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Consistent loss of functional transforming growth factor β receptor expression in murine plasmacytomas

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ABSTRACT **Murine plasmacytomas are tumors of Ig-secreting plasma cells that can be induced in genetically susceptible BALB/c mice. The deregulation of the c-*myc* protooncogene is a critical oncogenic event in the development of plasmacytomas (PCTs) although it is not sufficient for their malignant transformation. We have demonstrated that PCTs produce active transforming growth factor β (TGF-β) *in vitro*. Because TGF-β is a potent negative regulator of the proliferation and differentiation of B lymphocytes, we examined its role in plasmacytomagenesis by comparing responsiveness to TGF-β of nonneoplastic plasma cells and PCTs. The nontransformed plasma cells that accumulate in interleukin 6 transgenic mice undergo accelerated apoptosis upon treatment with TGF-β, but the 15 PCTs studied, including primary and transplanted tumors as well as established cell lines, were refractory to TGF-β-mediated growth inhibition and apoptosis. Although PCTs lack functional TGF-β receptors as demonstrated by chemical crosslinking to radiolabeled TGF-β1, they nonetheless contain mRNA and protein for both type I and II TGF-β receptors, suggesting a potential defect in receptor trafficking or processing. The results clearly show the consistent inactivation of TGF-β receptors in plasmacytoma cells, demonstrating for the first time that interruption of a tumor suppressor pathway contributes to plasmacytomagenesis.**

Transforming growth factor β (TGF-β) regulates cell proliferation, differentiation, and function in a cell type-specific manner (1). As a potent inhibitor of both epithelial and hematopoeitic cell growth, TGF-β causes reversible arrest at the G_1/S border of the cell cycle (2). This inhibition is linked to a direct effect of TGF-β on the expression and function of components of the cyclin–cdk complexes (3) and is frequently associated with terminal differentiation and apoptosis (4, 5). TGF-β also plays important roles in myeloid and lymphoid hematopoiesis, from the generation of these populations in the bone marrow to their differentiation and activation in peripheral tissues and lymphoid organs (5, 6).

In B lymphocytes, TGF-β controls specific aspects of normal maturation and function, including Ig production, expression of cell surface molecules, and activation through antigen receptor binding (7). Picomolar concentrations of TGF-β inhibit the proliferation of activated human tonsillar and peripheral blood B cells in a dose-dependent manner and also prevent their differentiation into plasma cells, as measured by decreased Ig production (8, 9). Activated B lymphocytes and B lymphomas also secrete biologically active TGF-β *in vitro* (8, 10), implicating a role for this cytokine in an autocrine-negative feedback loop controlling the expansion of these cells.

The effects of TGF-β on the mature, fully differentiated B cell or plasma cell have not been clearly defined, largely because of the difficulty of obtaining enriched populations from a single source. Most available data have come from the study of neoplastic murine plasmacytomas (PCTs). These tumors of Ig-secreting plasma cells are induced in genetically susceptible BALB/c mice by the i.p. injections of poorly metabolized alkanes such as pristane (2,6,10,14-tetramethylpentadecane). This system provides both *in vivo* and *in vitro* models to study the mechanisms associated with the development of plasma cell neoplasia because stable cell lines often can be generated from primary tumors (11). Plasmacytomas are characterized by a highly consistent oncogenic change in that they contain chromosomal translocations leading to the constitutive expression of the c-*myc* protooncogene. However, this deregulation is not sufficient for plasmacytomagenesis because B cells harboring these translocations also can be found in lymphoid tissue from normal BALB/c mice (12, 13). Additionally, Berg and Lynch have shown that a clone of the MOPC315 plasmacytoma secretes greater than 2-fold the amount of TGF-β produced by normal splenic B cells *in vitro* and that ascites from mice bearing this plasmacytoma contains a significant amount of TGF-β (14), indicative of a potential role for this cytokine in the pathogenesis of these tumors. However, the responsiveness of normal plasma cells to TGF-β and its role in plasmacytomagenesis remain to be determined.

The malignant transformation of many cell types is accompanied by a loss of sensitivity to TGF-β, often because of functional inactivation of TGF-β receptors or mutational inactivation of the newly identified TGF-β signaling intermediates Smad2 and Smad4 (15). The majority of these resistant cells are epithelial in origin, but this TGF-β-insensitivity has been described in lymphocytes as well (16), with some B cell lymphomas also exhibiting loss of TGF-β receptor expression (17). In the present study, we compared the responsiveness of nontransformed plasma cells, derived from the interleukin 6 (IL-6) transgenic mouse (18), to that of several primary murine plasmacytomas and established plasmacytoma cell lines. The predominant phenotype of the IL-6 transgenic mouse is polyclonal plasmacytosis in secondary lymphoid organs; IL-6 is essential for the terminal differentiation of B cells to plasma cells, and an accumulation of largely nonmitotic plasma cells develops in the spleen and lymph nodes of these mice (18). IL-6 transgenic mice that have been backcrossed onto the plasmacytoma-susceptible BALB/c background provided the source for the plasma cells used in this study.

