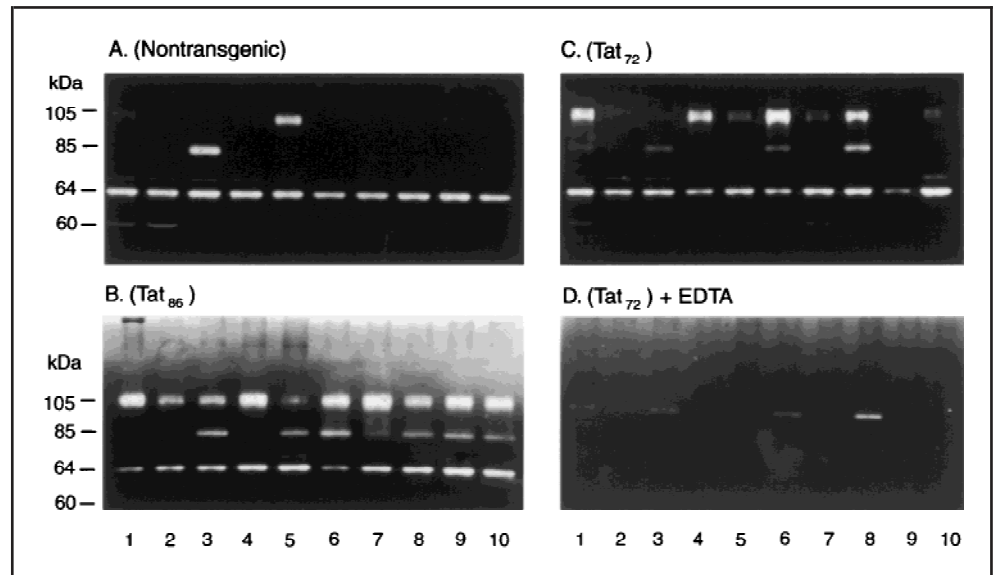


Fig. 6. A) Expression of 105-kd metalloproteinase (MMP) in mouse tissues. B) Identification of 105-kd MMP-producing cells in blood and bone marrow tissues of mice. The mononuclear cell (MNC) and polymorphonuclear leukocyte (PMN) fractions from blood are shown in lanes 1 and 2, respectively, and the MNC and PMN fractions from bone marrow cells are shown in lanes 3 and 4, respectively. Preparation of tissue extracts and gradient separation of blood and bone marrow cells into MNC and PMN fractions was carried out as described in the "Materials and Methods" section. Gelatin zymography was carried out with the use of 20 μ g of protein except that extracts containing 5 μ g of protein were used for bone marrow samples in panel A and for all of the samples in panel B. kDa = kilodalton.

activity in the MNC fraction obtained from bone marrow (Fig. 7, D) might also be attributed to segmented neutrophils, since these cells were abundantly present in this fraction. On the other hand, a limited presence (~4%) of segmented neutrophils in the MNC fraction from blood (Fig. 7, B) was associated with the negligible 105-kd MMP activity. In conclusion, the results of these experiments suggested that the 105-kd MMP activity predominantly observed in the tumors from Tat₈₆-transgenic mice might be associated with the infiltrating host neutrophils.

Extravasation of Circulating Leukocytes Into Lesions

The KS lesions contain a prominent infiltrate of blood cells. These cells in HIV-1-infected individuals could contribute to

tumor growth through the release of inflammatory cytokines and Tat protein. To determine whether the KS SLK cells attract and bind circulating leukocytes that secrete 105-kd MMP, we inoculated KS SLK cells mixed with Matrigel into nontransgenic mice and assessed the extent of leukocyte infiltration. Subcutaneously injected Matrigel alone was used as a control. As shown in Fig. 8 (lanes 1–4), the KS SLK cell pellet showed substantially higher gelatinase activity than the Matrigel pellet (compare lanes 3 and 1). The activity was further increased substantially (lane 4) by pretreatment of the KS cells in culture with PMA, whereas PMA alone in Matrigel had a minimal effect (lane 2). The changes in the MMP activity (lanes 3 and 4) were associated with the extravasation of leukocytes (not shown) into the SLK cell pellets. Since PMA, lipopolysaccharides, and certain cytokines, such as TNF- α , IL-1 β , and IL-8, are known to increase the chemotaxis and adhesion of leukocytes to primary endothelial cells and KS cells (41–43), our observations suggested that activation of KS SLK cells increased their adhesion for leukocytes that could, in turn, stimulate a local inflammatory response for the development of KS.

