MOLECULAR AND CELLULAR BIOLOGY, Nov. 1989, p. 4731–4737
0270-7306/89/114731-07$02.00/0

Vol. 9, No. 11

trans Activation of Granulocyte-Macrophage Colony-Stimulating Factor and the Interleukin-2 Receptor in Transgenic Mice Carrying the Human T-Lymphotropic Virus Type 1 *tax* Gene

JEFFREY E. GREEN,[1] C. GLENN BEGLEY,[2] DAVID K. WAGNER,[2,3] THOMAS A. WALDMANN,[2] AND GILBERT JAY[4]*

Laboratory of Molecular Virology[1] and Metabolism Branch,[2] National Cancer Institute, Bethesda, Maryland 20892; Westside Veterans Administration Medical Center, Chicago, Illinois 60612[3]; and Laboratory of Virology, Jerome H. Holland Laboratory, American Red Cross, Rockville, Maryland 20855[4]

Received 13 June 1989/Accepted 1 August 1989

Three lines of transgenic mice carrying the human T-cell lymphotropic virus type 1 *tax* gene have previously been reported to develop neurofibromas composed of perineural fibroblasts (S. H. Hinrichs, M. Nerenberg, R. K. Reynolds, G. Khoury, and G. Jay, Science 237:1340–1343, 1987; M. Nerenberg, S. H. Hinrichs, R. K. Reynolds, G. Khoury, and G. Jay, Science 237:1324–1329, 1987). Tumors from these mice and tumor cell lines derived from them expressed high levels of *tax* RNA and protein. They also expressed high levels of the granulocyte-macrophage colony-stimulating factor (GM-CSF) gene as measured by proliferative responses of FD-CP1 target cells using conditioned media from tumor cells and by Northern (RNA) blot analysis of RNA from tumors and tumor cell lines. Although other tissues, such as salivary glands and muscles, in the transgenic mice also expressed high levels of *tax*, they did not express the gene for GM-CSF. This indicates that tissue-specific cellular factors, in addition to *tax*, are required for GM-CSF gene expression. Systemic effects of excessive GM-CSF production were demonstrated by infiltration of polymorphonuclear leukocytes into tumor tissues which are not necrotic, by peripheral granulocytosis, and by splenomegaly resulting from myeloid hyperplasia. The interleukin-2 (IL-2) receptor was also found to be expressed by the tumors and tumor cell lines as measured by IL-2-binding and cross-linking studies. This is the first demonstration that the IL-2 receptor can be activated by *tax* in a nonlymphoid cell type. These in vivo findings are consistent with other reports which have demonstrated in vitro *cis*-regulatory elements within the 5′-flanking regions of the genes for GM-CSF and the IL-2 receptor which are responsive to *trans* activation by the *tax* gene.

Human T-lymphotropic virus type 1 (HTLV-1) is a human retrovirus etiologically associated with adult T-cell leukemia (18, 37, 39, 49) and tropical spastic paraparesis (5, 14, 22, 36, 40). It has previously been demonstrated that the product of the *tax* gene (Tax), a *trans*-acting protein encoded by the HTLV-1 genome, is able to *trans*-activate not only the viral long terminal repeat (10, 12, 21, 43, 46) but also specific cellular genes. The Tax protein has been shown to activate the transcription of the genes for granulocyte-macrophage (GM) colony-stimulating factor (CSF) (6, 31, 35, 44), interleukin-2 (IL-2) (19, 20, 45), the IL-2 receptor (1, 8, 20, 24, 41, 45), and c-*fos* (11). These effects of the Tax protein are similar to the events which occur as a result of T-cell activation by mitogens or phorbol esters (13, 19, 20).

It has been proposed that HTLV-1-induced leukemogenesis may be the result of *tax* expression through its stimulatory effects on genes involved in cellular proliferation (13, 20, 45). IL-2 is a potent growth factor for T-cell proliferation mediated by the membrane-localized IL-2 receptor (8, 23). Tax expression might begin a process of cellular proliferation, perhaps via an autocrine mechanism of IL-2–IL-2 receptor activation (8, 45), with subsequent events that result in malignancy (1, 13, 20, 45). Tax stimulation of other genes, such as that for GM-CSF, may not be directly involved in malignant transformation but may result in significant biologic effects. GM-CSF is a growth-regulating glycoprotein involved in differentiation, proliferation, and functional activation of neutrophils, macrophages, and eosinophils (4, 7, 27, 28, 32).