Here, we show that there is a significant amount of biologically active TGF-β present both in the ascites of plasmacytoma-bearing mice, as well as in media conditioned by pure cultures of these cells. More important to note, nondividing, nonneoplastic plasma cells of IL-6 transgenic mice undergo accelerated apoptosis after TGF-β treatment whereas murine primary PCTs and plasmacytoma cell lines are refractory to growth inhibition and apoptosis induced by TGF-β. Further-

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Abbreviations: TGF-β, transforming growth factor β; IL-6, interleukin 6; MACS, magnetic cell sorting; TβRI and TβRII, type I and II TGF-β receptors; PCT, plasmacytoma; IL-6-PC, plasma cells of the IL-6 transgenic mouse.

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more, although the plasma cells from IL-6 transgenic mice express both type I and II TGF-β receptors, these are absent from the cell surface of plasmacytomas, as determined by crosslinking analysis of radiolabeled TGF-β1. These results suggest that TGF-β is an important negative regulator of plasma cells and that their acquired resistance to TGF-β, combined with an increase in TGF-β production, may provide a strong selective advantage for transformed cells and be required for plasmacytomagenesis in BALB/c mice.

MATERIALS AND METHODS

Mice. Three strains of mice were used in these experiments: BALB/c AnPt (19), the congenic strain BALB/cAnPt.DBA/2-Idh1-Pep3 (C.D_2 I/P) (20), and the IL-6 transgenic strain BALB/c An.C57BL-H-2L^D-hu-IL-6 developed originally by Suematsu and Kishimoto (21). The IL-6 transgene was introduced onto BALB/c An N and is currently in the 12th N generation. Mice from N8–N12 were used in these experiments. The mice were maintained in closed colonies under conventional conditions. The experiments were carried out under National Cancer Institute protocols LG023, LG024, and LG025.

Cell Culture. Plasmacytoma and murine B lymphoma cell lines P388, L1210, and CH31 (kindly provided by David W. Scott, American Red Cross, Rockville, MD) were maintained in RPMI 1640 medium with heat-inactivated 10% fetal bovine serum (Biofluids, Rockville, MD), 4 mM L-glutamine, 1% penicillin/streptomycin, and 50 mM 2-mercaptoethanol. Murine recombinant IL-6 (Laboratory of Genetics, National Cancer Institute, Bethesda, MD) was added to factor-dependent plasmacytoma cell lines (TEPC 1165, TEPC 1198, BPC4, MOPC 21, MOPC 41, and TEPC 2027) at a concentration of 4 ng/ml (2000 B9 units). The TGF-β-sensitive Mv1Lu (CCL64) cells were maintained subconfluent in DMEM (high glucose) containing 10% fetal bovine serum, 1% penicillin/streptomycin, and 1% sodium pyruvate. All cells were incubated at 37°C in a 95% humidified atmosphere with 5% CO_2.

Conditioned Media Preparation. Plasmacytoma cells (1.5×10^6) were cultured for 24 h in 2 ml of serum-free medium, supplemented with 5 ml ITS+ (insulin, transferrin, selenium) Culture Supplement (Collaborative Biomedical Products, Bedford, MA, Lot 901837). Cultures were collected and then centrifuged at $10,000 \times g$ at 4°C to pellet cells, and supernatant was transferred to siliconized tubes with protease inhibitors (leupeptin, pepstatin, and aprotinin, 1 mg/ml) and stored at −70°C.

TGF-β Bioassay. Mv1Lu cells were plated at 1×10^4 cells per well in 96-well plates and incubated for 8–12 h at 37°C. Media was aspirated and replaced with serial dilutions of each conditioned medium in the presence or absence of 30 μg/ml of either the mouse monoclonal anti-TGF-β blocking antibody 1D11 (Genzyme) or control IgG in a total volume of 150 μl/well (each condition was performed in triplicate). Plates subsequently were incubated for an additional 24 h, and 0.2 μCi of ^{3}H-thymidine was added for the final 2 h of the incubation. Cells were released with trypsin–EDTA before harvesting onto 96-well filter plates, which were processed with a Top Count Microplate Scintillation Reader according to manufacturer's instructions (Packard).

