

PRODUCTION OF TRANSFORMING GROWTH FACTOR β BY HUMAN T LYMPHOCYTES AND ITS POTENTIAL ROLE IN THE REGULATION OF T CELL GROWTH

BY JOHN H. KEHRL,* LALAGE M. WAKEFIELD,[‡] ANITA B. ROBERTS,[‡]
SONIA JAKOWLEW,[‡] MELCHOR ALVAREZ-MON,[§] RIK DERYNCK,[¶]
MICHAEL B. SPORN,[‡] AND ANTHONY S. FAUCI*

*From the *Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, the [‡]Laboratory of Chemoprevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892; the [§]Service of Internal Medicine 1, Clinica Puerta de Hierro, Madrid, Spain; and the [¶]Department of Molecular Biology, Genentech, Incorporated, South San Francisco, California 94080*

Transforming growth factors (TGFs)¹ are polypeptides that reversibly induce certain nonneoplastic cells to express a transformed phenotype and to undergo anchorage-independent growth (1, 2). One of these, TGF- β , is a 25 kD homodimeric protein held together by disulfide bonds. TGF- β has been purified and partially sequenced, and its gene has been cloned (3–7). Using Northern blots, human TGF- β mRNA has been identified in a variety of cell types including mitogen-activated nonadherent PBMC (7). TGF- β binds to a unique receptor and has no known homology to other growth factors (8, 9). It was originally characterized by its ability to enhance the growth of normal rat kidney cells in soft agar when present with epidermal growth factor (EGF) (10). The precise mechanism of this effect is unknown, although TGF- β has been shown to upregulate EGF receptors in NRK cells (11). Subsequently, TGF- β has been shown to have both growth-enhancing and growth-inhibitory properties, and the effect that predominates is dependent upon the particular cell type and the other growth factors present (12, 13). For example, TGF- β synergized with platelet-derived growth factor to stimulate colony formation by a 3T3 cell line that had been transfected with *c-myc*, but inhibited colony formation by the same cell stimulated with EGF (12).

It has been clearly shown that a variety of soluble factors can influence the growth and effector function of murine and human lymphocytes (14). The present study examines the potential role of TGF- β in modulating human T lymphocyte function. We show that the addition of exogenous TGF- β to cultures of T lymphocytes inhibits IL-2-dependent T cell proliferation, and that activated T cells themselves synthesize and secrete TGF- β .

This work was partially supported by a grant from the Fondo de Investigaciones de la Seguridad Social. Address correspondence to J. Kehrl, National Institutes of Health, Building 10, Room 11B-13, Bethesda, MD 20892.

¹Abbreviations used in this paper: EGF, epidermal growth factor; NRK, normal rat kidney; TCE, T cell enriched; TGF, transforming growth factor.

Materials and Methods

Cell Preparation. Human tonsils were obtained at tonsillectomy from 7–20-yr-old patients with chronic tonsillitis, and were dispersed into single-cell suspensions. T cell-enriched (TCE) populations were obtained from tonsillar mononuclear cells by rosetting with 2-aminoethylisothiuronium bromide-treated sheep erythrocytes (15). The TCE fractions were further purified by passage over nylon wool columns. The Jurkat cell line, a human T cell leukemia, was kindly donated by Dr. Warner Greene.

Factors. TGF- β was purified from human platelets as previously described (3). The TGF- β was homogenous by analysis on SDS-polyacrylamide gels. rIL-2 (lot LP210) was kindly provided by Cetus Corp. (Emeryville, CA); it was 96% pure by SDS gel analysis and contained <0.04 ng endotoxin per 10^6 U (16). 1U IL-2 was defined as the amount of IL-2 required to induce half-maximal stimulation of an IL-2-dependent T cell line, HT2.

Culture Conditions. The TCE fractions were cultured with Con A (10 μ g/ml; Sigma Chemical Co., St. Louis, MO) for 12 h in RPMI 1640 plus 10% FCS (Dutchland Laboratories, Inc., Denver, PA), washed with RPMI containing 0.3 M methyl- α -D-mannopyranoside (Calbiochem-Behring Corp., La Jolla, CA), and recultured with various concentrations of TGF- β in the serum-free medium HB102 (Hana Media, Berkeley, CA), with 25–50 U/ml IL-2. Cells were cultured as 5×10^4 cells/well in 96-well plates (Costar, Cambridge, MA) for time periods as indicated. DNA synthesis was measured during the last 16 h of culture. Cells were pulsed with 1 μ Ci of [3 H]thymidine (6.7 Ci/mM; New England Nuclear, Boston, MA). Incorporation of [3 H]thymidine was measured by standard liquid scintillation counting techniques after harvesting by a Skatron harvester (Skatron, Inc., Sterling, VA). In other experiments, the TCE fractions were cultured with PHA (2 μ g/ml) in serum-containing media, and transferred to serum-free media at the indicated times.

Immunofluorescence. Cells (10^6 per 0.1 ml) to be analyzed for surface immunofluorescence were incubated for 30 min on ice with 5 μ l of either OKT3-FITC (Ortho Diagnostics, Inc., Raritan, NJ), anti-IL-2-R (Becton Dickinson Monoclonal Center, Mountain View, CA), or anti-transferrin receptor-phycoerythrin (Becton Dickinson Monoclonal Center). The cells were then washed twice with HBSS containing 0.1% human serum albumin and 0.02% azide, and analyzed on a FACS (FACS II.II; Becton Dickinson Immunocytometry Systems, Mountain View, CA). Control cells were unstained. For each sample, 20,000 or 30,000 light-scatter-gated viable cells were collected. Fluorescence data were collected with logarithmic amplification, and the specific cell immunofluorescence was expressed as channel numbers on a log scale. The mean fluorescence intensity was calculated by integrating the fluorescent histograms for the positive cells (compared to control cells).

