Immunoregulatory Role of Transforming Growth Factor β (TGF-β) in Development of Killer Cells: Comparison of Active and Latent TGF-β₁

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Summary

Using recombinant DNA technology, we have generated Chinese hamster ovary (CHO) cell lines that synthesize latent transforming growth factor β₁ (TGF-β₁) to study immune regulation by TGF-β₁. In vitro, latent TGF-β₁ synthesized by transfectants or added exogenously as a purified complex after activation inhibited CTL generation to a similar extent as seen with acid-activated recombinant human (rHu) TGF-β₁. In vivo, serum from nu/nu mice bearing CHO/TGF-β₁ tumors contained significant levels of latent TGF-β₁ in addition to depressed natural killer (NK) activity in spleens which paralleled that seen in C3H/HeJ mice treated with acid-activated rHuTGF-β₁. rHuTGF-β₁ treatment of mice receiving heart allografts resulted in significant enhancement of organ graft survival. Because of possible regulated tissue-specific activation, administration of latent rather than active TGF-β may provide a better route to deliver this powerful immunosuppressive agent in vivo.

Transforming growth factors encompass a family of polypeptides that regulate the growth and differentiation of both normal and transformed cells (1, 2). One of these, TGF-β₁, has been isolated and purified from various normal and neoplastic cells. Molecular cloning and sequencing of the cDNA for TGF-β₁ (3) in conjunction with protein structural data (4–6) showed that TGF-β₁ is synthesized as a precursor protein of 391 amino acid residues and subsequently processed after signal peptidase and proteolytic cleavage to a 112 residue (Mr, 12,500) form. The active or “mature” TGF-β₁ homodimer (25 kD) is noncovalently associated with the remainder of its precursor form (75-kD dimer), and in platelets covalently linked with one molecule of TGF-β₁ binding protein (135 kD) (5, 7, 8) or in serum associated with α2-macroglobulin (9) to form a high molecular weight inactive “latent” complex unable to bind TGF-β cellular receptors (10). TGF-β₁ can be activated by exposure to extremes of pH, heat, or by treatment with chaotropic agents, proteases, or glycosidases (11–14). However, the physiological processes by which latent TGF-β₁ is activated have not yet been fully elucidated.

Numerous studies have described the pleiotropic nature of the biologically active form of TGF-β₁. In addition to its potent effects on cell proliferation and differentiation, TGF-β₁ plays an active role in wound healing, tissue repair, and regulation of immune responses (1, 15, 16). The immunoregulatory properties of TGF-β₁ include inhibition of thymocyte proliferation (17–19), T and B cell proliferation, production of IgM or IgG (20–22), but not IgA synthesis which TGF-β enhances (23, 24), cytokine production (25), NK cell activity (26), cytotoxic T cell development (27–29), and lymphokine-activated killer (LAK) cell activity (28, 30). With regard to monocytes/macrophages, TGF-β displays both inhibitory and stimulatory effects. TGF-β can induce monocyte chemotaxis, and enhance mRNA expression for TNF-α and IL-1 (31, 32) while deactivating macrophages by reducing their capacity to release H₂O₂, cytotoxicity activity, class II and FcγR2 expression, as well as TNF-α and IL-1 production, thus perhaps imposing negative feedback and limiting the detrimental effects of toxic monokines (33–36).

Many of the studies that establish TGF-β as a regulator of immune function have examined the immunoregulatory effects of acid-activated TGF-β in vitro. However, the potential clinical usefulness of TGF-β as an immunosuppressant relies also on in vivo studies, few of which have been reported. Second, physiological TGF-β is first presented in vivo as a

Abbreviations used in this paper: CHO, Chinese hamster ovary cells; B6, C57BL/6; dhfr, dihydrofolate reductase; Hu, human; LAK, lymphocyte activated killer cell; LAP, latency-associated peptide.
in immune surveillance is discussed. Suppress immune function. The possible role of active TGF-β and latent TGF-β which in an autocrine or paracrine manner can include that mechanisms exist in vitro and in vivo to activate activities of latent and acid-activated rHuTGF-β. We conclude that mechanisms exist in vitro and in vivo to activate latent TGF-β which in an autocrine or paracrine manner can suppress immune function. The possible role of active TGF-β in immune surveillance is discussed.