Cell Purification. Plasma cells from IL-6 transgenic mice were extracted from peripheral lymph nodes by perfusion with cold 1× PBS through a 25-gauge needle. After lysis of red blood cells, pooled cells were resuspended in medium containing IL-6 and placed in 100-cm^2 plates at 37°C for 1–2 h to deplete adherent cells. Nonadherent cells were washed once in PBS and resuspended in 800 μl of cold magnetic cell sorting (MACS) buffer (1× PBS/0.5 mM EDTA/1% BSA) with anti-Thy 1.2 and anti-CD45 (B220) magnetic microbeads (100 μl each) (Miltenyi Biotec, Auburn, CA). T and B cells were removed using VarioMACS with BS depletion columns (Miltenyi Biotec) according to the manufacturer's instructions. Purification was determined by differential classification of eosin Y- and methylene blue-stained cytofuge preparations. To obtain PCT cells, ascites fluid was drained from the peritoneal cavity of tumor-bearing mice with an 18-gauge needle and processed as described above.

Growth Inhibition Assays. PCTs and B lymphoma cells (10^5) were cultured in 200 μl of serum-free medium supplemented with ITS+ (insulin, transferrin, selenium) in 96-well plates. Concentrations of human recombinant TGF-β1 (R & D Systems) ranging from 0.1 to 10 ng/ml were added, and cells were incubated for 24 h at 37°C. ^{3}H-Thymidine (0.5 μCi/well) was added for the last 4 h of incubation, and cells were harvested directly onto Packard filter plates and processed as above. Results are the averages of triplicate cultures.

Flow Cytometry. Viability of control and TGF-β-treated cultures of purified normal plasma cells and primary PCTs was assessed by flow cytometry of propidium iodide-permeable (nonviable) cells (22). Cells cultured for 48 h with 1 ng/ml TGF-β were harvested, washed, and incubated in buffer containing propidium iodide and fluorescein diacetate and analyzed by using a FACScan flow cytometer (Becton Dickinson).

TGF-β Receptor Crosslinking. Cells (10^7) were washed three times in ice-cold binding buffer (RPMI 1640 medium/25 mM Hepes, pH 7.4/1 mg/ml BSA), resuspended in 2 ml of the same buffer, and divided into two 1-ml aliquots in siliconized screw-capped tubes. ^{125}I-TGF-β1 (0.35 μCi; DuPont/NEN) was added to both tubes, with one tube also receiving an excess of unlabeled TGF-β1 (120 ng) to compete for specific binding of radiolabeled ligand. Samples were incubated on a rocker at 4°C for 2.5 h, washed three times in cold wash buffer (RPMI 1640 medium/5 mM Hepes, pH 7.4) and resuspended in 1 ml of the same buffer containing 3 mM disuccinimidyl suberate (Pierce). After incubating for 1 h at 4°C, cells were washed three times in cold sucrose buffer (250 mM sucrose/10 mM Tris, pH 7.4/1 mM EDTA) and lysed in 400 μl of 1× RIPA buffer (1× PBS/1% Nonidet P-40/0.5% sodium deoxycholate/0.1% SDS) with protease inhibitors (200 nM AEBSF [4-(2-aminoethyl)-benzenesulfonyl fluoride hydrochloride]/1 μg/ml leupeptin, pepstatin, and aprotinin) added immediately before cell lysis. Immunoprecipitations were performed overnight at 4°C with 1 μg of the rabbit polyclonal anti-TGF-β type II receptor antibody designated C16 (Santa Cruz Biotechnology). Antibody was captured by incubating lysates with 35 μl of 50% protein A Sepharose (Pharmacia) for 1 h. Immunoprecipitates were washed three times in 1× RIPA buffer, eluted by boiling for 2 min in gel loading buffer, and separated on 4–12% SDS/PAGE gels (NOVEX, San Diego). Gels were dried and exposed to Kodak X-Omat film for 4–5 days or analyzed by PhosphorImager (Molecular Dynamics).

Reverse Transcriptase PCR. Total RNA was extracted from 2×10^7 suspension cells by using Trizol (Life Technologies, Grand Island, NY). RNA (1 μg) was converted to cDNA by random priming by using a 1st Strand cDNA Synthesis Kit for RT-PCR (Boehringer Mannheim). cDNA (3 μl) was used in a subsequent PCR reaction (Expand High Fidelity PCR System, Boehringer Mannheim) by using the following primers: type I TGF-β receptor (TβRI)-35–5′ TCC GCA GCT CCT CAT CGT GTT-3′, TβRI-796–3′ CTG CGT CCA TGT CCC ATT CTC TT-3′, type II TGF-β receptor (TβRII)-763–5′ TTG CCA TAG CTG TCA TCA TCA TCT TCT-3′, TβRII-1624–3′ GGG GCT CGT AAT CCT TCA CTT CTC-3′. Primers specific for murine TβRI amplified a 761-bp product encoding the N-terminal region of the molecule, and those for TβRII amplified a 961-bp fragment encoding part of the C-terminal kinase region. PCR was performed on a MJ Research (Cambridge, MA) thermal cycler.