Binding Assay. TGF- β was iodinated to a specific activity of 2–4 mCi/nmol using a modified chloramine T method (8). T cells were incubated for 2 h at 37°C in serum-free media to remove bound endogenous TGF- β . After washing, cells were resuspended in binding buffer (DMEM with 0.1% BSA, 25 mM Hepes, pH 7.4) containing 1–500 pM 125 I-TGF- β at 10^6 cells per 0.2 ml. Nonspecific binding was determined in the presence of 10 nM cold TGF- β . Samples were incubated for 2 h at 25°C in a CO₂ atmosphere with constant agitation. The cells were then spun down and the supernatants were counted in a gamma counter to determine the free ligand concentration. The cell pellet was resuspended in binding buffer and spun through a silicone oil/paraffin oil layer, and the resulting pellet was counted to determine bound ligand.

TGF- β Assays. Supernatants to be assayed for TGF- β were obtained from T cells cultured from serum-free media in the presence or absence of PHA. After harvesting, 1 mM PMSF (Pierce Chemical Co., Rockford, IL) was added to each supernatant. Subsequently, the supernatants were dialyzed against 1 M acetic acid, lyophilized to dryness, and reconstituted in a solution of one part 4 mM HCl plus three parts DMEM. The bioassay for TGF- β depends upon the ability of TGF- β to induce anchorage-independent growth of normal rat kidney (NRK) fibroblasts grown in the presence of 5 ng/ml EGF in soft agar. The soft agar assay was performed as previously described by measuring colony formation in 0.3% agar (10). Colonies were stained after 7 d of incubation, and those >62

μM in diameter were counted using an Omnicon image analysis system programmed to count a 5-cm^2 area of a 35-mm-diam plate.

The radioreceptor assay for TGF- β , a competitive binding assay that uses A549 cells, was performed as previously described (8). A549 cells were incubated with ^{125}I -TGF- β for 2 h at 25°C in 5% CO_2 in the presence of various dilutions of the test supernatant. After incubation, the cells were washed four times with cold HBSS (Gibco Laboratories, Grand Island, NY) containing 0.1% BSA. The cells were solubilized with 0.6 ml Triton solution (20 mM Hepes, 2% Triton X-100, 10% glycerol, 0.01% BSA, pH 7.4) by a 20-min incubation at room temperature, and counted on a gamma counter. A TGF- β standard curve was constructed using serial dilutions of a known amount of TGF- β .

Northern Blots. Total RNA was isolated from T cells using a guanidine thiocyanate method. The purified total RNA was electrophoresed in agarose-formaldehyde gels and transferred to either nitrocellulose (Schliecher and Schuell, Keene, NH) or Gene Screen II (New England Nuclear). The size-fractionated RNA was probed using a ^{32}P -labelled Eco R1 insert from $\lambda\beta\text{C1}$ under high stringency conditions (7). As previously reported (7), the TGF- β probe recognized an mRNA of ~ 2.5 kb.

Results

TGF- β Suppresses IL-2-dependent T Cell Proliferation and Partially Inhibits IL-2-R and Transferrin Receptor Expression. Since serum is a source of TGF- β and a variety of binding proteins for TGF- β could be present in serum, all of the experiments examining the effects of TGF- β were performed in serum-free media. T cells were stimulated with Con A for 8–12 h in serum-containing media, washed, and recultured in serum-free media. This duration of stimulation is sufficient for cellular activation and IL-2-R expression, but not for subsequent progression into S phase unless exogenous IL-2 is present (17, 18). Fig. 1 (*top*) shows the effect of TGF- β on DNA synthesis by Con A-activated tonsillar T lymphocytes that were cultured in the presence of IL-2. Optimal concentrations of TGF- β suppressed DNA synthesis by 60–80%. This degree of suppression was present 3, 4, and 5 d after the addition of IL-2 and TGF- β (Fig. 1, *bottom*). TGF- β was not toxic to the T cells, as judged by trypan blue dye exclusion.

Additionally, the effects of 2 ng/ml of TGF- β on IL-2-dependent T cell proliferation were examined as a function of IL-2 concentrations (Table I). TGF- β was most suppressive at the lower IL-2 concentrations. At higher IL-2 concentrations (100 and 300 U/ml), the degree of suppression decreased, although some suppression was present even at 300 U/ml. The degree of suppression was greater at day 5 than at day 3. Thus, by regulating the amount of IL-2 and TGF- β present, the amount of T cell proliferation can be regulated.

The interaction of IL-2 with its receptor on T cells has been shown (19) to be of primary importance in T cell growth, and satisfies the criteria for an autocrine system. One of the consequences of this interaction is an upregulation in cell-surface membrane IL-2-R (20, 21). IL-2 has also been shown (22–24) to be an important signal in the upregulation of another receptor, the transferrin receptor, which is thought to be important in cellular growth and is found on rapidly dividing cells. A potential mechanism whereby TGF- β might inhibit the growth of T cells is by interfering with the normal expression of either of these receptors. In Fig. 2, the effects of TGF- β on IL-2 and transferrin receptor levels are examined using immunofluorescence with a mAb against the transferrin receptor and an anti-IL-2-R mAb. Exposure of previously activated T cells to IL-2 for 16 h was sufficient to result in enhanced expression of both the IL-2-R and the

