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Transforming Growth Factor Beta 1 (TGF-β1) Receptor Expression on Resting and Mitogen-Activated T Cells

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Transforming growth factor β1 (TGF-β1) is a potent autocrine growth inhibitor of lymphocytes. In this study, the expression of TGF-β1 binding proteins was characterized on murine splenic T cells. With an affinity cross-linking method and by neutralizing antibodies to TGF-β1, [^{125}I] TGF-β1 was found to bind to three cell surface–binding proteins (280–200 kD, 95–85 kD, 65 kD) that were differentially expressed on resting and mitogen-stimulated T cells. Freshly prepared (resting) T cells were found to constitutively express the 95–85-kD form of these binding proteins, whereas mitogenic stimulation by either concanavalin-A (Con-A), interleukin-1 (IL-1), interleukin-2 (IL-2), or 12-tetradencanoyl-phorbol-13-acetate (TPA) for 12–72 h induced the appearance of all forms of the TGF-β1 binding proteins (280–200 kD, 95–85 kD, and 65 kD). Furthermore, antibodies that neutralized the biologic action of TGF-β1 also blocked the binding of [^{125}I] TGF-β1 to all three binding proteins, suggesting that these binding proteins are involved with signal transduction. These results suggest that the expression of the TGF-β1 receptor on T cells is regulated by T cell mitogenic signals and that a regulatory relationship may exist between T cell growth-promoting cytokines (IL-1 and IL-2) and the T cell growth inhibitor, TGF-β1.

Key words: receptor regulation, neutralizing antibodies, immunosuppression, autocrine growth regulation

The transforming growth factors were originally characterized as protein factors that function to impart a transformed phenotype to normal fibroblasts [1–6]. On the basis of this activity, two classes of protein factors were isolated and found to act synergistically to promote the anchorage independent growth of normal fibroblasts. These factors were named transforming growth factor alpha (TGF-α) and transforming growth factor beta (TGF-β). TGF-α was subsequently found to be structurally and functionally related to epidermal growth factor [7–8], whereas TGF-β was found to represent a unique class of growth factor [9–11].

It is now known that TGF-β is actually a family of structurally conserved hormone-like peptides. At least two forms of TGF-β, TGF-β1 and TGF-β2, have been

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isolated from bovine [12,13], porcine [14], monkey [15], and human [16,17] sources. TGF-β1 and TGF-β2 are homodimeric proteins that have M_r of 25,000 daltons; the monomeric subunits have 70% amino-acid identity [17]; and both forms appear to be equipotent growth inhibitors in several in vitro assays [12,13,18].

Although the physiological roles of TGF-β1 and TGF-β2 remain to be determined, it is known that these factors are potent growth inhibitors of developing and mature immune and hematopoietic cells, including thymocytes [18,28], pre-B cells [21], myeloid progenitor cells [22,29], and mature T and B cells [19,20,30]. Kehrl et al. [19,20] have shown that mitogen-stimulated T and B cells can be induced to secrete a TGF-β-like factor(s) and that these cells have high-affinity receptors for TGF-β1. Interestingly, however, there appears to be a temporal delay (2–4 days) between mitogenic stimulation and secretion of TGF-β by lymphocytes, suggesting that TGF-β1 may function as an autocrine factor to limit the extent of clonal growth of T and B cells.

Although lymphocytes are known to have high-affinity receptors (kd of 5 pM) for TGF-β1 [19,20], nothing is currently known regarding the regulation of the TGF-β1 receptor on lymphocytes. In this study, we show that TGF-β1 binds to three cell-surface glycoproteins (280–200 kD, 95–85 kD, and 65 kD) on T cells and that these binding proteins are differentially expressed on resting and mitogen-activated T cells. Furthermore, antibodies that neutralize the antiproliferative actions of TGF-β1 also blocked the binding of TGF-β1 to these binding proteins, suggesting a role in signal transduction. These observations are consistent with the proposed autocrine role of this factor.

MATERIALS AND METHODS

Animals

Six- to ten-week-old BALB/c mice were purchased from Bantin and Kingman (Fremont, CA).

TGF-β1 and TGF-β2

Bone-derived TGF-β1 and TGF-β2 were purified from the noncollagenous, guanidine-HCl–soluble proteins of bovine demineralized metatarsal bone. These factors were purified by a combination of gel filtration, CM-cellulose cation exchange chromatography, and C18 reverse-phase high-pressure liquid chromatography with previously described methods [24]. The biological activity of TGF-β1 and TGF-β2 was determined by the NRK colony-forming assay method as described by Roberts et al. [25], with NRK clone 49F (CRL 1570) used as the indicator cell.