Several studies have characterized the *tax*-responsive regulatory domains of the genes for GM-CSF and the IL-2 receptor in vitro (1, 6, 8, 20, 24, 31, 35, 41, 44, 45). We have studied the *trans* activation of these genes in vivo by using a transgenic mouse model expressing the *tax* gene which has previously been described (17, 33). Marked recruitment of neutrophils into the tumors from all three transgenic lines has previously been noted to occur before development of any signs of tumor necrosis. We demonstrated that this results from Tax activation of GM-CSF in tumors of perineural fibroblasts but not in other tissues which produce high levels of Tax. In addition, GM-CSF production led to systemic effects in the animals. We demonstrated that Tax also stimulated IL-2 receptor production in these nonlymphoid tumor cells.

MATERIAL AND METHODS

Derivation of transgenic mice. Derivation of transgenic animals containing the HTLV-1 long terminal repeat *tax* gene has previously been described (33).

Preparation of tissues from animals. Tissues were dissected from sacrificed animals and frozen at −70°C for subsequent RNA extraction, placed in Formalin or B-5 fixatives for histologic studies, or placed in sterile media for tissue culture studies. Blood samples were obtained from live animals through the tail artery.

Establishment of tumor cell lines. Cell lines or peripheral tumors were derived from two founder series of transgenic

* Corresponding author.

mice by standard techniques. Tissues were excised under sterile conditions and finely minced with a scalpel blade. Tissues were further dissociated between two abrasive glass plates, trypsinized in 0.05% trypsin–0.1% EDTA, and diluted to various cellular densities in Dulbecco modified Eagle medium with 20% fetal calf serum (FCS) (GIBCO Laboratories, Grand Island, N.Y.), 100 U of penicillin per ml, and 100 μg of streptomycin per ml (Quality Biological, Inc., Gaithersburg, Md.). Once established, cultures were passaged every 3 to 7 days by trypsinization and maintained in Dulbecco modified Eagle medium with 10% FCS and antibiotics. A number of cell lines were established, and two derived from founder series 6-2 (33) were designated PX-1 and PX-3, respectively.

Northern (RNA) blot analysis. RNA was extracted by the isothiocyanate-cesium chloride procedure (25). Total and poly(A)$^+$ RNAs were separated by electrophoresis through 1% agarose–formaldehyde gels, transferred to nitrocellulose membranes (Schleicher & Schuell, Inc., Keene, N.H.), and hybridized to ^{32}P-labeled cDNA probes prepared by random oligolabeling (Pharmacia, Piscataway, N.J.). The probe for murine GM-CSF (2) was kindly provided by N. Gough (15).

Histologic preparation of tissues. Tissue sections were stained with hematoxylin and eosin. Blood smears were stained with Wright-Giemsa stain.

Measurement of peripheral blood indices. Blood samples were collected in Capiject T-MQ tubes (Terumo, Elkton, Md.), and complete blood counts and differentials were performed (Medpath, Rockville, Md.).

Bioassays for CSF. Agar cultures of 75,000 C57BL/6 bone marrow cells were prepared in 35-mm-diameter plastic petri dishes by using 1-ml volumes of Dulbecco modified Eagle medium in a final concentration of 20% preselected FCS and 0.3% agar. Cultures were stimulated by addition of 0.1 ml of serial twofold dilutions of the test supernatant and serial dilutions of known CSFs. After gelling, cultures were incubated for 7 days at 37°C in a fully humidified atmosphere of 10% CO_2 in air. Colony formation was scored with a dissection microscope (magnification, ×35) by scoring colonies as clones containing more than 50 cells. Units of CSF activity were determined by assigning 50 U/ml to the concentration stimulating the formation of half-maximal numbers of GM colonies (26).

To determine whether the test supernatants were able to induce differentiation in WEH1-3BD$^+$ myelomonocytic leukemic colonies, 0.1-ml volumes of serial dilutions of test supernatant were added to agar cultures containing 300 WEH1-3BD$^+$ cells. The percentage of colonies exhibiting differentiation was determined after 7 days of incubation (34). Control cultures contained serial dilutions of known CSFs.

Microwell assays using the continuous hemopoietic cell lines 32Dc1.3 and FD-CP1 were performed in microtiter trays by using 200 cells per well in 100 μl of Dulbecco modified Eagle medium with 20% FCS. Serial dilutions of the test supernatant were added, and viable cell counts were performed after 48 h of incubation (29). Control cultures contained serial dilutions of known CSFs. Units of CSF activity in this assay were determined by assigning 1 U to the lowest detectable concentration allowing viable cells after 48 h by using previously described criteria (3).

The control CSFs used in these studies were recombinant murine GM-CSF (Genzyme, Boston, Mass., and the kind gift of D. Rennick, DNAX Research Institute, Palo Alto, Calif.), recombinant murine mutli-CSF (IL-3; Genzyme and a gift from D. Rennick), and recombinant human granulocyte (G)-CSF (Genzyme; Amgen, Thousand Oaks, Calif.).