Immunoblotting. Asynchronously growing cells were lysed for 30 min at 4°C in 1× RIPA buffer with protease inhibitors. Lysates were centrifuged at 4°C for 15 min at $10,000 \times g$ in a tabletop Eppendorf centrifuge (5415 C), supernatants were removed, and protein was quantitated by using the BCA Protein Assay (Pierce). Protein (100 μg) was added to 400 μl of 1× RIPA buffer (with protease inhibitors), precleared for 1 h with 10 μg of nonimmune

IgG (Zymed) and 50 μl of protein A Sepharose, then immunoprecipitated overnight at 4°C with 1 μg of the C16 anti-TβRII antibody. Precipitates were run on 4–12% SDS/PAGE gradient gels and transferred onto nitrocellulose membranes. Blots were incubated for 2.5 h with a 1:1000 dilution of anti-TβRII (Upstate Biotechnologies, Lake Placid, NY) followed by incubation with a secondary horseradish peroxidase-conjugated goat anti-rabbit IgG (1:7000 dilution) for 40 min. Signal was detected by chemilumenescence (Pierce).

RESULTS

Response of IL-6 Plasma Cells and Transformed Plasma Cells to TGF-β. Because TGF-β inhibits the growth of activated B cells and certain B cell lymphomas and induces apoptosis in resting B lymphocytes, we compared the effects of TGF-β on the viability of plasma cells of the IL-6 transgenic mouse (IL-6-PC) and of primary PCTs. Purified plasma cells from an IL-6 transgenic mouse (Fig. 1*A*) and isolated PCTs were analyzed by flow cytometry to determine cell viability in response to TGF-β, with nonapoptotic cells appearing negative for propidium iodide and positive for fluorescein diacetate (found in bottom right quadrant of each panel in Fig. 1*B*). The number of viable IL-6-PC remaining 48 h after treatment with 1 ng/ml TGF-β (Fig. 1*B Lower Right*), was reduced to 26% of that present in untreated cultures (Fig. 1*B Upper Right*). The same concentration of TGF-β did not affect viability of the primary PCTs (Fig. 1*B Left*). We next examined the ability of TGF-β to inhibit the proliferation of several plasmacytomas *in vitro* (Fig. 1*C*). All PCTs were resistant to growth inhibition at concentrations of TGF-β up to 10 ng/ml whereas thymidine incorporation in the murine B cell lymphoma CH31 was reduced by >50% at the same concentration, an effect that is associated with induction of apoptosis (10). The effect of TGF-β on IL-6-PC proliferation cannot be assessed because these plasma cells are a terminally differentiated and nondividing population.

Evaluation of TGF-β Receptor Expression in Plasmacytomas. In a variety of tumors, the inability to respond to TGF-β has been associated with either an absence or a decrease in expression of the plasma membrane signaling receptors TβRI and TβRII (15). We examined TGF-β receptor expression on normal plasma cells and plasmacytomas by receptor crosslinking to ^{125}I-labeled TGF-β1, followed by immunoprecipitation of receptor–ligand complexes (Fig. 2). The nontransformed IL-6-PCs showed strong crosslinking to both TβRI and TβRII (Fig. 2*A*). However, we failed to detect either receptor on the cell surface of any of the TGF-β-insensitive PCT lines (Fig. 2*B*). The fact that these receptors also were absent in the primary and transplanted tumor cells (Fig. 2*C*) suggests that this defect exists before adaptation of these tumors to cell culture conditions and is not an artifact resulting from tissue culture. Several early (P388, L1210, and CH31) B cell lymphomas that displayed TGF-β receptors were used as controls. Results from all cell lines and tumors tested are shown in Table 1.

To assess whether this defect in TGF-β receptor expression was at the transcriptional level, reverse transcriptase PCR was performed for both TβRI and TβRII mRNA from total RNA extracted from PCT lines and from IL-6-PC (Fig. 3). In striking contrast to the crosslinking analysis, all of the plasmacytoma cell lines tested contained transcripts for both receptors and were indistinguishable from IL-6-PC in this regard. DNA sequencing confirmed the validity of the PCR (data not shown). These results suggest that our inability to detect receptors by crosslinking of radiolabeled ligand reflects either a translational block in receptor expression or possibly a mutation affecting either the localization of receptors to the cell surface or their ability to bind ligand.

To address the issue of translational inhibition of TβRI and TβRII messages, Western blots were performed on immunoprecipitates prepared from whole cell lysates from various PCT lines, by using antibodies to either receptor. It was surprising that the results revealed the appropriate 70-kDa band for TβRII in all cell lines examined (Fig. 4), indicating that loss of receptor expression at the cell surface is not because of translational inefficiency. Although all plasmacytoma cells also contained the 53-kDa TβRI protein product by Western analysis (data not shown), it remains possible that this receptor is functional and can localize to the membrane but that it cannot bind TGF-β because of a defect in TβRII.