Mitogenic Assay

Spleen cell suspensions were enriched for T cells with a nylon wool column by a method described elsewhere [26]. The T cell–enriched spleen cells were suspended to 2×10^6 cells/ml in RPMI 1640 (Irvine Scientific, Santa Ana, CA) supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, and 10% fetal calf serum (Sterile Systems, Logan, UT). The cells (2×10^5 cells/well) were plated into 96-well microculture plates (Costar 3596). The cell cultures were stimulated with 2.5 μg/ml concanavalin-A (Con-A, Sigma, St. Louis, MI) and/or the indicated amount of human interleukin-2 (IL-2, Boehringer Mannheim, Indianapolis, IN).

Experimental cultures were supplemented with either TGF-β1 or TGF-β2, as indicated in each experiment. Unless otherwise indicated, these cells were cultured for 72 h in a humidified incubator in 5% CO_2 at 37°C. These cultures were pulsed with 1.0 μCi of [^{3}H]-thymidine (Amersham, Chicago, IL) for 18 h before being harvested with a semiautomatic cell harvester (Otto Hiller CO., Madison, WI). The amount of [^{3}H]-thymidine incorporation was determined by standard liquid scintillation methods.

IL-2–Dependent T Cells

IL-2–dependent T cells were preactivated in bulk cultures (4×10^6 cells/ml) for 24 h in culture medium supplemented with 2.5 μg/ml of Con-A. The Con-A–activated cells were washed with culture medium containing 20 mg/ml methyl-α-D-manno-pyranoside in order to remove the mitogen. The cells were then recultured at 2×10^5 cells/well in 96-well microculture plates in medium containing 2.5 U/ml to 100 U/ml of human IL-2 and the indicated amounts of TGF-β1 or TGF-β2. These cultures were incubated for an additional 72 h and pulsed with 1.0 μCi of [^{3}H]-thymidine for the final 18 h of the culture period.

Antibodies to TGF-β1

A New Zealand white rabbit was immunized by multiple intramuscular injections of highly purified TGF-β1. The injections were given monthly over 6–9 months with 100 μg of TGF-β1 per injection. The initial immunization was in Freund's complete adjuvant and the subsequent boosts were in Freund's incomplete adjuvant (Gibco Laboratories, Grand Island, NY). Serum was obtained by cardiac puncture and the antiserum IgG (anti-TGF-β1 IgG) or normal rabbit serum IgG was purified with a protein-A sepharose affinity column (Pharmacia Inc., Piscataway, NJ). The bound IgG was eluted with 0.1 M glycine-HCl (pH 2.0), neutralized with 4.0 M Tris-base, dialyzed against 0.1 M phosphate buffered saline (pH 7.0), and sterile filtered.

Antibody Neutralization

Anti-TGF-β1 IgG was evaluated for neutralizing activity by the T cell proliferation assay described above. In these experiments, normal rabbit IgG (30 μg/ml) or anti-TGF-β1 IgG (30 μg/ml) were combined simultaneously with either TGF-β1 or TGF-β2 at the initiation of the culture.

Radioiodination

TGF-β1 was radiolabeled with 1 mCi of $Na^{125}I$ (13–16 mCi/μg Amersham) with a previously described method [11]. The specific activity of the radiolabeled peptide ranged between 50 to 100 Ci/μg.

Affinity Cross-Linking

TGF-β1 binding proteins were identified by covalently cross-linking [^{125}I] TGF-β1 to cell surface with either freshly prepared (resting) T cells or T cells (2×10^6 cells/ml) that had been stimulated for 72 h with either 2.5 μg/ml Con-A, 8 U/ml of human interleukin-1 (IL-1, Cistron Technology, Pine Brook, NJ), 100 U/ml of IL-2, or 10 ng/ml of TPA. [^{125}I] TGF-β1 was cross-linked to the receptors with disuccinimidyl suberate (DSS, Pierce no. 21555). Briefly, 100 pM of [^{125}I] TGF-β1 was incubated for 2–3 h at 4°C with 5×10^6 T cells in Dulbecco's minimal essential medium (Irvine Scientific) containing 25 mM (4-[2-hydroxyethyl]-1-piperazine-ethane) sul-

fonic acid (HEPES) and 0.1% (w/v) bovine serum albumin (Sigma). DSS was added to the cell suspensions at a final concentration of 0.3 mM and incubated for 15 min at 4°C. The cells were washed three times with 10 mM Tris-HCl (pH 7.4) containing 250 mM sucrose and 1 mM ethylenediaminetetraacetic acid (sucrose buffer) to remove the nonbound radiolabel and excess DSS. The cells were lysed in 100 μl of sucrose buffer containing 1% Triton, 1 mM phenylmethylsulphonyl fluoride, and 5 mM benzamidine. The nuclei were pelleted (10,000 rpm Beckman Microfuge) for 1 min. The solubilized cell extracts were reduced with sample buffer containing 100 mM DL-dithiothreitol (Sigma). The cell extracts were electrophoretically separated in a 4–10% linear SDS-polyacrylamide gel by standard methods [27]. The dried gels were exposed for 48–72 h to Kodak XAR-5 film at −80°C. The specificity of binding was determined by mixing either excess (20 nM) unlabeled TGF-β1 or anti-TGF-β1 IgG with the [^{125}I] TGF-β1 before cross-linking with DSS.