IL-2 receptor-binding assays. The method used to determine IL-2 binding has been described previously (38, 47). Briefly, ^{125}I-labeled human recombinant IL-2 was purchased from Dupont, NEN Research Products (Boston, Mass.) (NEX 229; specific activity, 28.6 to 44.4 μCi/μg). Cell samples were first washed for 30 s in 10 mM sodium citrate (pH 4.0)–0.14 M NaCl to remove IL-2 before the binding studies were performed. Cells (1×10^6 to 4×10^6) were incubated with serial dilutions of [^{125}I]IL-2 (final concentration, 1.8 to 7,500 pM) for 1 to 2 h at 4°C in RPMI with 10% FCS in a final volume of 100 μl. Cells were centrifuged through an underlayer of a mixture of 20% olive oil–80% di-*n*-butylphthalate (Sigma Chemical Co., St. Louis, Mo.) for 2 min at 10,000 × *g*. Nonspecific binding was determined in the presence of a 200-fold molar excess of unlabeled IL-2. Specific binding was obtained by subtracting nonspecific binding from total binding. Free ^{125}I-labeled IL-2 was measured, and the data were analyzed by Scatchard analysis. The derived values for the dissociation constant (K_d) and the average number of binding sites per cell are given.

IL-2 cross-linking studies. The technique used to study IL-2 cross-linking has been described previously (47). Cells (40×10^6 to 80×10^6) were washed and suspended in 200 μl of phosphate-buffered saline and 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES; pH 7.3). They were incubated at 4°C for 2 h with 5 nM ^{125}I-labeled IL-2 (Amersham Corp., Arlington Heights, Ill.) (1M197; specific activity, 22.2 to 33.3 TBq/mmol). Cold competition was performed by preincubating cells for 1 to 2 h with a 100-fold molar excess of unlabeled IL-2. Cross-linking was performed by addition of 2 mM disuccinimidyl-suberate (Pierce Chemical Co., Rockford, Ill.). After 30 min of incubation, the reaction was quenched by adding 705 μl of 10 mM Tris hydrochloride (pH 7.4)–1 mM EDTA–0.14 M NaCl. Cells were solubilized with 100 μl of phosphate-buffered saline containing 2% Nonidet P-40 and 1 mM phenylmethylsulfonyl fluoride. After centrifugation (10,000 × *g*, 5 min), the supernatants were mixed with an appropriate volume of 4× concentrated sodium dodecyl sulfate sample buffer containing 10% (vol/vol) 2-mercaptoethanol and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

RESULTS

GM-CSF production by tumor cells. Histologic examination of tumors revealed marked infiltration of neutrophils before development of any signs of tumor necrosis (see Fig. 3A), consistent with tumor cell production of a hematopoietic growth factor. GM-CSF was detected in all supernatants from each of the five different tumor cell lines tested. Supernatants were taken randomly from cell cultures over a period of 18 months and assays for CSF activity. The culture supernatants stimulated colony formation of normal murine bone marrow cells in agar cultures. The total number of colonies stimulated was equivalent to that seen in cultures maximally stimulated by GM-CSF or IL-3 and, as with such cultures, included neutrophil, macrophage, and eosinophil colonies. These supernatants, however, did not stimulate differentiation of WEH1-3BD$^+$ cells, an assay for G-CSF (data not shown).

To further characterize the CSF produced by the tumor cells, hematopoietic factor-dependent cell lines were used. All 40 of the supernatants tested stimulated proliferation of FDCP-1 cells, cells that respond to both GM-CSF and IL-3,

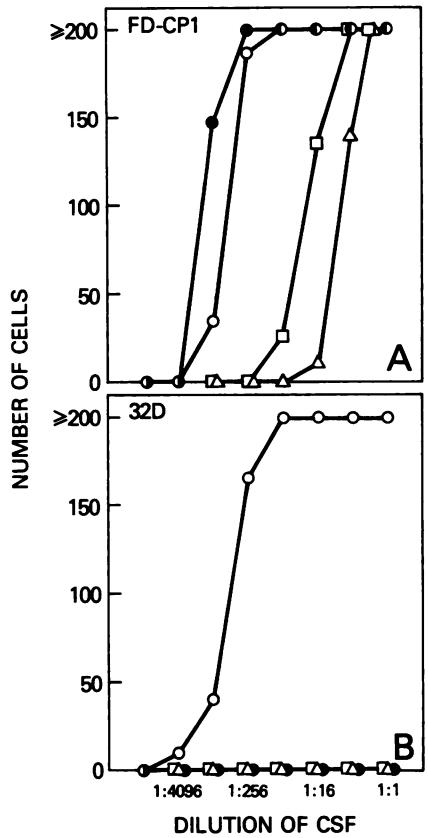

FIG. 1. Cell proliferation of hemopoietic cell lines stimulated by recombinant CSFs and *tax* transgenic tumor cell supernatants. (A) Proliferation of FDCP-1 cells stimulated by recombinant GM-CSF (●) and IL-3 (○) and two tumor supernatants (□, PX-1 cells; △, PX-3 cells). (B) Stimulation of 32D cell proliferation by recombinant IL-3 but not GM-CSF or tumor cell supernatants.