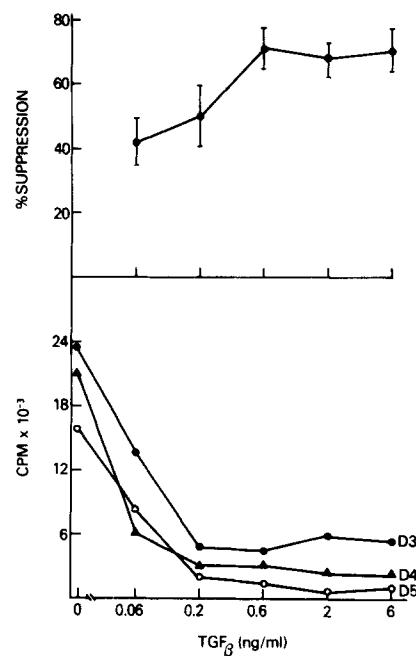


FIGURE 1. Suppression of T cell DNA synthesis by TGF- β . The TCE fractions were cultured with Con A, 10 μ g/ml, for 12 h in RPMI 1640 plus 10% FCS; washed with RPMI containing 0.3 M methyl- α -D-mannopyranoside; and recultured with IL-2 and various concentrations of TGF- β in HB102 media. Data in the upper panel are the mean \pm SEM for four experiments that were harvested on day 3 after a 16-h pulse with [3 H]thymidine. Percent suppression is calculated as $(1 - [\text{cpm in presence of TGF-}\beta] - [\text{background}]) / ([\text{cpm in absence of TGF-}\beta] - [\text{background}])$. Data in the lower panel are [3 H]thymidine incorporation from one experiment harvested on different days (days 3, 4, and 5).

TABLE I
Effect of TGF- β on IL-2-dependent T Cell Proliferation as a
Function of IL-2 Concentration

IL-2 (U/ml)	[3 H]Thymidine incorporation day 3 (cpm/ 10^5 cells)		[3 H]Thymidine incorporation day 5 (cpm/ 10^5 cells)	
	No TGF- β	TGF- β *	No TGF- β	TGF- β *
0	7,302	3,721	9,175	1,523
3	10,531	3,954	9,581	1,464
10	34,241	11,474	22,440	3,942
30	82,930	45,411	79,251	9,620
100	92,766	79,442	170,323	96,688
300	102,645	88,109	189,512	161,306

T cells were activated with Con A and cultured in the presence or absence of IL-2. [3 H]Thymidine incorporation was measured as described in the Materials and Methods.

* TGF- β present at 2 ng/ml.

transferrin receptor. If TGF- β was present in the culture together with the IL-2, the increase in density of both receptors was ablated. Additionally, the presence of TGF- β in culture reduced the number of IL-2R $^+$ cells from 99 to 76%. TGF-

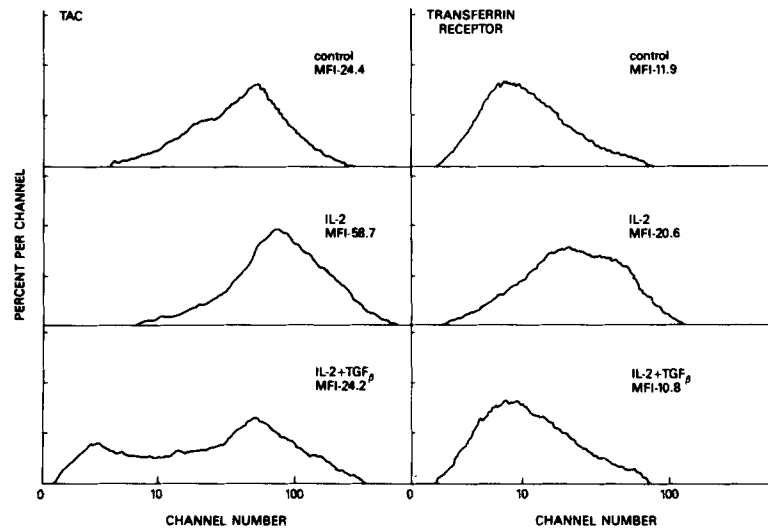


FIGURE 2. Regulation of the IL-2-R and transferrin receptors on human T cells by IL-2 and TGF- β . T cells were purified from tonsillar mononuclear cells and activated with 2 μ g/ml of PHA for 4 d in RPMI with 10% FCS. The cells were harvested and replated in 24-well Costar plates at 10^6 cells/well in HB102 media in the presence of media alone, 25 μ /ml of IL-2, or 2 ng/ml TGF- β plus IL-2. 16 h later, the cells were harvested and immunofluorescently stained with an anti-IL-2-R mAb, and an anti-transferrin receptor mAb. The cells were analyzed with a FACS. The mean fluorescent intensity (MFI) of the positive cells is indicated in the upper right portion of each FACS pattern. The IL-2-R is referred to as Tac.

β did not modulate T3-R expression (data not shown). Thus, a possible mechanism whereby TGF- β suppresses T cell proliferation is by interfering with the normal regulation of IL-2-R. The diminished expression of the transferrin receptor may be a secondary effect related to the effects of TGF- β on the IL-2-R.