RESULTS

The effects of TGF-β1 and TGF-β2 on the Con-A–induced T cell proliferative response were determined and are shown in Figure 1A and 1C, respectively. When added at the initiation (time 0) of the culture, TGF-β1 and TGF-β2 were found to inhibit the proliferative response in a dose-dependent fashion between 1 pM and 100 pM. Both forms of TGF-β maximally inhibited (78%) proliferation at culture concentrations of 100 pM. The half-maximal inhibitory dose (ID_{50}) for TGF-β1 was 20 pM and the ID_{50} for TGF-β2 was approximately 10 pM.

The antiproliferative effects of TGF-β1 and TGF-β2 were partially reversed with exogenous IL-2. In these experiments, T cell cultures were mitogenically stimulated with Con-A and IL-2 (20 U/ml). Various amounts (10^{-12} to 10^{-9} M) of either TGF-β1 or TGF-β2 were added with the mitogens at the initiation of the cultures. As seen in Figure 1, the IL-2 containing cultures were also growth inhibited by TGF-β1 (Fig. 1A) or TGF-β2 (Fig. 1C) in a dose-dependent fashion between 10^{-12} and 10^{-9} M. In contrast to the cultures without exogenous IL-2, however, 10^{-10} M TGF-β1 or TGF-β2 maximally inhibited the proliferative response by only 50%. These results suggest that TGF-β1 and TGF-β2 effectively suppress the T cell proliferative response when added at the initiation of the cultures and that exogenous IL-2 can partially reverse the suppressive actions of these factors.

The effects of TGF-β1 and TGF-β2 upon IL-2–dependent T cell growth was next determined. In these experiments, the T cells were preactivated for 24 h with Con-A, washed, and then recultured for an additional 72 h in medium supplemented with 2.5 to 100 U/ml of IL-2 and different amounts (10^{-12} to 10^{-9} M) of TGF-β1 or TGF-β2. As seen in Figure 1, higher amounts (900 pM) of TGF-β1 (Fig. 1B) or TGF-β2 (Fig. 1D) were required in order to obtain maximal growth inhibition (32% to 38%) of IL-2–dependent T cells. Taken as a whole, these results suggest that TGF-β1 and TGF-β2 are potent T cell inhibitors and that the antiproliferative actions of these factors can be modulated by the T cell growth factor IL-2.

Having determined that TGF-β1 and TGF-β2 are equally potent growth inhibitors of T cells and that the inhibitory action appears to be regulated by IL-2, we next determined whether or not T cell mitogens could regulate the expression of TGF-β1 binding proteins on T cells. These binding proteins have been previously characterized on normal fibroblasts as consisting of three glycoproteins with M_r of 280–200 kD,