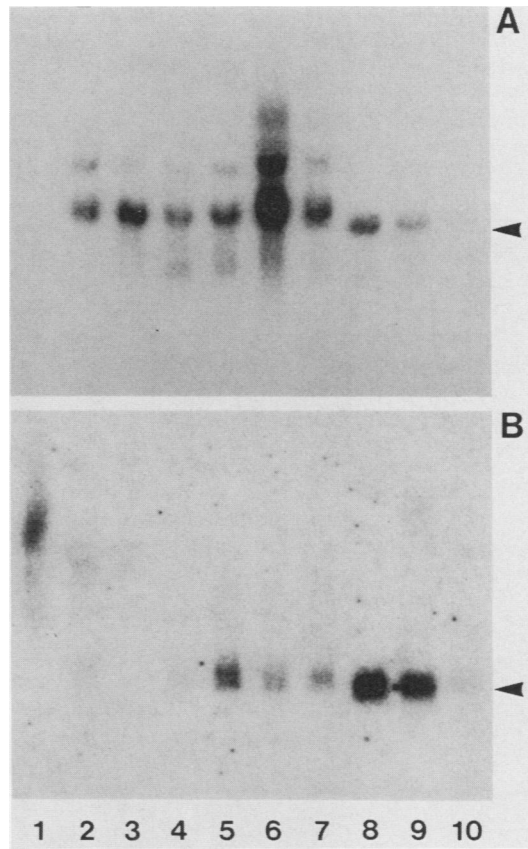

FIG. 2. Northern analysis of tissues from transgenic mice and tumor cell lines. Lanes: 1 to 5, founder series 6-2; 6 and 7, founder series 12-2; 1, brain; 2, submandibular gland; 3, skeletal muscle; 4 and 6, tail tumor; 5 and 7, ear tumor; 8 and 9, PX-1; 10, PX-3. (A) Blot probed with a ^{32}P-labeled *tax* probe. Arrowhead, 2.0-kilobase *tax* transcript. (B) Same blot labeled with ^{32}P-labeled GM-CSF gene probe. Arrowhead, 1.3-kilobase GM-CSF gene transcript. A 20-μg sample of total RNA was electrophoresed in a 1% agarose–formaldehyde gel and transferred to nitrocellulose.

but did not stimulate IL-3-responsive 32D cells (Fig. 1), thus demonstrating that the CSF activity was GM-CSF. The level of GM-CSF in tumor supernatants ranged between 160 and 15,000 U/ml and was related to the density of the tumor cells. Levels were lowest 24 h after passage of the tumor cells. Supernatants from PX-3 tumor cells consistently showed two- to fivefold less GM-CSF activity than did those from PX-1 tumor cells, even when cultures were of equivalent cell density, and this was also evident on Northern blot analysis (see below). Of 15 supernatants from PX-3 tumor cells, 3 had low levels of IL-3 in addition to GM-CSF, but the level was 20-fold lower than that of GM-CSF. All supernatants, however, contained GM-CSF, including supernatants from cells which had been in culture for over 18 months.

Tissue-specific *tax*-induced transcription of the gene for GM-CSF. Production of GM-CSF mRNA was assayed in several tissues from transgenic mice by Northern blot analysis (15). Phorbol myristate acetate-stimulated EL-4 thymoma cells were used as a positive control for production of GM-CSF mRNA. Unstimulated EL-4 cells produced undetectable message levels, whereas phorbol myristate acetate-treated cells produced abundant quantities of the expected 1.2-kilobase transcript when hybridized with a ^{32}P-labeled mouse GM-CSF cDNA probe (2) (specific activity, $>1 \times 10^9$ cpm/μg of DNA; data not shown). No mRNA for GM-CSF was detected in brain, submandibular gland, or muscle tissue (Fig. 2B, lanes 1 to 3). GM-CSF transcripts, however, were detected in peripheral tumors from both lines of transgenic mice tested (Fig. 2B, lanes 4 to 7). The tumor cell lines also expressed GM-CSF mRNA (Fig. 2B, lanes 8 to 10).