Secretion of Active TGF-β by Plasmacytoma Cells. Loss of TGF-β responsiveness has been associated with increased production of TGF-β in a variety of tumor cell lines. Moreover, although most normal cells secrete TGF-β in a latent, inactive form, many malignant cells have acquired the capacity to secrete biologically active TGF-β, which enhances tumor progression through suppression of the host immune response. This process already has been suggested for murine plasmacytomas; the *in vivo*

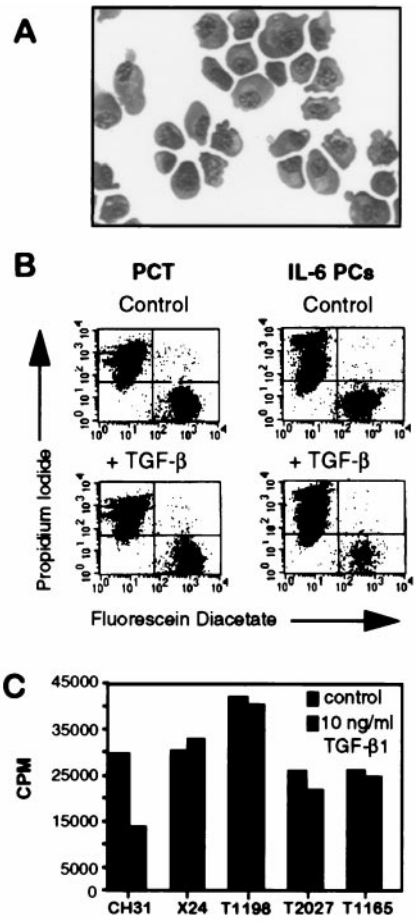

FIG. 1. (*A*) Stained cytofuge preparation of purified plasma cells from an IL-6 transgenic mouse. Pooled lymph nodes were perfused, and cells were incubated at 37° to remove adherent cells. The remaining cell suspension was depleted of B220+ and Thy1.2+ cells by using MACS. (*B*) FACS analysis of cell viability in response to treatment with TGF-β. Comparison of MACS-purified, untreated primary PCT cells (*Upper Left*) with those treated with 1 ng/ml TGF-β (*Lower Left*) shows that TGF-β has no effect on the viability of PCTs after 48 h. The percentages of viable untreated vs. treated cells are 21.11 and 22.43, respectively (*Lower Right*). Treatment of purified IL-6-PCs with 1 ng/ml TGF-β decreased their viability from 20% in the control (*Upper Right*) to 5.22% after 48 h (*Lower Right*). This procedure was repeated two more times. (*C*) Proliferation assay of PCT cell lines upon treatment with TGF-β. Cells were plated in serum-free media and treated with or without TGF-β for 24 h. Samples were pulsed with ^{3}H-thymidine for 4 h and harvested, and counts per minute (CPM) were determined. PCT cell lines were not inhibited by TGF-β compared with the control, CH31, a B lymphoma that is 50% inhibited by TGF-β.