Materials and Methods

Cell Culture and cDNA Transfection. Chinese hamster ovary (CHO) cells deficient in dihydrofolate reductase (dhfr) activity (37) were grown in DME/F12 (50:50) medium (Gibco Laboratories, Grand Island, NY) supplemented with 1 g/liter glucose, 10% FCS (HyClone, Logan, UT), 2 mM L-glutamine, penicillin (100 U/ml) and streptomycin (100 μg/ml) (Gibco) and 0.133 mM glycine, 0.11 mM hypoxanthine, 0.02 mM thymidine (GHT). CHO dhfr- cells (2 x 10⁶) were cotransfected with 1 μg of the expression vector p(SB4) containing the TGF-β1 cDNA (3) driven from the cytomegalovirus immediate early promoter in pRK5 (kindly provided by R. Kleml and D.V. Goeddel, Genentech, Inc.) and 100 ng of pFD11 which encodes the dhfr gene. Cells stably expressing the dhfr gene were selected in DME/F12 lacking GHT and 10% diazacylated FCS. Individual transfectant clones were selected, expanded in culture, and screened for TGF-β1 production by either RIA (6) or bioassay (described below). Confirmation of rHuTGF-β1 production was also determined by SDS-PAGE (6). The integrated plasmid copy number was amplified by culturing cells in the presence of up to 1-2 μM methotrexate in selective medium. For some experiments we used active rHuTGF-β1 produced by CHO transfectant cells, purified to 0.8 mg/ml (1 pg endotoxin/μg protein) and acid-activated by dialysis against 20 mM sodium acetate, pH 4.0. Latent rHuTGF-β1, in CHO transfectant-conditioned medium was purified by sequential cation exchange and hydrophobic chromatography (12). The latency of purified rHuTGF-β1 was confirmed both before and after its isolation by inactivity in bioassay or TGF-β receptor binding studies. The structure of purified latent rHuTGF-β1 is representative of the small latent TGF-β complex composed of the active 25-kD TGF-β dimer noncovalently associated with the 75-kD precursor remnant, but lacking the 135-kD binding protein found in the platelet large latent complex (6). For ease in reading the text the following abbreviations are used: CHO for parental CHO dhfr- cells, CHO dhfr+ and CHO/TGF-β1 refer to CHO dhfr- mutant cell line that has been transfected and expresses dhfr, dhfr and rHuTGF-β1 activities, respectively.

TGF-β Bioassay. A subclone of the Mv1Lu mink lung cell line (American Type Culture Collection, Rockville, MD) isolated in our laboratory was used to measure TGF-β activity (38). Conditioned medium or serum were assayed either untreated or after treatment with 0.12 N HCl for 15 min at room temperature followed by neutralization with 0.1M HEPES buffer containing 0.144 M NaOH (acid-activated). Samples diluted in MEM supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, 0.1 mM nontoxic amino acids, 100 U/ml penicillin, 100 μg/ml streptomycin (complete MEM [CMEM]) (all reagents from Gibco Laboratories) and 0.1% FCS (HyClone) were added to cultures in 96-well flat-bottomed microtiter plates (Costar, Cambridge, MA). The plates were incubated at 37°C with 5% CO₂ for 24 h and pulsed during the last 4 h with 1 μCi of [3H]thymidine in CMEM (6.7 Ci/mmol; Amersham Corp., Arlington Heights, IL). Cells were harvested using a PHD Cell Harvester (Cambridge Technology, Inc., Watertown, MA) and [3H]TBR incorporation was measured with a liquid scintillation counter. Results (picograms/milli liter of TGF-β) were calculated based on percent decrease in [3H]TBR incorporation compared to a rHuTGF-β1 standard. The lower detection limit of the bioassay was 10 pg/ml TGF-β. Samples containing rHuTGF-β1 that show negligible bioactivity without acid-activation are considered "latent," i.e., biologically inactive.

Animals. 6-8-wk-old BALB/c nu/nu female mice and C57BL/6 (B6) female mice were purchased from Charles River Breeding Laboratories, Wilmington, MA, C3H/HeJ female mice were obtained from Jackson Laboratories, Bar Harbor, ME. For the transplantation studies adult (8-10 wk) male C3H/km mice and neonatal (24-48 h) unsexed BALB/c mice were obtained from the Department of Radiobiology, Stanford University Medical Center, Stanford, CA.

Animals Studies. BALB/c nu/nu mice were injected subcutaneously with 10⁵ CHO or CHO/TGF-β transsected cells (>95% viable as judged by Trypan blue exclusion) washed with PBS (0.15 M NaCl, pH 7.0) and tumor size determined based on the average of the two cross-sectional diameters of each tumor. Serum samples were withdrawn for quantitation of TGF-β1 by bioassay and spleens were assayed for NK activity. C3H/HeJ mice were injected by various routes with various doses of acid-activated rHuTGF-β1 and spleen cells were assayed for NK activity.