The same Northern blot was hybridized with a labeled cDNA probe of *tax* (33) (specific activity, $>1 \times 10^9$ cpm/μg of DNA). All of the above-mentioned mouse tissues except brain tissue produced strong bands corresponding to *tax* mRNA (Fig. 2A, lanes 2 through 10). The level of GM-CSF production in the tumor tissue generally correlated with the level of *tax* expression (Fig. 2A and B). Northern blot studies using poly(A)$^+$ RNA produced similar results (data not shown). Production of GM-CSF mRNA was greater in PX-1 than in PX-3 tumor cells, which also correlated with the amount of *tax* transcripts produced by each cell line (Fig. 2A and B, lanes 9 and 10). These results were consistent with the levels of GM-CSF produced by these cell lines as measured in a biologic assay (Fig. 1A). Although significant *tax* message levels were produced in submandibular gland and muscle tissues, GM-CSF mRNA was not detectable by Northern blot analysis.

Systemic effects of *tax*-induced GM-CSF production in transgenic mice. Transgenic animals were studied for evidence of biologic effects of GM-CSF. Blood smears from animals with advanced disease revealed peripheral granulo-

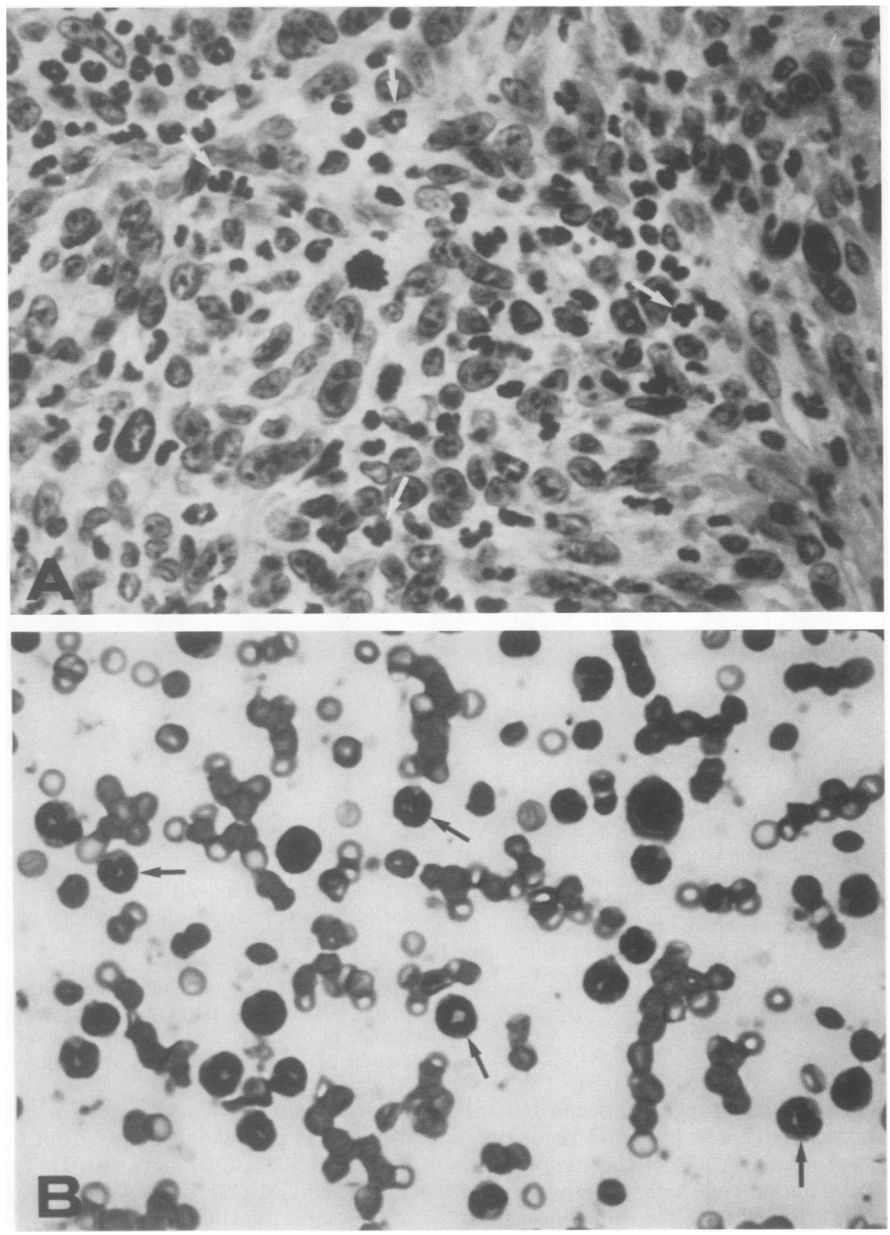

FIG. 3. Histologic analysis of transgenic tissues. (A) Peripheral neurofibroma with granulocytic infiltration (arrows) (hematoxylin and eosin stain; magnification, ×400). (B) Blood smear with peripheral granulocytosis (arrows) (Wright-Giemsa stain; magnification, ×1,000).