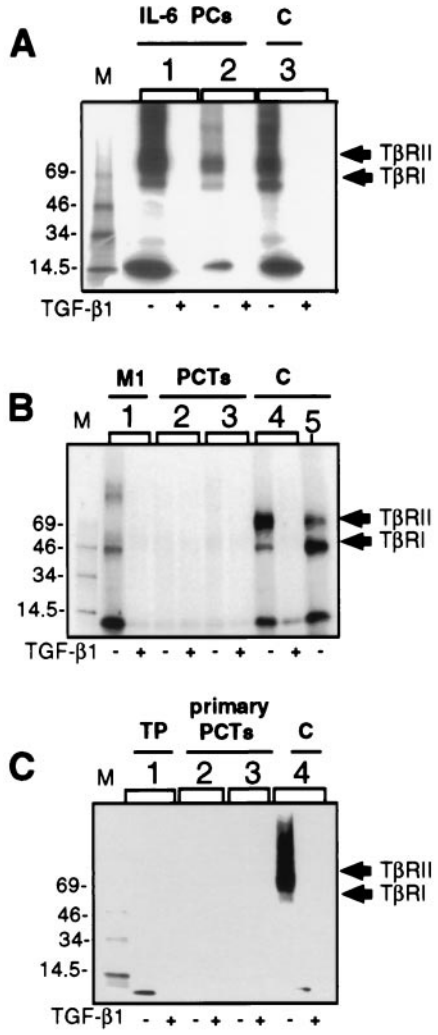

FIG. 2. Chemical crosslinking of receptor-bound ^{125}I-TGF-β was performed to determine the presence or absence of the receptors on normal plasma cells (*A*) and PCT cells (*B* and *C*). SDS/PAGE of receptor immunoprecipitates of purified (*A*, lane 2) and unpurified (*A*, lane 1) plasma cells from an IL-6 transgenic mouse demonstrates the presence of TβRI and TβRII at levels comparable to the control B cell lymphoma P388 (*A*, lane 3). Loss of expression occurs in the *in vitro* PCT cell lines X24 and 1165 (*B*, lanes 2 and 3, respectively), again compared with pre-B lymphomas P388 and L1210 (*B*, lanes 4 and 5, respectively) and the M1 myeloid leukemic cell line (B, lane 1). Crosslinking of purified primary PCTs (*C*, lanes 2 and 3) and a transplanted PCT (TP) (*C*, lane 1) demonstrates that loss of receptor expression occurs *in vivo*, before cell culture adaptation; here compared with B cell lymphoma CH31 (*C*, lane 4). (+) and (-) indicate the addition of unlabeled TGF-β1 to compete for specific binding.

growth of the PCT line MOPC315 leads to increased TGF-β levels in secondary lymphoid organs (14), and PCT-bearing mice exhibit increased circulating levels of active TGF-β that are directly responsible for defects in neutrophil function and their susceptibility to bacterial infection (23). To investigate whether plasmacytomas secrete active TGF-β, we assayed culture supernatants from several PCT lines, including MOPC315, for TGF-β activity by using the standard Mv1Lu bioassay. Fig. 5 shows results from two representative PCT cell lines (T1165 and T2027); 1×10^6 cells per 1.5 ml produced active TGF-β in the range of 120 pg/ml over 24 h, with total TGF-β (active plus latent) after heat activation of latent TGF-β found to be >1 ng/ml. This activity is specific because it can be reversed with the anti-TGF-β mAb 1D11 but not by nonimmune control IgG. Cell-free ascites from a mouse bearing transplanted PCT MOPC467 also contained a substantial amount of active TGF-β (780 pg/ml); ascites from several mice were assayed for total TGF-β by ELISA and contained from 2 ng/ml to >20 ng/ml (mean 10.5 $\pm$ 5.4 ng/ml for eight mice).

DISCUSSION

We show that the loss of cell surface TGF-β receptor expression is a consistent lesion in mouse plasmacytomas, rendering them insensitive to TGF-β-mediated apoptosis. A series of PCT cell lines, as well as primary and transplanted tumors (*ex vivo*), demonstrated an insensitivity to TGF-β and failed to express either type I or type II TGF-β cell-surface receptors as detected by chemical crosslinking of receptor-bound, radiolabeled ligand. In contrast, nontransformed plasma cells of IL-6 transgenic mice exhibited TGF-β receptor expression and decreased viability in response to TGF-β, confirming that the receptor complex on these plasma cells was functional. The failure of PCTs to bind TGF-β contrasted with the murine pre-B cell lymphomas P388 and L1210, which also were resistant to TGF-β but demonstrated a normal crosslinking pattern for TGF-β receptors.

A heteromeric interaction between TβRI and TβRII is essential for signaling by TGF-β (24). The loss of growth inhibition by TGF-β often results from mutation of the genes encoding these receptors. Specific mutations in TβRII have been identified in many colon (25–27) and gastric carcinomas (28), two squamous cell carcinomas (29, 30), and several breast cancer lines (31, 32). Mutations of TβRI are less common but have been identified in the LnCAP human prostate cancer cell line (33) and in a nephritogenic T cell clone (34). Additionally, reduced receptor expression at the cell surface has been reported in many TGF-β-resistant tumors. As examples, numerous retinoblastomas (35) and small cell lung carcinomas (36) and several B (16) and T (37) cell lymphomas have been found to lack TβRII; reduced levels of TβRII also were reported in T cells from patients with Sézary syndrome (38) and in progenitor cells in patients with myelofibrosis with myeloid metaplasia (39).

The presence of mRNA and protein for both TβRI and TβRII in all PCT lines examined suggests that the defect in these cells is unlikely the result of inefficient transcription or translation but may instead represent a mechanism by which PCTs escape effects of TGF-β within the TGF-β-rich microenvironment of the oil granuloma. There have been only a few instances in which loss of cell surface expression of TβRI or TβRII has not been accompanied by transcriptional down-regulation of the receptor gene. DeCoteau *et al.* (40) showed that primary tumor cells from several patients with chronic lymphocytic leukemia have become refractory to inhibition by TGF-β because of loss of TβRI expression although mRNA of the expected size for TβRI were found in these cells (40). Similarly, it has been proposed that the TGF-β-resistant MCF-7 breast cancer cell line, which does not display surface TβRII but has cytosolic TβRII protein, may have a pH defect compromising the regulation of endosome acidification and impeding trafficking of the receptors to the cell surface (32). Finally, Knaus *et al.* (41) identified a dominant negative TβRII in a cutaneous T cell lymphoma, which is coded for by one allele and can oligomerize with the wild-type TβRII, preventing it from reaching the cell surface (41).