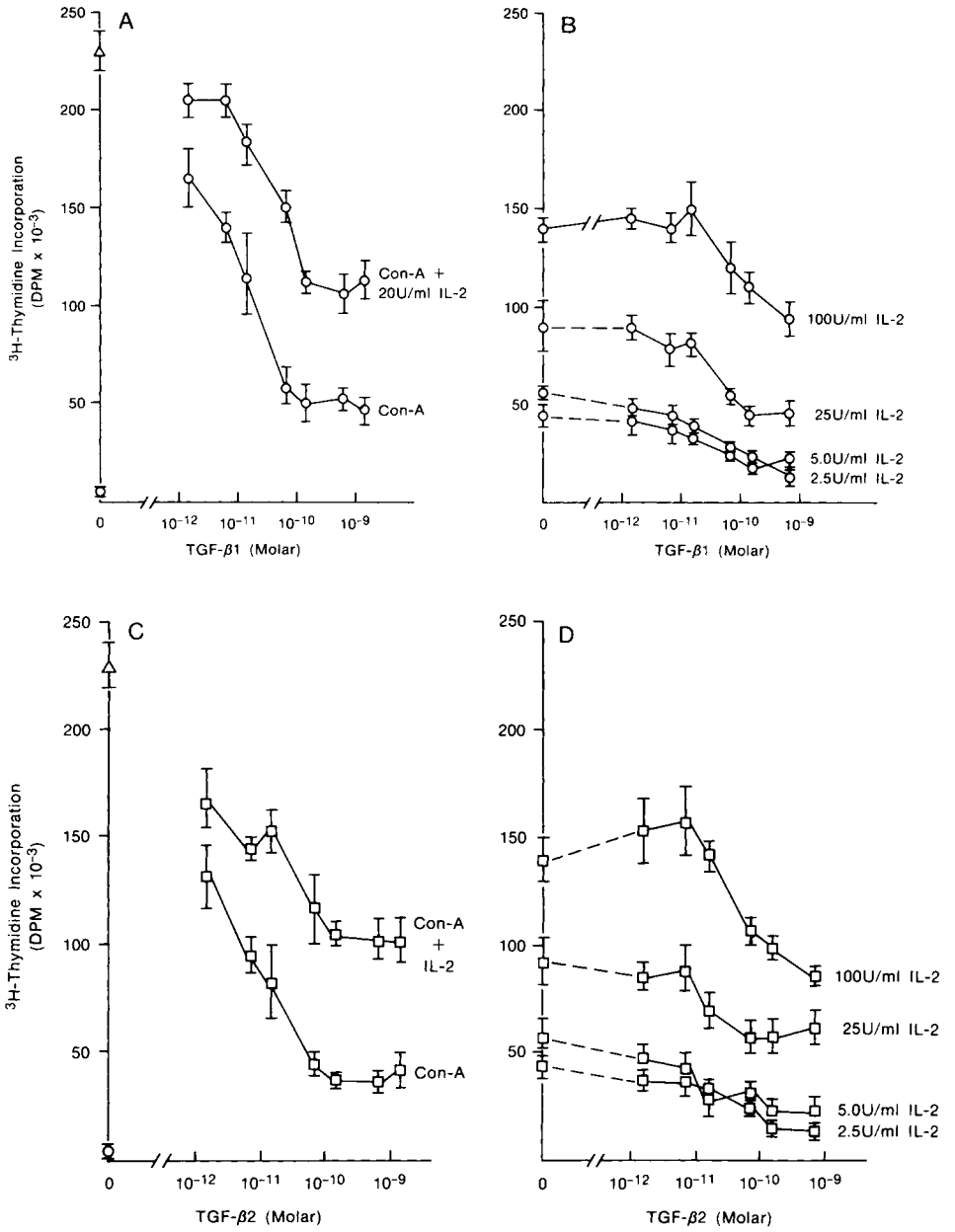

Fig. 1. Effect of TGF-β1 and TGF-β2 on the proliferative responses of BALB/c splenic T cells. In **panels A** and **C**, T cells (2×10^5 cells/well) were cultured in medium containing concanavalin-A (2.5 μg/ml) with or without 20 U/ml IL-2. To these cultures, TGF-β1 (panel A) or TGF-β2 (panel C) were added with the mitogens at the initiation of the cultures. The cells were incubated for 72 h at 37°C and pulsed for the final 18 h with 1 μCi of [^{3}H] thymidine per culture. In **panels B** and **D**, the T cells (4×10^6 cells/ml) were preactivated for 24 h with 2.5 μg/ml of concanavalin-A, washed with 20 mg/ml of methy-α-D mannopyranoside, and recultured at 2×10^5 cells/well in medium containing 2.5 to 100 U/ml of IL-2 and either TGF-β1 (panel B) or TGF-β2 (panel D). These cells were cultured for an additional 72 h at 37°C and pulsed for the final 18 h with 1 μCi of [^{3}H] thymidine per culture.

95–85 kD, and 65 kD [14,23]. In this study, an affinity cross-linking method was used to characterize the expression of TGF-β1 binding proteins on resting and mitogenically stimulated cells. The autoradiograph in Figure 2 shows the binding of [^{125}I] TGF-β1 to these binding proteins on the T cells. Lane 1 shows that resting T cells appear constitutively to express predominantly the 95–85 kD form of the TGF-β1 binding protein. In contrast to resting T cells, however, mitogenically stimulated T cells (lane 3) were found to express all forms (280–200 kD, 95–85 kD, and 65 kD) of these binding proteins. Coincubation with an excess (20 nM) of unlabeled TGF-β1 (lanes 2, 4) specifically blocked the labeling of these binding proteins, indicating that