cytosis (Fig. 3B). Complete and differential blood counts of transgenic and normal mice were measured (Fig. 4). While transgenic mice ($n = 10$) had slightly elevated total leukocyte counts compared with normal mice ($n = 5$), i.e., $12.7 \times 10^3 \pm 5.0 \times 10^3$ (standard deviation) versus $8.5 \times 10^3 \pm 3.5 \times 10^3$ cells per mm^3, this was not statistically significant. There was no significant difference in lymphocyte counts between the two groups ($9.3 \times 10^3 \pm 5.0 \times 10^3$ versus $7.4 \times 10^3 \pm 3.2 \times 10^3$ cells per mm^3). There was, however, a significant difference between the absolute neutrophil counts ($P \leq 0.05$; t test of unpaired samples with unpooled variances). Transgenic mice had, on average, 2.5 times as many peripheral neutrophils as did control mice ($3.2 \times 10^3 \pm 1.6 \times 10^3$ versus $1.1 \times 10^3 \pm 0.3 \times 10^3$ cells per mm^3). This finding was consistent with the stimulation of myeloid precursors by GM-CSF in the bone marrow and spleens of these animals (see below). Other hematopoietic indices remained within normal limits, although several transgenic mice had low normal hemoglobin values, slightly increased total erythrocyte counts, and mildly elevated mean corpuscular volumes.

Massive splenomegaly was observed in most transgenic animals, and splenic weights were 2.6 times higher than those of control animals (201 ± 45 versus 76 ± 8 mg). Histologic examination of the enlarged spleens demonstrated significant myelomonocytic hyperplasia of the red pulp with normal germinal centers and white pulp.

IL-2 receptor gene expression by tumor tissues and cell lines. Because of the known ability of Tax to *trans*-activate the IL-2 receptor in lymphoid cells, the presence of the IL-2 receptor on Tax-transgenic perineural fibroblasts was assessed. The two transgenic tumor cell lines were initially examined for expression of the IL-2 receptor. Early-passage

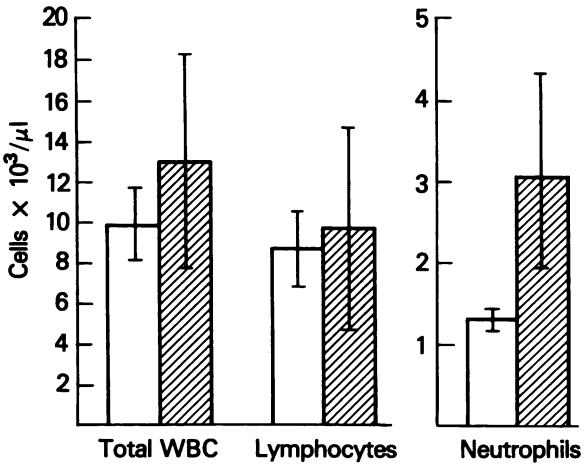

FIG. 4. Peripheral leukocyte counts. Open bar, control mice; hatched bar, transgenic mice. Brackets represent one standard deviation.

tumor cells showed specific binding of [^{125}I]IL-2 under binding conditions of both high and low affinity (Fig. 5A). The estimated number of high-affinity binding sites was between 35 and 350 (mean of seven experiments, 162), and the K_d was between 12 and 90 pM (mean, 36 pM). The number of low-affinity sites was between 4,000 and 8,500