DISCUSSION

Several lines of experimental evidence now suggest that HIV-1 plays an active role in the pathogenesis of AIDS-KS through the cooperative effects of inflammatory cytokines produced by infected and activated immune cells and the HIV-1-encoded Tat protein [reviewed in (4)]. In this article, we provide the first evidence that Tat expression is associated with growth of KS SLK cell-induced tumors in Tat-transgenic mice. The tumor growth in Tat₈₆ mice was sixfold to sevenfold higher than that in the nontransgenic animals. An earlier study (44) has shown that picomolar concentrations of Tat released into the extracellular medium by infected cells can promote the growth of cells derived from KS lesions of AIDS patients. Subsequently, it was found that Tat can significantly enhance the growth-promoting effects of inflammatory cytokines that are growth factors for AIDS-KS spindle cells (10). Extracellular Tat stimulates growth and induces spindle morphology in normal endothelial cells that have been exposed to inflammatory cytokines. Inflammatory cytokines also induce AIDS-KS cells to

Fig. 7. Light microscopic identification of polymorphonuclear leukocytes (PMNs) (arrows) in the PMN and mononuclear cell (MNC) fractions of blood and bone marrow. The PMN and MNC fractions from blood are shown in panels A and B, respectively, and those from bone marrow are shown in panels C and D, respectively.

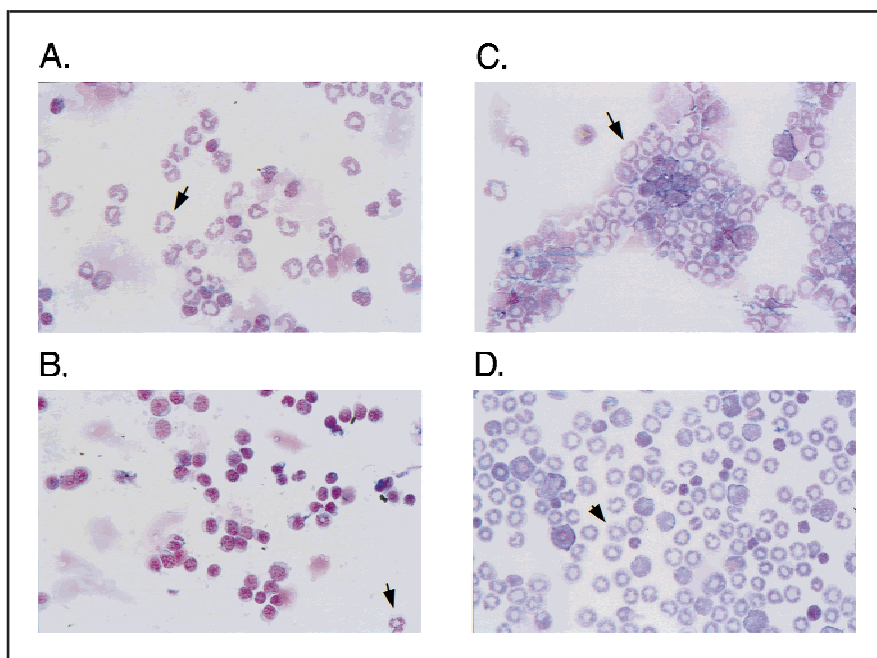
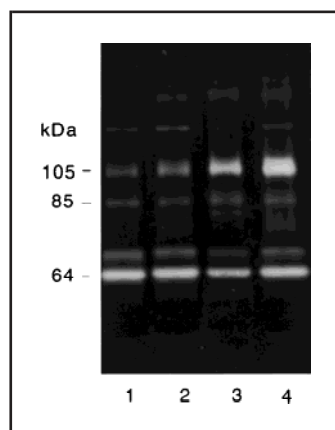


Fig. 8. Effect of Kaposi's sarcoma SLK cells on the infiltration of polymorphonuclear leukocytes (PMNs) in Matrigel implants. Matrigel, either alone (lane 1) or with phorbol 12-myristate 13-acetate (PMA) (lane 2), SLK cells (lane 3), or PMA-treated SLK cells (lane 4), was subcutaneously injected into nontransgenic mice as described in the "Materials and Methods" section. After 24 hours, the implants were removed for light microscopic examination and gelatin zymography. kDa = kilodalton.



release bFGF, a potent autocrine growth factor that promotes growth and angiogenesis of AIDS-KS cells (45). *In vivo*, the proliferative and angiogenic responses of bFGF are synergistically enhanced by purified Tat (11).