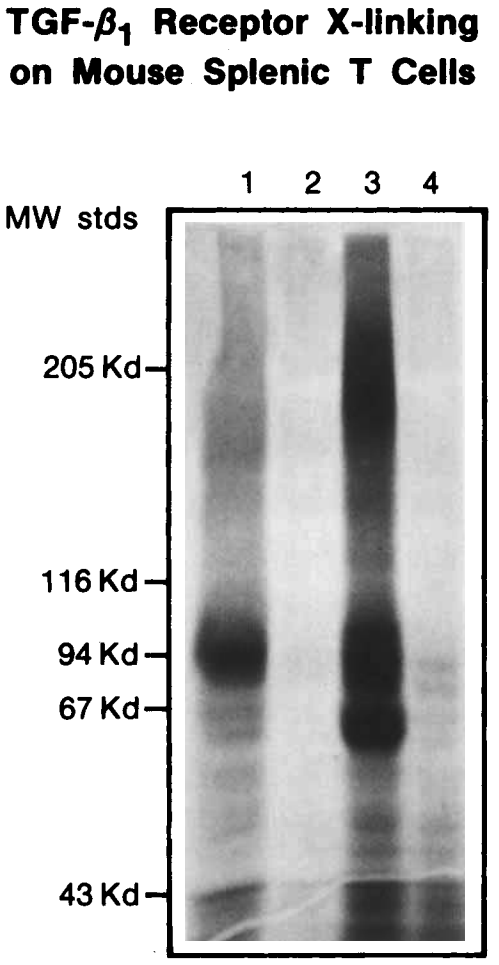

Fig. 2. Comparison of TGF-β1 binding proteins on resting and mitogen-activated BALB/c splenic T cells. [^{125}I] TGF-β1 (100 pM) was cross-linked with 0.3 mM disaccinimidyl suberate. The radiolabeled factor was cross-linked to approximately 5×10^6 freshly prepared (resting) T cells (**lane 1**) or 5×10^6 concanavalin-A–stimulated (72 h) T cells (**lane 3**). Specific binding was determined by competition with 20 nM of unlabeled TGF-β1 (**lanes 2** and **4**). The cell extracts were separated by SDS-polyacrylamide gel electrophoresis methods in a 4% to 10% linear gradient gel.

the binding of [^{125}I] TGF-β1 was specific. Furthermore, kinetic studies showed that the appearance of these binding proteins can be initially detected at 12 h after mitogenic stimulation, but they were not maximally expressed until 24–72 h after stimulation (data not shown), suggesting that a temporal relationship may exist between inductive signals and the expression of these TGF-β1 binding proteins. This apparent delay in receptor expression, therefore, is consistent with the concept that this factor may function temporally to control clonal growth of T cells.

The autoradiograph in Figure 3 shows that these TGF-β1 binding proteins can be specifically induced by such T cell mitogens as Con-A (lanes 3, 4), IL-1 (lanes 5, 6), IL-2 (lanes 7, 8) and TPA (lanes 9, 10).