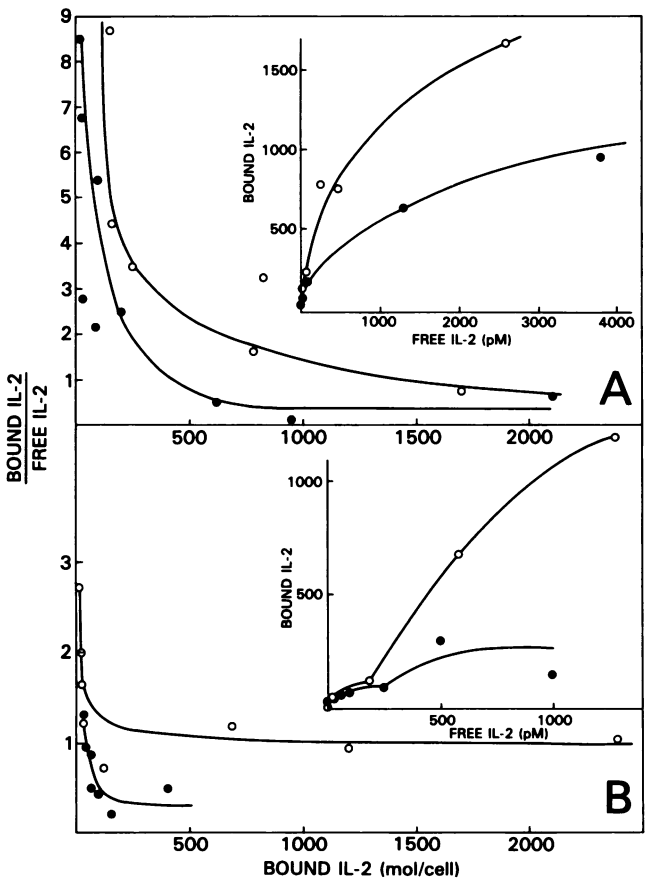

FIG. 5. Scatchard analysis and equilibrium binding data (insets) for [^{125}I]IL-2 binding to two *tax*-transgenic tumor cell lines (A) and two freshly isolated tumors (B).

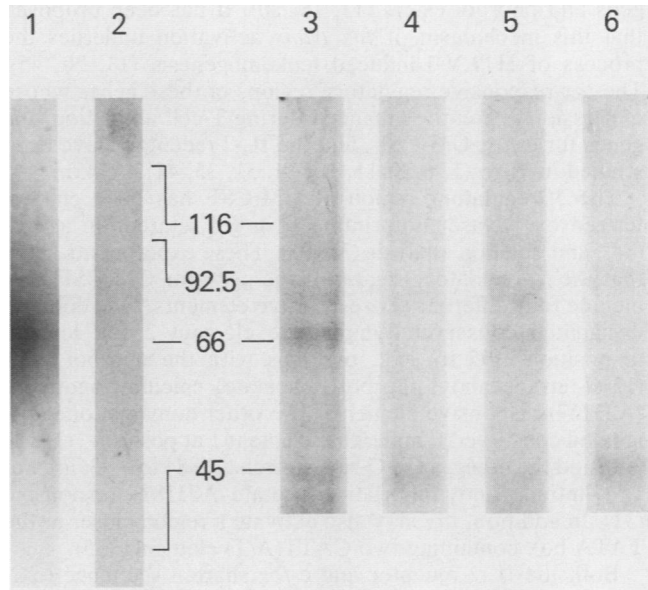

FIG. 6. Cross-linking of [^{125}I]IL-2 to *tax*-transgenic tumor cells. Lanes: 1, cross-linking of early-passage PX-1 cells (note the 70-kilodalton band consistent with p55 Tac peptide cross-linked to p15 IL-2); 3 to 6, later-passage cells (note the 90- and 100-kilodalton bands as previously reported for murine T cells [lanes: 3 and 4, PX-1; lanes 5 and 6, PX-3]). Lanes 2, 4, and 6 show inhibition of binding with excess unlabeled IL-2.

(mean of four experiments, 6,100), and the K_d was 2.5 to 6 nM (mean, 3.6 nM). While specific binding was observed in early-passage cells, the level of IL-2 binding decreased and eventually disappeared after 20 passages in culture.

IL-2 binding was also assessed by using freshly isolated tumor tissue (Fig. 5B). In two experiments, specific binding was observed under conditions of high- and low-affinity binding (K_d, 32 and 56 pM and n = 90 and 80; K_d, 9.3 and 12 nM and n = 3,000 and 8,000, respectively). In control experiments, there was no increased IL-2 binding in other tissues (thymus, spleen, and bone marrow) from these transgenic mice compared with those of nontransgenic littermates, nor was there any specific IL-2 binding in the control tissues. Specific binding of IL-2 was observed in both freshly isolated tumor tissue and in tumor-derived cell lines.

IL-2-binding proteins from transgenic perineural fibroblasts were assessed. Figure 6 shows cross-linking of ^{125}I-labeled IL-2 to receptors from tumor cells. In early passages, the predominant cross-linked band was 70 kilodaltons, consistent with the low-affinity p55 Tac-binding peptide. The presence of the p55 Tac peptide was also confirmed by using the murine enzyme-linked immunosorbent assay for soluble p55 Tac (48), which showed significant levels of soluble p55 Tac in the supernatants of these tumor cells (control, <31.3 U/ml; tumor supernatant, 143 U/ml). In addition to the p55 Tac peptide, two additional faint bands were observed at 90 and 110 kilodaltons, consistent with the p70-75 and p100 peptides that have also been reported on murine T cells (16, 42).