T Cell Activation Results in Upregulation of TGF- β -R. A binding assay using 125 I-labeled TGF- β has been previously developed and used to demonstrate the presence of TGF- β receptors on a variety of cell lines (8, 9). We examined the expression of TGF- β receptors on unstimulated and activated T cells. The unstimulated T cells had low numbers of high-affinity TGF- β -R. When the T cells were cultured in the presence of PHA for 36 h, the number of TGF- β -R per cell increased approximately sixfold compared to cells cultured in the absence of PHA (Fig. 3). The affinity of the TGF- β -R for its ligand was not significantly changed when the T lymphocytes were stimulated. Although the number of TGF- β -R present on activated T cells is less than almost all the cell lines previously tested, the affinity of the receptor on T cells is higher by 5–10-fold (8, 9). The effect of IL-2 on TGF- β binding was also examined. T cells were activated for 12 h with Con A, washed with α -methyl mannoside, and recultured for 24 h in the presence or absence of IL-2. The presence of IL-2 did not alter the number or the affinity of the TGF- β -R present (data not shown). Thus, resting T cells express low numbers of TGF- β -R, and cellular activation results in a substantial increase in their number.

T Cell Activation Results in Appearance of TGF- β mRNA in T Cells and Increased

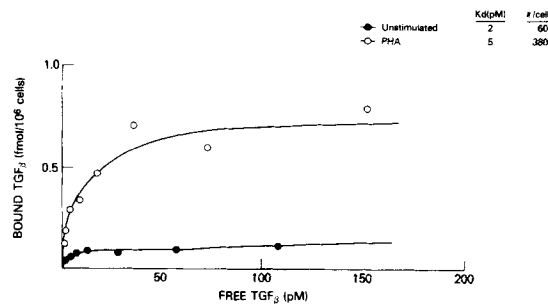


FIGURE 3. T cell activation results in an increase in the number of TGF- β binding sites per cell. Typical curves for the specific binding of ^{125}I -TGF- β to activated and unactivated tonsillar T lymphocytes. Scatchard analysis of the binding curves revealed the indicated affinity and number of binding sites. Unstimulated (●), $K_d = 2$ pM; sites/cell, 58. PHA-stimulated (○), $K_d = 5.1$; sites/cell, 380. 1 pM of TGF- β corresponds to 25 pg/ml.

Production of TGF- β in Culture Supernatants. The previous data showed that T cells have receptors for TGF- β , that exogenous TGF- β can modulate T cell DNA synthesis, and that exogenous TGF- β interferes with the normal regulation of IL-2 and transferrin receptors. The likelihood that TGF- β is an important regulator of T cell function would be considerably enhanced if T cells themselves made TGF- β . Previous experiments (7) showed the induction of TGF- β mRNA in mitogen-stimulated PBMC. To ascertain whether stimulated T cells actively secreted TGF- β , we determined whether supernatants conditioned by either unstimulated or stimulated T cells contained TGF- β biologic activity. We also examined purified T cells for the presence of TGF- β mRNA and examined the temporal relationship between induction of TGF- β mRNA and the secretion of TGF- β by activated T cells.

To show TGF- β biologic activity in conditioned media, T cells were either cultured in serum-free media in the presence or absence of PHA, or initially cultured in serum-containing media in the presence or absence of PHA, and after extensive washing, transferred to serum-free conditions. The collected supernatants were assayed for TGF- β biologic activity using a soft agar assay. This assay relies upon the ability of TGF- β to enhance the growth of NRK cells in agar in the presence of EGF (10). Supernatants conditioned by unactivated T cells had low levels of TGF- β -like biologic activity (0.6–1.6 pM), while supernatants from PHA-activated T cells had levels 10–50-fold higher (20–74 pM, Table II). To confirm that the TGF- β like biologic activity was indeed due to TGF- β , the supernatants were also assayed in a competitive radioreceptor assay (8). This assay detects TGF- β in an unknown sample by its ability to inhibit the binding of radiolabeled TGF- β to a cell line that expresses large numbers of TGF- β -R. The results from the radioreceptor assay strongly correlated with the results from the soft agar assay, and confirms the presence of TGF- β in the T cell supernatants (Table II). We have also tested supernatants conditioned by several different T cell lines and found TGF- β present in them. Table II shows that the human T cell leukemia cell line, Jurkat, constitutively secretes a moderate amount of TGF- β .

To confirm the presence of TGF- β mRNA in activated T lymphocytes, and to examine the time course of expression of TGF- β mRNA, human tonsillar T cells

TABLE II
TGF- β Production by Human T Lymphocytes

Exp.	Sample	Soft agar assay (pM)	Binding assay (pM)
1	Unstimulated	2.3	4.9
	PHA	23 (10)*	21 (4.3)
2	Unstimulated	1.6	0.62
	PHA	74 (46)	32 (56)
3	Jurkat	14	26

In the first experiment, tonsillar T cells were cultured for 4 d in serum-free media at a concentration of 2×10^6 cells/ml in the presence or absence of PHA. In the second experiment, tonsillar T cells were cultured in serum containing media in the presence or absence of 2 μ g/ml of PHA. After 48 h, the cells were harvested, washed three times, and recultured in serum-free media for 4 d. In the third experiment, Jurkat cells were cultured in serum-free media for 2 d at an initial concentration of 2×10^6 cells/ml.

* Number in parentheses is the fold stimulation.