In addition to other sources of inflammatory cytokine production in HIV-1-infected individuals [reviewed in (4)], Tat itself has been shown to modulate the expression of a number of cytokines. Lafrenie et al. (46) have shown that treatment of monocytes with purified Tat in culture enhances the production of IL-1 β , IL-6, IL-8, and TNF- α and their respective mRNAs. In other studies, induced cellular expression of Tat in cells has been shown to increase the expression of IL-2 (47), IL-6 (48), and TNF- β (49,50). Tat expression in transgenic mice has been shown to increase the expression of TNF- β and TGF- β (51) as well as IL-6 and IL-10 (52,53). In our study, the HIV-1 Tat protein could promote the growth of SLK cell-derived tumors in Tat-transgenic mice in a number of ways. Since the tumors contain a prominent infiltrate of mouse blood cells, these cells could provide exogenous Tat. As shown in Fig. 4, Tat mRNA is abundantly expressed in the tumors from Tat₈₆ mice. We have previously reported that these mice also show readily detectable levels of Tat protein in most tissues, including the bone marrow

cells (24). In addition to bFGF expression, these tumors also show substantial increases in the mRNA levels for TNF- α , IL-6, IL-8, and GM-CSF. These are some of the cytokines that have an autocrine/paracrine role in the proliferative activity of AIDS-KS, and their activity is further augmented in the presence of Tat protein [reviewed in (4)].

Several reports (36,37,54) have demonstrated that extracellular Tat is associated with activation of transcription factor NF- κ B. The major form of NF- κ B is composed of a p50 and p65 heterodimer. In this study, we found that the expression of p50 transcripts is significantly increased in tumors from Tat mice but not in tumors from nontransgenic mice. Although we have not examined the expression of the p65 subunit, its stoichiometric appearance with p50 (55) suggests that the induction of the native NF- κ B complex might be increased as well in the tumors from Tat mice. NF- κ B is involved in the transcriptional regulation of a number of inflammatory cytokines [reviewed in (56,57)]. Aberrant NF- κ B activity is also associated with enhanced cell proliferation and oncogenesis. For example, constitutive NF- κ B activation is required for proliferation and survival of many cells, including breast cancer cells (58) and Hodgkin's lymphoma cells (59). Consistent with these observations, it is conceivable that NF- κ B might participate in tumor progression in Tat transgenic mice.

Tat contains several functional domains. Previous studies (7,15) have shown that binding of extracellular Tat to the cell surface is mediated by two distinct domains: a basic domain (amino acids 42–64) and a C-terminal domain (amino acids 65–80) containing the RGD sequence that binds cell-surface integrin receptors (6). The same integrins are highly expressed by KS spindle cells and are inducible by inflammatory cytokines on endothelial cells (7). The basic domain of Tat is similar to that of several growth factors [reviewed in (60)] and mediates binding of Tat to Flk-1/KDR, a receptor for vascular endothelial growth factor-A, which is a potent growth factor for AIDS-KS cells (61). Interaction of extracellular Tat with these receptors can induce the growth and migration of endothelial and KS cells (7,11,17,60). In our study, the selective presence of RGD in

Tat₈₆ might explain the enhanced tumor growth in Tat₈₆ mice over that in Tat₇₂ mice.

Numerous studies (20–22) have suggested that, in addition to providing inflammatory cytokines and viral Tat, the infiltrating HIV-1-infected leukocytes in KS lesions could also be a source of MMPs, which are essential for tumor invasion and metastasis. MMP secretion has been shown to be increased in HIV-1-infected T lymphocytes, monocytes, and macrophages (20–22). In addition, treatment of monocytes with soluble Tat in culture has been shown to increase the production of MMP-9 (23). In this study, we found that the mouse neutrophil-associated 105-kd gelatinase activity was significantly increased in all of the tumors from Tat₈₆ mice. Although the mechanism of its activation remains to be explored, our observations suggest that this MMP might also play a role in the accelerated growth of KS SLK cell-derived tumors in Tat₈₆ mice.

In conclusion, our *in vivo* study strengthens the role of extracellular Tat and inflammatory cytokines on the pathogenesis of AIDS-KS and may provide one of the explanations for why KS is a more aggressive disease in HIV-infected individuals. Our transgenic mouse model may be useful to study the efficacy of the anti-KS therapies, especially those targeting Tat activity.

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Supported in part by a grant from the AIDS Crisis Trust (O. Prakash).

We thank John and Mary Lou Ochsner and Marguerite Littman for supporting this study. We also thank Peng Zhou for technical assistance, Nicholas Lanson for assistance in the phosphoimaging analysis of the reverse transcription-polymerase chain reaction data, and Robert Gallo for his encouragement in this work and for his careful reading of the manuscript.

Manuscript received November 16, 1999; revised January 27, 2000; accepted February 13, 2000.