DISCUSSION

Previous in vitro transfection studies have suggested that HTLV-1 *tax* is capable of stimulating the regulatory regions of several genes (1, 6, 8, 11, 19, 20, 24, 31, 35, 41, 44, 45) which are also expressed during T-cell activation by mito-

gens and phorbol esters (13, 18, 20). It has been proposed that this mechanism of *tax trans* activation underlies the process of HTLV-1-induced leukemogenesis (13, 20, 45). The *tax*-responsive regulatory regions of these genes whose expression is greatly enhanced during T-cell activation, the genes for c-*fos*, GM-CSF, and the IL-2 receptor, have been studied in vitro (1, 6, 8, 11, 20, 24, 31, 35, 41, 44, 45).

The 5′-regulatory region of GM-CSF has been characterized by DNase footprinting (35), gel retardation assays (44), and deletion analysis (31, 35). These experiments show that the 5′-regulatory region of the gene for GM-CSF may include four different *tax*-responsive elements. One domain, designated conserved lymphokine element 2 and located at position −95 to −72, overlaps with the phorbol ester (12-*O*-tetradecanoyl-phorbol-13-acetate)–calcium ionophore (A23187)-responsive element. Two other domains, one designated conserved lymphokine element 1 at position −113 to −96 and the other, a G+C-rich stretch at −84 to −73, are not 12-*O*-tetradecanoyl-phorbol-13-acetate–A23187 responsive (31). In addition, *tax* may also activate a region closer to the TATA box containing two CATT(A/T) elements (35).

Both the IL-2 receptor and c-*fos* share a sequence (CC-A+T rich-GG) that is essential for *trans* activation by *tax* (8, 11). In addition, *trans* activation of the IL-2 receptor promoter by *tax* may also occur through an NF-κB-like transcription factor that is distinct from other 12-*O*-tetradecanoyl-phorbol-13-acetate-responsive elements in the IL-2 receptor control region (1, 9, 24, 45). Thus, these in vitro investigations have demonstrated *tax*-responsive regulatory domains which stimulate genes that are important during T-cell activation by HTLV-1 infection. There is no evidence that Tax acts directly on regulatory elements through DNA binding. Rather, *tax*-induced *trans* activation most likely occurs through stimulation of other cellular transcription factors (1, 24, 41).

The results of this investigation using transgenic mice containing the HTLV-1 long terminal repeat *tax* gene provides in vivo evidence that Tax is able to stimulate GM-CSF and IL-2 receptor production. GM-CSF production was found in tumors and tumor cell lines by Northern blot analysis, as well as by biologic assays. GM-CSF production was correlated with Tax production in the tumors and tumor cell lines but not in other tissues producing high levels of *tax* (salivary gland and muscle). Thus, Tax alone is insufficient for stimulation of GM-CSF and most likely requires other specific cellular factors or the absence of particular cellular inhibitors.

GM-CSF production was correlated with several biologic effects in these transgenic animals. Recruitment of polymorphonuclear granulocytes was demonstrated in GM-CSF-producing tumors which had significant early infiltrations of polymorphonuclear leukocytes unrelated to tumor necrosis. In addition, animals with significant tumor burdens had extensive peripheral granulocytosis and massive splenomegaly with myelomonocytic hyperplasia of the red pulp. The results of these experiments correlated well with those of another study describing the hematopoietic responses of mice injected with recombinant murine GM-CSF (30). Mice injected with GM-CSF developed a twofold increase in blood neutrophils and splenomegaly with monocytic hyperplasia. Production of the IL-2 receptor in tumor tissue cell lines was demonstrated by IL-2 receptor cross-linking studied and IL-2 receptor-binding studies. In addition, the presence of IL-2 receptors was also shown by measuring soluble receptors in the conditioned media of tumor cell lines.

Although the IL-2 receptor regulatory region has been demonstrated to be *tax* responsive in vitro, this is the first in vivo study to show Tax activation of the IL-2 receptor in cells of nonlymphoid origin. Further studies to explore the mechanism of this activation of the IL-2 receptor promoter by Tax would be of great interest. Measurable amounts of the IL-2 receptor diminished after multiple passages of these tumor cell lines until it could not be demonstrated after passage 20. Whether expression of the IL-2 receptor is an important event in the early transformation process of these cells remains unclear.

Transgenic animals containing the HTLV-1 *tax* gene demonstrate in vivo the stimulatory effect of *tax* on GM-CSF and IL-2 receptor production. This animal model provides the opportunity to study further the molecular regulation of *tax*-responsive genes and their biologic effects in vivo.

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