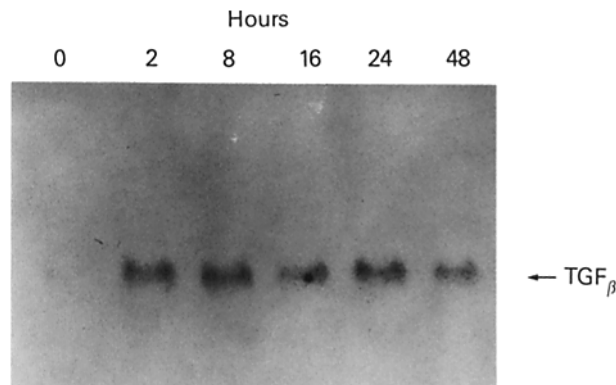


FIGURE 4. TGF- β mRNA is rapidly induced in stimulated T cells. Northern blot of RNA obtained at various time intervals after stimulation with 2 μ g/ml of PHA in serum-containing media. The time 0 point was cultured for 2 h in the absence of PHA. RNA was extracted from 10^8 cells for each time point. 15 μ g of total cytoplasmic RNA was loaded per lane.

were cultured with PHA for various periods of time. Total RNA from each group of cells was isolated and analyzed using Northern blots with the TGF- β cDNA as a hybridization probe. Fig. 4 shows the induction of TGF- β mRNA in T cells at various times after stimulation. Unstimulated T cells had low to undetectable levels of TGF- β mRNA, while PHA-activated T cells expressed substantial levels of TGF- β mRNA. The message appeared rapidly after stimulation, and thereafter the level of message was relatively constant. In contrast to the rapid induction and plateauing of TGF- β mRNA, the rate of secretion of TGF- β into culture supernatants gradually increased during the culture and did not reach maximal levels until 72–96 h after stimulation (Fig. 5).

Human T cells can be subdivided into two major subsets on the basis of the expression of either the T4 or the T8 surface marker. We examined whether

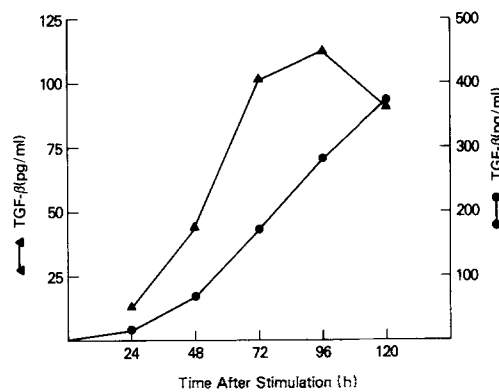


FIGURE 5. Kinetic of TGF- β production by activated T lymphocytes. Tonsillar T lymphocytes were activated with PHA (2 μ g/ml) in the presence of serum. 24 h before termination of the culture, the cells were harvested, washed extensively, then recultured in serum-free media. At the end of the culture period, the supernatants were assayed for TGF- β activity using the soft agar assay. For the 0–24 h points, the cells were cultured for 2 h in the presence of serum, washed, and recultured in serum-free media in the presence or absence of PHA for 24 h. The unstimulated culture contained 4 pg/ml of TGF- β . (◆), TGF- β secreted in the previous 24 h (left y axis); (●), calculated accumulation of TGF- β to that time point (right y axis).

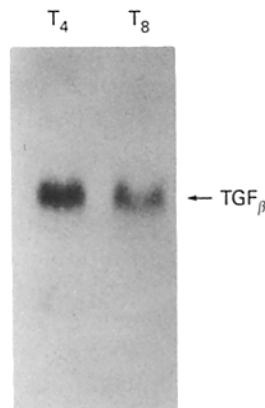


FIGURE 6. TGF- β mRNA is present in both T4 and T8 lymphocytes. Peripheral blood T lymphocytes were separated into a T4 and T8 population using an affinity rosetting technique. Each fraction was >90% pure, as judged by immunofluorescence. The T4 and T8 populations (5.0×10^7 cells for each fraction) were stimulated with 2 μ g/ml of PHA in serum containing media for 24 h, and after extraction of the RNA from each fraction, they were analyzed for the presence of TGF- β mRNA.

T4 or T8 lymphocytes preferentially expressed TGF- β message. T cells were separated into T4 and T8 fractions using immune rosetting, and each fraction was stimulated with PHA for 24 h. Total RNA from each fraction was purified, and a Northern blot was performed (Fig. 6). Although both the T4 and T8 subsets were induced to express TGF- β mRNA, the T4 subset expressed a slightly greater amount. Thus, both activated T4 and T8 cells contain mRNA for TGF- β , and significant quantities of TGF- β are secreted by activated T cells.

Discussion

This report examines the effect of TGF- β on human T cell proliferation and proposes that TGF- β production by T lymphocytes may be important in lymphocyte immunoregulation and T cell interactions with other cell types. We showed that T lymphocytes have receptors for TGF- β , which are upregulated after T cell activation, that the addition of exogenous TGF- β impairs IL-2-dependent T cell proliferation, and that it inhibits IL-2-induced upregulation of the IL-2-R and the transferrin receptor. Additionally, we confirmed the presence of TGF- β mRNA in activated T cells, and determined the kinetics of TGF- β mRNA induction after T cell activation. Finally, we have shown that TGF- β biologic activity is present in culture supernatants conditioned by human T cells, and that PHA activation results in a 10–46-fold increase in TGF- β production.

We think it is unlikely that the TGF- β detected in the conditioned supernatants is related to a cell type other than T cells. The experiments described here used T cells purified from tonsillar mononuclear cells. These preparations were <0.5% esterase positive. Although there is sufficient accessory cell function to induce a PHA response, there are numerically very few monocytes present. Thus, it is unlikely that PHA would stimulate the few remaining accessory cells to make significant levels of TGF- β mRNA and to secrete the quantities of TGF- β we have detected in the conditioned supernatants. Also arguing that T cells are a source of TGF- β is the production of TGF- β by a human T cell leukemia cell line.