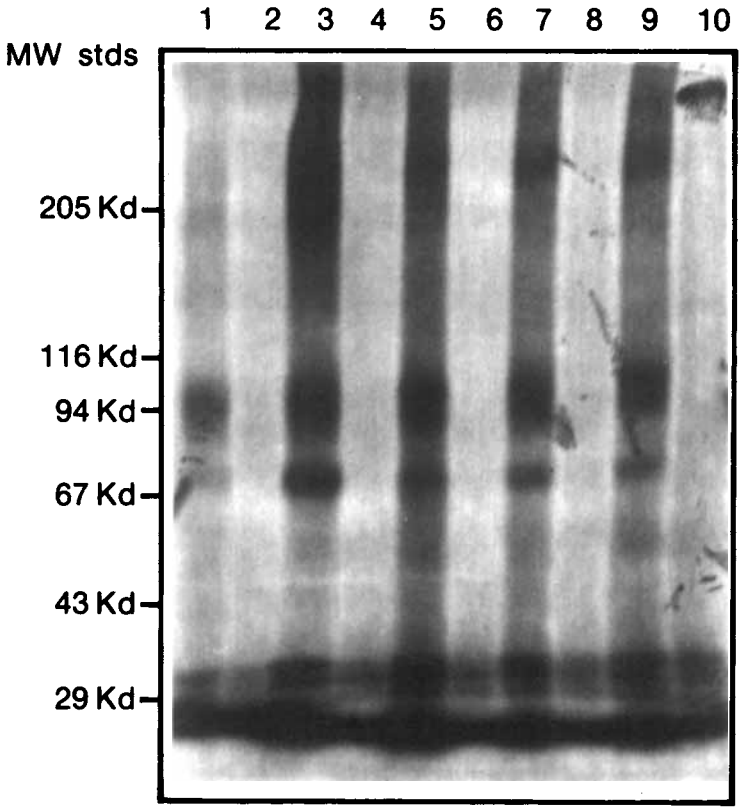

Fig. 3. Induction of TGF-β1 binding proteins on BALB/c splenic T cells. **Lane 1:** [^{125}I] TGF-β1 cross-linked to resting T cells; **lane 2:** [^{125}I] TGF-β1 competed with unlabeled TGF-β1 on resting T cells; **lane 3:** [^{125}I] TGF-β1 cross-linked to concanavalin-A–stimulated (72 h) T cells; **lane 4:** [^{125}I] TGF-β1 competed with unlabeled TGF-β1 on concanavalin-A–stimulated (72 h) T cells; **lane 5:** [^{125}I] TGF-β1 cross-linked to IL-1 (8 U/ml)–stimulated (72 h) T cells; **lane 6:** [^{125}I] TGF-β1 competed with unlabeled TGF-β1 on IL-1 (8 U/ml)–stimulated (72 h) T cells; **lane 7:** [^{125}I] TGF-β1 cross-linked to IL-2 (100 U/ml)–stimulated (72 h) T cells; **lane 8:** [^{125}I] TGF-β1 competed with unlabeled TGF-β1 on IL-2 (100 U/ml)–stimulated (72 h) T cells; **lane 9:** [^{125}I] TGF-β1 cross-linked to TPA (10 ng/ml)–stimulated (72 h) T cells; **lane 10:** [^{125}I] TGF-β1 competed with unlabeled TGF-β1 on TPA (10 ng/ml)–stimulated (72 h) T cells.

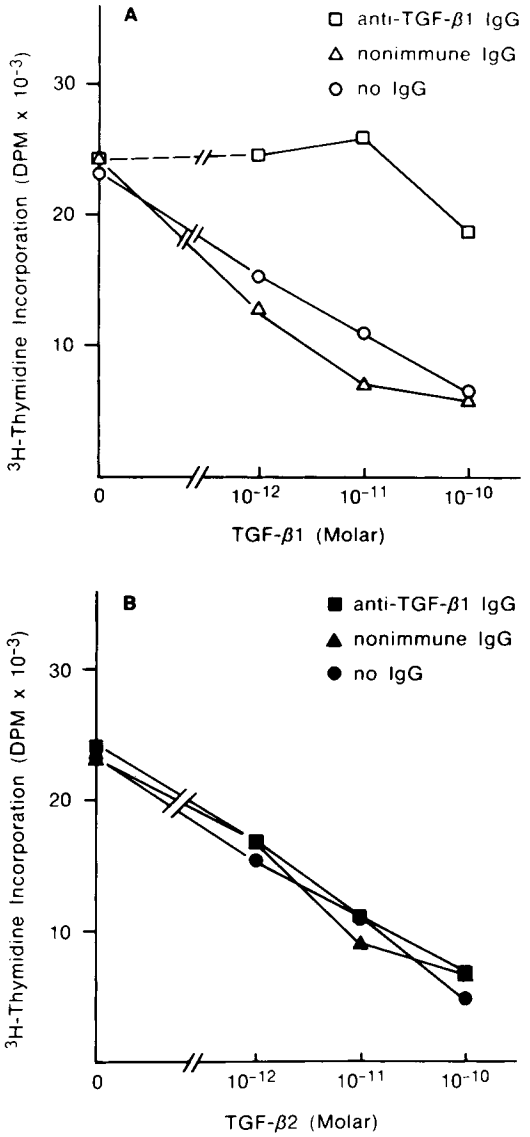

Fig. 4. Antibody neutralization of the growth inhibiting activity of TGF-β1. BALB/c splenic T cells (2×10^5 cells/well) were mitogen stimulated with 2.5 μg/ml of concanavalin-A. To these cultures, TGF-β1 (**panel A**) or TGF-β2 (**panel B**) was added with either 30 μg/ml of anti-TGF-β1 IgG, nonimmune rabbit IgG, or no antibody (control). These cultures were incubated for 72 h at 37°C and pulsed for the final 18 h with 1 μCi of [^{3}H] thymidine.

The specificity of TGF-β1 receptor binding was further characterized with antibodies that neutralize the antiproliferative action of TGF-β1. As seen in Figure 4, anti-TGF-β1 IgG specifically blocked the antiproliferative action of TGF-β1 (Fig. 4A), but it did not block the antiproliferative action of TGF-β2 (Fig. 4B). Since the antiserum neutralizes the biologic action of TGF-β1, we next determined whether this

antiserum also blocked the binding of [^{125}I] TGF-β1 to the binding proteins on resting and mitogen-stimulated T cells. The autoradiograph in Figure 5 shows that unlabeled TGF-β1 (lane 2) and anti-TGF-β1 IgG (lane 3) specifically blocked the binding of [^{125}I] TGF-β1 to the 95–85 kD binding protein on resting T cells. In a similar fashion, unlabeled TGF-β1 (lane 6) and anti-TGF-β1 IgG (lane 7) also blocked the binding of [^{125}I] TGF-β1 to the 280–200-kD, 95–85-kD, and 65-kD binding proteins on mitogen-stimulated T cells, suggesting that these binding proteins are responsible for TGF-β1 signal transduction to the T cell.