The deficiency of surface expression of these receptors in mouse PCTs was uniform in the cell lines and in the primary and transplanted tumors examined. Although other tumors, such as adenocarcinomas of the colon, appear to escape from regulation by TGF-β by a variety of mechanisms including microsatellite instability (26, 42) and mutation of Smad signaling intermediates (43), this occurrence in PCTs instead may be attributed to defects in the regulation of both TGF-β signaling receptors. It recently has been demonstrated in Mv1Lu epithelial cells that the metabolic half-lives of TβRI and TβRII are substantially different (44, 45). Treatment with TGF-β was shown to reduce the stability of the Golgi-processed forms of both receptors, implying the existence of metabolic regulatory mechanisms in response to autocrine or paracrine TGF-β. Both receptors are highly glycosylated, and it

Table 1. TGF-β receptor expression in mouse plasmacytomas

Tumor	Type	Source	Strain	Induction agent	Receptor
P388	Pre-B Lymphoma	TC	DBA	MCA	+
L1210	Pre-B Lymphoma	TC	DBA	MCA	+
CH31	B Lymphoma	TC	B10.H-2a	N/A	+
XRPC24	PCT	TC	BALB/c	Mineral oil	−
TEPC1165	PCT	TC	BALB/c	Pristane	−
TEPC1198	PCT	TC	BALB/c	Pristane	−
TEPC1033	PCT	TC	BALB/c	Pristane	−
TEPC2027	PCT	TC	BALB/c	Pristane	−
MOPC21	PCT	TC	BALB/c	Mineral oil	−
MOPC315	PCT	TC	BALB/c	Mineral oil	−
BPC4	PCT	TC	C57 BL/6	Pristane	−
AP	PCT	PR	BALB/c	Pristane	−
S25	PCT	PR	c.D2-I/p	Silicone gel	−
SNIHPC2	PCT	PR	c.D2-I/P	Silicone gel	−
SNIHPC 4	PCT	PR	c.D2-I/P	Silicone gel	−
MOPC195	PCT	TP	BALB/c	Mineral oil	−
MOPC467	PCT	TP	BALB/c	Mineral oil	−
TEPC3609	PCT	TP	BALB/c	Pristane	−

TC, tissue culture; PR, primary; TP, transplanted PCT; MCA, methylcholanthrene; N/A, not applicable.

has been observed that altered glycosylation may affect turnover and degradation of the receptors. Two differentially glycosylated forms of TβRII have been identified in cytoplasmic and membrane fractions from Mv1Lu CCL-64 cells and the MDA-231 breast cancer line, both of which express receptors, whereas only the cytoplasmic form was present in the TβRII cell surface-deficient MCF-7 breast carcinoma line mentioned above (45).

It is likely that the high level of TGF-β produced in the inflammatory environment of the oil granuloma acts as a positive selection factor, allowing only for the growth of those cells that have escaped from negative regulation by TGF-β. Although it is unclear whether resistance to TGF-β precedes B cell differentiation into a plasma cell or occurs at the plasma cell stage, the resulting escape from TGF-β-induced apoptosis is a critical prerequisite for the persistence of these cells and plasmacytoma development. The uniformity of the lesion supports this hypothesis. Whereas in other models, such as colon carcinoma, loss of TGF-β receptors indirectly results from an earlier defect in DNA repair machinery and may not occur in every tumor, their absence is a primary event in plasmacytomagenesis and one required for neoplastic development. Additionally, we have demonstrated that several PCT lines produce a combination of biologically active and latent TGF-β *in vitro*, indicating that PCT cells are a contributing source of the abundant TGF-β present in ascites resulting from their growth *in vivo*. Therefore, in BALB/c mice, PCT cells that are resistant to TGF-β may then acquire a malignant phenotype *in vivo*, and their progression may be enhanced by an immunosuppression of host defenses by means of the TGF-β they secrete.

The results presented here are strongly supported by recent studies of TGF-β1-null mice. Although targeted disruption of the gene encoding this TGF-β isoform typically leads to severe autoimmunity, systemic inflammation, and early death, these mice will invariably develop a severe plasmacytosis if bred onto a background that obviates the autoimmune process and extends their survival (46). Although this model is clearly distinguished from the receptor-deficient PCT model by the absence of expressed ligand, it is functionally equivalent to the loss of receptor function.