For NK assays, spleen effector cells harvested from nu/nu or C3H/HeJ mice were washed three times in CMEM and seeded at various concentrations into 96-well round-bottomed tissue culture plates (CoStar in CMEM supplemented with 10% FCS). YAC-1 target cells were labeled with 150 μCi of Na¹¹¹CrO₄ (5 mCi/ml; Amersham Corp.) for 45 min and after three washes with CMEM, 10⁵ target cells were added to effector cells at various concentrations in 96-well round-bottomed microtiter plates (Costar). After 4 h of incubation at 37°C, culture supernatants were harvested (Skatron, Sterling, VA) and radioactivity was quantitated using an automatic gamma counter (Micromedic Systems, Hersham, PA). Percent specific lysis was calculated as 100 x [(cpm of combined effector and target cells supernatants (experimental release) − cpm of supernatants of target alone (spontaneous release)]/[cpm after lysis of targets with 2% NP-40 (maximum release) − spontaneous release]. Results are expressed as mean of triplicate determinations ± SEM. Spontaneous release of target cells alone was <10% of maximum for all experiments.

Cytotoxic T Lymphocyte Generation. CTLs were generated in 5-d cultures by incubation in T25 culture flasks of 2.5 x 10⁶ B6 responding spleen cells and 2.5 x 10⁶ stimulator CHO or CHO/TGF-β1, transfectant cells (irradiated 5,000 rad) in 10 ml of CMEM + 10% FCS. It should be noted that CHO/TGF-β1 cells used in the one-way MLC still accumulate TGF-β1 into culture medium after 5,000-rad irradiation. For some experiments B6-anti-CHO CTLs were generated in the presence of various concentrations of acid-activated rHuTGF-β1, purified latent rHuTGF-β1, or acid-activated purified latent rHuTGF-β1. After 5 d of culture effector cells were assayed for cytolytic activity against various ¹⁵⁴Cr-labeled, CHO cell lines that were labeled with ¹⁵⁴Cr as indicated for YAC-1 cells, and cytotoxicity assays were performed as described (27).

Mouse Heterotropic Cardiac Transplantation Model. A mouse pinna (ear)-heart transplant model was used in which a newborn BALB/c
donor heart is transplanted into a surgically constructed subdermal pocket on the left dorsal pinna of an adult C3H/km recipient. Recipient mice were anesthetized via intraperitoneal injection of chloral hydrate dissolved in saline (25 mg/kg) and a 2-mm epidermal/dermal incision was made parallel to the body axis and 2–3 mm distal to the ear-skull junction. An oval subcutaneous pouch measuring 3–4 mm in diameter was formed on the ear by inserting one arm of a small curved forceps into the incision separating the epidermal/dermal layer from the underlying cartilage. The donor heart was extracted en bloc via thoracotomy and both atria were removed. Excess air and fluid were expressed from the pouch with a cotton swab to facilitate maximal contact between donor and recipient tissues.

Treatment groups received intraperitoneal injection of either activated rHuTGF-β1 at various doses or normal saline scheduled at various intervals for 13 d after surgery. On the sixth postoperative day and on every other day thereafter until graft failure, the grafts were visually examined for contractions by using a stereomicroscope (Sterezoom No. 4; Bausch and Lomb Co., Rochester, NY) at 10–2-fold magnification. Graft viability was evaluated in a binary fashion (+ or −) based upon the presence or absence of graft contractions. All animals were weighed periodically to monitor drug toxicity.

Results

Production of Latent TGF-β1 by CHO Transfectants. Our initial studies used CHO cells lines 12/20/6 and 61-1/7C3C12A engineered to produce recombinant TGF-β1 (CHO/TGF-β1). To demonstrate that the TGF-β1 secreted from the CHO/TGF-β1 transfectants was latent, conditioned medium from CHO/TGF-β1 cells was analyzed for bioactivity with or without acid-activation. The data indicate that clones 12/20/6 and 61-1/7C3C12A produced significant quantities of recombinant latent TGF-β1 after 2 d of in vitro culture of which >90–98% was detectable by bioassay only after acid-activation (Table 1); the rHuTGF-β1 secreted by the higher producing clone 12/20/6 was also incapable of binding TGF-β1 cellular receptors as determined by radioreceptor assay (Dr. Lalage Wakefield, personal communication). Media obtained from parental CHO cells showed background levels of TGF-β. SDS-PAGE analysis showed that the rHuTGF-β1 synthesized by CHO transfectants formed a high molecular weight complex with molecular species of Mr 100,000 and 75,000, consistent with the presence of processed precursor and precursor remnant, respectively, similar in structure to platelet latent complex but lacking the 135-kD binding protein (data not shown, reference 6).