DISCUSSION

It has been proposed that TGF-β1 functions as an autocrine factor to regulate the clonal growth of T cells [19] and B cells [20]. This hypothesis is based upon several observations: 1) TGF-β1 is a potent (picomolar) growth inhibitor of lymphocytes; 2) mitogenic stimulation induces the expression of TGF-β1 mRNA in lymphocytes; 3) mitogenically stimulated lymphocytes do not secrete TGF-β until 2–4 days after mitogenic stimulation; and 4) lymphocytes have high-affinity cell-surface receptors for TGF-β1.

The autocrine regulatory actions of TGF-β1 and TGF-β2 appear to be mediated through three cell-surface glycoproteins. With affinity cross-linking methods, it has been shown previously that [^{125}I] TGF-β1 binds to glycoproteins that have M_r of 280–200 kD, 95–85 kD, and 65 kD [14]. The 280–200-kD form appears to have high affinity for both TGF-β1 and TGF-β2 and appears to be associated as a dimer with an apparent M_r of 600 kD. It is not clear whether these various binding proteins associate into a receptor complex on the cell surface. These binding proteins, however, are presumably responsible for signal transduction to the cell, since antibodies which neutralize the antiproliferative action of TGF-β1 (Fig. 4) also block the binding of [^{125}I] TGF-β1 to these three binding proteins (Fig. 5).

Although the biological actions of TGF-β1 appear to be mediated through specific receptors, nothing is known regarding the regulation of the receptor. In the present study, an affinity cross-linking method was used to characterize the expression of the TGF-β1 binding proteins on resting and mitogen-activated murine splenic T cells. These results show that resting T cells constitutively express predominantly the 95–85 kD binding protein, whereas mitogen (i.e., Con-A, TPA, IL-1, or IL-2)-stimulated T cells were induced to express all three TGF-β1 binding proteins (280–200 kD, 95–85 kD, and 65 kD). Interestingly, kinetic studies show that the 280–200-kD and 65-kD binding proteins were not detectable by the cross-linking method until 12–24 h after mitogenic stimulation (data not shown), which correlates well with the observation that T cells do not secrete TGF-β until 2–4 days after mitogenic stimulation [19]. These results suggest that a regulatory relationship may exist between the mitogenic signal, clonal growth of the T cell, and subsequent expression of the TGF-β1 receptor. Identical observations also have been made with human peripheral blood T cells (unpublished observation), indicating that this regulatory scheme is operative across species. These observations are consistent with the concept that TGF-β1 functions naturally as an autocrine factor to regulate the clonal growth of T cells.

The mechanism whereby TGF-β1 and TGF-β2 suppress T cell growth remains unclear at this time. It is unlikely, however, that these factors directly inhibit the induction of IL-2 or IL-2 receptor mRNAs in mitogenically stimulated T cells. With