The means by which resting T cells are activated and induced to undergo clonal expansion has been intensively studied. The major pathway of T cell activation occurs via the interaction of appropriately processed and presented antigen with the T cell receptor (25–27). This interaction results in the expression of IL-2-R, and the synthesis and secretion of IL-2 (28, 29). Once a critical number of high-affinity IL-2-R on the T cell membrane have bound ligand, DNA synthesis and cell division occurs. Additionally, the interaction of IL-2 with its receptor is a positive feedback signal for the cell to further increase its number of IL-2-R (20, 21, 30). Whether predominantly low-affinity IL-2-R or both high- and low-affinity IL-2-R are increased by this interaction is controversial (30, 31). Once T cell mitosis occurs, T cells continue to express IL-2-R and to proliferate in response to IL-2. However, even in the presence of exogenous IL-2, the T cell proliferation gradually decreases. Accompanying this decrease in proliferation is a decrease in IL-2-R expression (32). In this pathway of T cell activation and proliferation, the major regulatory signals are antigen in conjunction with Ia, IL-2, and IL-2-R expression. The observations reported here suggest that TGF- β may also be an important physiologic regulator of T cell growth.

The evidence supporting a role for TGF- β in the regulation of T cell growth includes the demonstration that the addition of exogenous TGF- β to cultures inhibits IL-2-dependent T cell proliferation and blocks IL-2-induced IL-2-R upregulation, that receptor levels for TGF- β are dependent upon the state of cellular activation, that T cells upon activation produce mRNA for TGF- β , and that TGF- β is secreted by PHA-activated T cells in concentrations in the range of those capable of inhibiting T cell proliferation.

The early synthesis of TGF- β mRNA at a time when the cells are being induced

to proliferate appears to be contradictory to this hypothesis that TGF- β is a growth inhibitor. However, despite the early appearance of TGF- β mRNA, significant quantities of the processed TGF- β protein were not secreted until 2–4 d later in culture. Thus, a prolonged interval sufficient for the gradual accumulation of TGF- β may be required to trigger an inhibitory signal. Furthermore, the inhibitory effect of exogenous TGF- β on IL-2-dependent T cell proliferation can be partially overcome by the addition of a higher concentration of IL-2. This suggests that the balance between IL-2 and TGF- β may be important in regulating T cell proliferation.

It has been shown (33) that TGF- β can be secreted by certain cells in a biologically inert form that is unmasked by acid treatment of the culture media. The platelet-derived TGF- β used in the functional studies described here has been acid treated during the purification process. This leaves open the possibility that lymphocyte-derived TGF- β does not impart an inhibitory signal unless it is activated in some manner. Experiments are in progress to examine this possibility.

There is a precedent for TGF- β being an autocrine growth inhibitor. In this regard, TGF- β and a growth inhibitor from green monkey kidney cells (BSC-1) have been shown to have nearly identical biologic activity, and to compete for binding to the same membrane receptor (13, 34). The BSC-1 cells produce the inhibitor when arrested at saturation density. The growth inhibitor has been purified from media conditioned by density-arrested cells, and has been shown to inhibit the proliferation of the same cells at a low cell density (34).

The production of TGF- β by T lymphocytes may not only be important in the regulation of lymphocyte function, but also may be important in the interaction of T cells with other cell types. For example, TGF- β has been shown to accelerate wound healing (35). Collagen deposition in a healing wound generally begins 5–7 d after the injury (36). This is preceded by an influx of mononuclear cells. Although the macrophage is the predominant cell type in this cellular infiltrate, lymphoid cells are also present (36). T cell production of lymphokines such as TGF- β during this early phase of wound healing may be an important signal for fibroblasts to synthesize collagen (37, 38). Whether macrophages also produce TGF- β is currently being investigated.

While a resolving acute inflammatory response such as a healing wound results in minimal fibrosis, chronic inflammation can cause considerable scarring with subsequent tissue deformation. Areas of chronic inflammations are characterized by a mononuclear cell infiltrate, fibroblast proliferation, and fibroblast collagen production. The continued production of TGF- β by activated T cells in these inflammatory sites may be important in maintaining fibroblast collagen synthesis. Furthermore, aberrant TGF- β production by T lymphocytes may be important in certain diseases that are characterized by excessive collagen production.

Conversely, TGF- β production by other cells may influence immune function. The exposure of T cells to large amounts of TGF- β would, very likely, severely limit normal T cell function. Certain tumor and virally transformed cell lines have been shown (2) to produce large amounts of TGF- β . The production of TGF- β by tumors *in vivo* could enhance a tumor's ability to escape immune-mediated destruction. In addition to the effects of TGF- β on T cell function, we have also shown that TGF- β inhibits NK activity (39) and B cell function (Kehrl,

J. H., A. B. Roberts, L. M. Wakefield, S. Jakowlew, M. B. Sporn, and A. S. Fauci. TGF- β is an important immunomodulatory protein for human B lymphocytes, manuscript in preparation).

Thus, TGF- β production by T lymphocytes may have two roles in inflammatory foci: the first to limit T and B cell clonal expression and subsequent effector function by a feedback mechanism, and second, to stimulate fibroblast proliferation and collagen production. By limiting T cell clonal expansion and presumably further T cell TGF- β secretion, the production of TGF- β by T lymphocytes may be an important signal in the regulation of inflammation.