This study shows that TGF-β is important in the elimination of the terminally differentiated plasma cell by apoptosis. Abrogation of response to TGF-β clearly is associated with malignant lymphoid disorders, and these may potentially be distinguished by the differentiation stage at which the resistance is acquired. The disruption of TGF-β signaling in neoplastic plasma cells of plasmacytoma-bearing mice because of loss of cell surface expression of TGF-β receptors supports

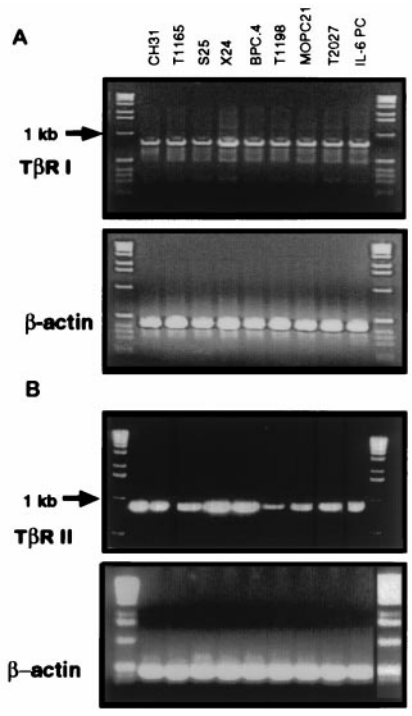

FIG. 3. Reverse transcriptase PCR in PCT cell lines and IL-6-PC. (*A* and *B*) Products using primers specific for TβRI and TβRII, respectively (see *Materials and Methods*), indicating that mRNA is being produced in PCT cells that do not express functional surface receptors. Negative control using RNA instead of cDNA was performed to confirm specificity of the bands (data not shown).

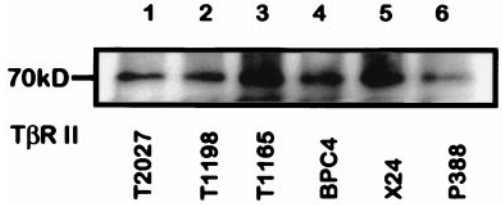

FIG. 4. Immunoprecipitation and Western blots for TβRII. Shown are protein products of the expected size in whole cell lysates from PCT cell lines, indicating that the lack of surface expression is not due to translational repression.

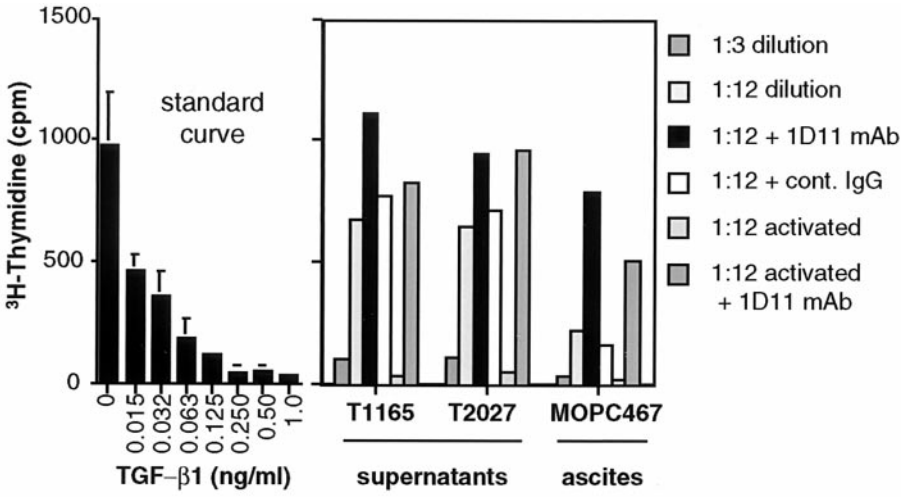

FIG. 5. Production of bioactive TGF-β by murine plasmacytomas. Cell-free supernatants from 24-h cultures of plasmacytomas (T1165 and T2027 shown here) and ascites removed from mice bearing the plasmacytoma MOPC467 were diluted and added to cultures of TGF-β-sensitive mink lung epithelial cells (CCL64 cells). A 1:3 dilution of each resulted in an inhibition of CCL64 cells $\geq$ 0.125 ng/ml TGF-β1. Inhibitory activity present in 1:12 dilutions could be reversed specifically by the addition of the monoclonal anti-TGF-β antibody 1D11. The substantial increase in this specific activity after heat activation of culture supernatants indicated that they produced a mixture of active and latent forms. This difference was less pronounced in ascites samples, suggesting that other mechanisms may be contributing to activation of TGF-β *in vivo*.

this hypothesis. Finally, although this acquired resistance to TGF-β in PCTs may not be unique to plasma cells, it clearly contributes to plasmacytomagenesis in BALB/c mice.

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