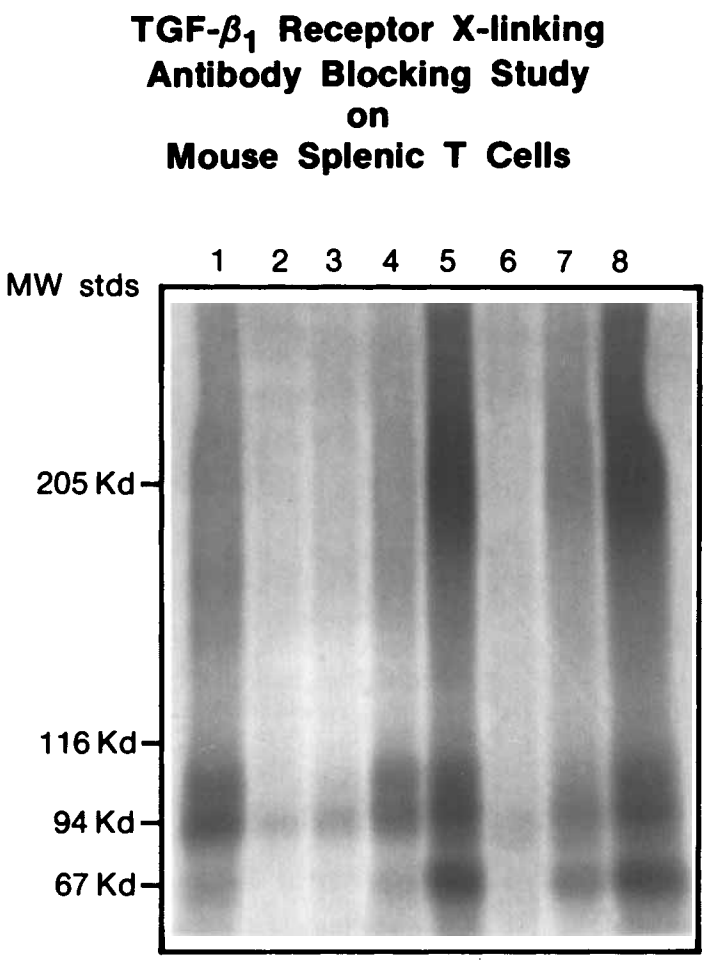

Fig. 5. Antibody blocking of [^{125}I] TGF-β1 to binding proteins on freshly (resting) prepared (lanes 1–4) and mitogen-stimulated (lanes 5–8) BALB/c splenic T cells. [^{125}I] TGF-β1 (100 pM) was mixed with 30 μg/ml of either anti-TGF-β1 IgG or nonimmune IgG for 30 min at room temperature before addition to the cells. The mixture was cross-linked to 5 × 10^6 T cells for 2 h at 4°C. **Lane 1:** resting T cells cross-linked with [^{125}I] TGF-β1; **lane 2:** resting T cells cross-linked with [^{125}I] TGF-β1 and competed with 20 nM of unlabeled TGF-β1; **lane 3:** resting T cells cross-linked with [^{125}I] TGF-β1 and competed with anti-TGF-β1 IgG; **lane 4:** resting T cells cross-linked with [^{125}I] TGF-β1 and competed with nonimmune IgG; **lane 5:** mitogen-stimulated (72 h) T cells cross-linked with [^{125}I] TGF-β1; **lane 6:** mitogen-stimulated (72 h) T cells cross-linked with [^{125}I] TGF-β1 and competed with 20 nM of unlabeled TGF-β1; **lane 7:** mitogen-stimulated T cells cross-linked with [^{125}I] TGF-β1 and competed with anti-TGF-β1 IgG; **lane 8:** mitogen-stimulated T cells cross-linked with [^{125}I] TGF-β1 and competed with nonimmune IgG. The cell extracts were separated by SDS-polyacrylamide gel electrophoresis methods in a 4% to 10% linear gradient.

Northern blot analysis methods, the relative amounts of these mRNAs appear not to be affected by immunosuppressive amounts of TGF-β1 or TGF-β2 (unpublished observation). Kehrl et al. [19], however, have shown that TGF-β1–treated human tonsillar T cells do express reduced amounts of IL-2 receptor and transferrin receptor, suggesting that TGF-βs may inhibit T cell proliferation by inhibiting IL-2 or IL-2 receptor mRNA translation.

TGF-β1 and TGF-β2 have been shown to be potent growth inhibitors of thymocytes and may have a regulatory role in T lymphopoiesis [18,28,31]. Using TGF-β1 monospecific antibodies and immunoperoxidase staining methods, Ellingsworth et al. [31] have shown that TGF-β1 is associated with accessory cells (Hassal corpuscles and macrophages) within the medullary portion of the thymus. It is within this region of the thymus that thymocyte proliferation is greatly reduced and that T cell maturation is taking place. Furthermore, the antiproliferative actions of TGF-β1 and TGF-β2 also appear to be mediated through binding proteins that have M_r of 280–200 kD, 95–85 kD, and 65 kD. Like mature T cells, these binding proteins are differentially expressed on resting and mitogen (IL-1)-activated thymocytes [18].

The antiproliferative actions of TGF-β1 and TGF-β2 are not limited to cells of the T cell lineage. These factors have been shown also to inhibit the proliferation and differentiation of pre B cells [21]. Furthermore, these studies show that TGF-β treatment inhibited kappa light chain gene expression, but not mu chain gene expression, and augmented Ia antigen expression on pre B cells. More recent studies have shown that the TGF-βs are also potent antagonists of interleukin-3–induced myeloid progenitor cell growth and differentiation and, therefore, appear to have a broad role in controlling the growth and differentiation of lymphocyte and hematopoietic progenitor populations [22,29].

The results presented here are consistent with the concept that TGF-β1 and TGF-β2 function as negative growth regulators of immune and hematopoietic cell types. In this study, the regulation of the expression of TGF-β1 binding proteins was assessed. These results show that these binding proteins are differentially expressed on resting and mitogen-activated T cells, suggesting that T cell mitogenic signals induce the expression of these binding proteins in a temporal fashion, further supporting the concept that TGF-β1 has an important role in the regulation of the clonal growth of T cells.

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