Summary

This study examines the potential role of transforming growth factor β (TGF- β) in the regulation of human T lymphocyte proliferation, and proposes that TGF- β is an important autoregulatory lymphokine that limits T lymphocyte clonal expansion, and that TGF- β production by T lymphocytes is important in T cell interactions with other cell types. TGF- β was shown to inhibit IL-2-dependent T cell proliferation. The addition of picograms amounts of TGF- β to cultures of IL-2-stimulated human T lymphocytes suppressed DNA synthesis by 60–80%. A potential mechanism of this inhibition was found. TGF- β inhibited IL-2-induced upregulation of the IL-2 and transferrin receptors. Specific high-affinity receptors for TGF- β were found both on resting and activated T cells. Cellular activation was shown to result in a five- to sixfold increase in the number of TGF- β receptors on a per cell basis, without a change in the affinity of the receptor. Finally, the observations that activated T cells produce TGF- β mRNA and that TGF- β biologic activity is present in supernatants conditioned by activated T cells is strong evidence that T cells themselves are a source of TGF- β . Resting T cells were found to have low to undetectable levels of TGF- β mRNA, while PHA activation resulted in a rapid increase in TGF- β mRNA levels (within 2 h). Both T4 and T8 lymphocytes were found to make mRNA for TGF- β upon activation. Using both a soft agar assay and a competitive binding assay, TGF- β biologic activity was found in supernatants conditioned by T cells; T cell activation resulted in a 10–50-fold increase in TGF- β production. Thus, TGF- β may be an important antigen-nonspecific regulator of human T cell proliferation, and important in T cell interaction with other cell types whose cellular functions are modulated by TGF- β .

We thank Ms. Dee Goodrich for her fine editorial assistance, and thank Dr. Richard Assoian for generously providing the purified TGF- β . We also thank Dr. Ulrich Siebenlist and Ms. Nicola Salvatore for assistance in performing the Northern blots.

Received for publication 20 November 1985 and in revised form 30 December 1985.

References

1. Roberts, A. B., C. A. Frolik, M. A. Anzano, and M. B. Sporn. 1983. Transforming growth factors from neoplastic and nonneoplastic tissues. *Fed. Proc.* 42:2621.
2. Anzano, M. A., A. B. Roberts, J. M. Smith, M. B. Sporn, and J. E. DeLarco. 1983. Sarcoma growth factor from conditioned medium of virally transformed cells is

- composed of both type α and type β transforming growth factors. *Proc. Natl. Acad. Sci. USA*. 80:6264.
3. Assoian, R. K., A. Komoriya, C. A. Meyers, and M. B. Sporn. 1983. Transforming growth factor- β in human platelets: identification of a major storage site, purification, and characterization. *J. Biol. Chem.* 258:7155.
 4. Frolik, C. A., L. L. Dar, C. A. Meyers, D. M. Smith, and M. B. Sporn. 1983. Purification and initial characterization of a type β transforming growth factor from human placenta. *Proc. Natl. Acad. Sci. USA*. 80:3676.
 5. Roberts, A., M. Anzano, C. Meyers, J. Wideman, R. Blacher, Y. E. Pan, S. Stein, S. R. Lehrman, J. M. Smith, L. C. Lamb, and M. B. Sporn. 1983. Purification and properties of a type β transforming growth factor from bovine kidney. *Biochemistry*. 22:5692.
 6. Childs, C. B., J. A. Proper, R. F. Tucker, and H. L. Moses. 1982. Serum contains a platelet-derived transforming growth factor. *Proc. Natl. Acad. Sci. USA*. 79:5312.
 7. Derynck, R., J. A. Jarret, E. Y. Chen, D. H. Eaton, J. R. Bell, R. K. Assoian, A. B. Roberts, M. B. Sporn, and D. V. Goeddel. 1985. Human transforming growth factor- β complementary DNA sequence and expression in normal and transformed cells. *Nature (Lond.)*. 316:701.
 8. Frolik, C. A., L. M. Wakefield, D. M. Smith, and M. B. Sporn. 1984. Characterization of a membrane receptor for transforming growth factor- β in normal rat kidney fibroblasts. *J. Biol. Chem.* 259:10995.
 9. Tucker, R. F., E. L. Branum, G. D. Shipley, R. J. Ryan, and H. L. Moses. 1984. Specific binding to cultured cells of ^{125}I -labeled type β transforming growth factor from human platelets. *Proc. Natl. Acad. Sci. USA*. 81:6757.
 10. Roberts, A. B., M. A. Anzano, L. C. Lamb, J. M. Smith, and M. B. Sporn. 1981. New class of transforming growth factors potentiated by epidermal growth factor: Isolation from non-neoplastic tissues. *Proc. Natl. Acad. Sci. USA*. 78:5339.
 11. Assoian, R. K., C. A. Frolik, A. B. Roberts, D. M. Miller, and M. B. Sporn. 1984. Transforming growth factor- β controls. Receptor levels for epidermal growth factor in NRK fibroblasts. *Cell*. 36:35.
 12. Roberts, A. B., M. A. Anzano, L. M. Wakefield, N. E. Roche, D. F. Stern, and M. B. Sporn. 1985. Type β transforming growth factor. A bifunctional regulator of cellular growth. *Proc. Natl. Acad. Sci. USA*. 82:119.
 13. Tucker, R. F., G. D. Shipley, H. L. Moses, and R. W. Holley. 1984. Growth inhibitor from BSC-1 cells closely related to platelet Type β transforming growth factor. *Science (Wash. DC)*. 226:705.
 14. Smith, K. A. 1984. Lymphokine regulation of T cell and B cell function. In *Fundamental Immunology*. W. E. Paul, editor. Raven Press, New York. pp. 559.
 15. Falkoff, R. J. M., L. P. Zhu, and A. S. Fauci. 1982. Separate signals for human B cell proliferation and differentiation in response to *Staphylococcus aureus*: evidence for a two-signal model of B cell activation. *J. Immunol.* 129:97.
 16. Rosenberg, S., E. Grimm, M. McGrogan, M. Doyle, E. Kawasaki, K. Koths, and D. Mark. 1984. Biological activity of recombinant interleukin-2 produced by *Escherichia coli*. *Science (Wash. DC)*. 223:1412.
 17. Larsson, E.-L., and A. Coutinho. 1979. The role of mitogenic lectins in T cell triggering. *Nature (Lond.)* 280:239.
 18. Larsson, E.-L. 1981. Mechanisms of T cell activation. II. Antigen- and lectin-dependent acquisition of responsiveness to TCGF is a non-mitogenic, active response of resting T cells. *J. Immunol.* 126:1323.
 19. Meuer, S. C., R. E. Hussey, D. A. Cantrell, J. C. Hodgdon, S. F. Schlossman, K. A. Smith, and E. L. Reinherz. 1984. Triggering of the $\text{T}_3\text{-T}_1$ antigen receptor complex

- results in clonal T cell proliferation through an interleukin-2 dependent autocrine pathway. *Proc. Natl. Acad. Sci. USA*. 81:1509.
20. Reem, G. H., and N.-H. Yeh. 1984. Interleukin-2 regulates expression of its receptor and synthesis of gamma interferon by human T lymphocytes. *Science (Wash. DC)*. 225:429.
 21. Welte, K., M. Andreff, E. Platzter, K. Holloway, B. Y. Rubin, M. A. S. Moore, and R. Mertelsmann. 1984. Interleukin-2 regulates the expression of Tac antigen on peripheral blood T lymphocytes. *J. Exp. Med.* 160:1390.
 22. Sutherland, R., D. Delia, C. Schneider, R. Newman, J. Kemshead, and M. Greaves. 1981. Ubiquitous cell-surface glycoprotein on tumor cells is proliferation-associated receptor for transferrin. *Proc. Natl. Acad. Sci. USA*. 78:4515.
 23. Dillner-Centerlind, M.-L., S. Hammarström, and P. Perlmann. 1979. Transferrin can replace serum for in vitro growth of mitogen-stimulated T lymphocytes. *Eur. J. Immunol.* 9:942.
 24. Trowbridge, I. S., and F. Lopez. 1982. Monoclonal antibody to transferrin receptor blocks transferrin binding and inhibits human tumor cell growth in vitro. *Proc. Natl. Acad. Sci. USA*. 79:1175.
 25. Rosenthal, A. S., and E. M. Shevach. 1973. Function of macrophages in antigen recognition by guinea pig T lymphocytes. I. Requirement for histocompatible macrophages and lymphocytes. *J. Exp. Med.* 138:1194.
 26. Katz, D. H., and B. Bennacerraf. 1975. The function and interrelationship of T cell receptors, Ir genes, and other histocompatibility gene products. *Transplant. Rev.* 22:175.
 27. Davis, M. D., Y. Chien, N. R. J. Gascoigne, and S. M. Hedrick. 1984. A murine T cell receptor gene complex: Isolation structure and rearrangement. *Immunol. Rev.* 81:235.
 28. Smith, K. A. 1980. T-cell growth factor. *Immunol. Rev.* 51:337.
 29. Robb, R. J., A. Munck, and K. A. Smith. 1981. T-cell growth factor receptors: quantitation, specificity, and biological relevance. *J. Exp. Med.* 154:1455.
 30. Smith, K. A., and D. A. Cantrell. 1985. Interleukin-2 regulates its own receptors. *Proc. Natl. Acad. Sci. USA*. 82:864.
 31. Depper, J. M., W. J. Leonard, C. Drogula, M. Kronke, T. A. Waldmann, and W. C. Greene. 1985. Interleukin-2 (IL-2) augments transcription of the IL-2 receptor gene. *Proc. Natl. Acad. Sci. USA*. 82:4230.
 32. Cantrell, D. A., and K. A. Smith. 1983. Transient expression of IL-2 receptors. Consequences for T cell growth. *J. Exp. Med.* 158:1895.
 33. Krycève-Martinerie, C., D. A. Lawrence, J. Crochet, P. Jullien, and P. Vigier. 1985. Further study of β -TGF released by virally transformed and non-transformed cells. *Int. J. Cancer*. 35:553.
 34. Holley, R. W., R. Armour, and J. H. Baldwin. 1978. Density-dependent regulation of growth of BSC-1 cells in cell culture: growth inhibitors formed by these cells. *Proc. Natl. Acad. Sci. USA*. 75:1864.
 35. Sporn, M. B., A. B. Roberts, J. H. Shull, J. M. Smith, J. M. Ward, and J. Sodek. 1983. Polypeptide transforming growth factors isolated from bovine sources used for wound healing in vivo. *Science (Wash. DC)*. 219:1329.
 36. Ross, R. 1968. Wound healing. *Biol. Rev.* 43:51.
 37. Postlethwaite, A. R., G. N. Smith, C. L. Mainardi, J. M. Seyer, and A. H. Kang. 1984. Lymphocyte modulation of fibroblast function in vitro: Stimulation and inhibition of collagen production by different effector molecules. *J. Immunol.* 132:2470.
 38. Roberts, A. B., M. B. Sporn, R. K. Assoian, J. M. Smith, N. S. Roche, L. M. Wakefield, U. I. Heine, L. A. Liotta, V. Falanga, J. H. Kehrl, and A. S. Fauci. 1986.

- Transforming growth factor-beta: rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro. *Proc. Natl. Acad. Sci. USA*. In press.
39. Rook. A. H., J. H. Kehrl, L. M. Wakefield, A. B. Roberts, D. B. Burlington, H. C. Lane, and A. S. Fauci. 1986. Effects of transforming growth factor β on the functions of natural killer cells: depressed cytolytic activity and blunting of interferon responsiveness. *J. Immunol.* In press.