rHuTGF-β1 Inhibits CTL Generation In Vitro. We have shown previously that activated rHuTGF-β1 can inhibit the generation of CTL in vitro but not their cytolytic activity (27). The studies presented now further examined whether (a) latent TGF-β–secreting CHO cell lines can inhibit CTL generation in vitro and (b) such CHO cells can serve as targets for B6 anti-CHO–specific CTL. The data in Fig. 1 indicate that B6 spleen cells generated potent CTL against the CHO parental and CHO dhfr+ cells but not CHO/TGF-β1 stimulator cells. In contrast, B6 anti-CHO–specific CTL exhibited similar cytolytic activity against the CHO parental cells, CHO dhfr+ cells and CHO/TGF-β1 cells in 4-h CTL cytolytic assays (Fig. 1). These results indicate that transfection per se did not affect the ability of CHO cells to serve as stimulators or targets for CTLs and that CHO cells that secrete TGF-β are poor stimulators for CTL generation. Since latent TGF-β itself cannot bind cellular receptors, mechanisms

| Testsamples of conditioned media (CM) were assayed either untreated or acid-activated as described in Materials and Methods. Results were obtained with 10⁴ cells incubated for 48 h in CMEM and 10% FCS. Data are from one representativexperiment. | 
|---------------------------------------------------------------|
| **Table 1. rHuTGF-β1 Synthesized by CHO Transfectant Is Biologically Latent** |
| **TGF-β1 concentration** | Untreated CM | Acid-treated CM |
|---------------------------|--------------|-----------------|
| CMEM | <161 pg/ml | 206 ± 6 |
| CHO | 230 ± 50 | 340 ± 77 |
| CHO/TGF-β1, clone 12/20/6 | 394 ± 18 | 17,783 ± 1,269 |
| CHO/TGF-β1, clone 61-1/7C3C12A | <161 | 1,813 ± 359 |

* Test samples of conditioned media (CM) were assayed either untreated or acid-activated as described in Materials and Methods. Results were obtained with 10⁴ cells incubated for 48 h in CMEM and 10% FCS. Data are from one representative experiment.
exist in vitro to convert latent TGF-β into a biologically active form that can regulate antitumor responses.

We next investigated whether latent/activatable rHuTGF-β1 was as effective in suppressing CTL generation in vitro as acid-activated rHuTGF-β1. Both untreated and acid-activated latent rHuTGF-β1 inhibited CTL generation that was dose-dependent and in a manner similar to that of activated rHuTGF-β1 (Fig. 2). The concentration necessary to inhibit to 50% (ID50) the maximal CTL response was 0.63, 0.75 and 0.83 ng/ml acid-activated, latent/acid-activated, and latent rHuTGF-β1, respectively, for the experiment shown (Fig. 2). These data suggest that latent TGF-β is effectively converted to its active form in a manner quantitatively similar to activation by acid pH. Although the latent preparation contained ~5% active fraction (as determined by radioreceptor assay), the [ID50] for latent, latent/activated, and activated rHuTGF-β1 suggest that the inhibitory activity is not the result of pre-existing activated TGF-β but rather the conversion of latent rHuTGF-β1 to active polypeptide.

Tumor Growth and TGF-β Production In Vivo Tumor growth was observed in nu/nu mice injected subcutaneously with 1 × 10^7 CHO/TGF-β1 cells (Table 2). Over the 30-d examination period, the tumors grew progressively but were nonlethal. Tumor formation was observed in some animals as early as the first day after cell inoculation, with all animals demonstrating measurable tumors by day 5 after the study start. Elevated TGF-β levels were observed in the CHO/TGF-β1 bearing nu/nu mice compared with baseline levels of untreated controls (Table 2). Serum levels of activatable TGF-β increased as the study progressed, and tumors increased in size. In the two animals, which regressed the CHO tumor (one on day 14 and the other on day 30) the serum TGF-β levels were significantly lower in comparison with those obtained for the tumor bearing mice (data not shown). These results suggest that the CHO/TGF-β1 cells lines continue to synthesize rHuTGF-β1 in vivo which circulates in a latent but potentially activatable form.

Effect of rHuTGF-β1 on Murine Lymphoid Functions Acid-activated human platelet-derived TGF-β1 has been shown to inhibit NK activity in vitro (26). However, the effects of both latent and acid-activated TGF-β1 on NK activity in vivo have not been reported. As shown in Fig. 3 A, the i.p. injection of 1 μg acid-activated rHuTGF-β1 suppressed NK activity even after a single administration. Maximum inhibition was observed when rHuTGF-β1 was administered daily. Less sup-

Table 2. Serum TGF-β Levels and Growth of CHO/TGF-β1 Cells in nu/nu Mice

| Day of study | Average serum levels TGF-β (pg/ml)* | Tumor incidence | Average tumor size (mm)† |
|--------------|-------------------------------------|----------------|-------------------------|
| CHO/TGF-β1 clone | 5 | 23,256 ± 11,574 | 3/3 | 3.25 ± 0.48 |
| 61-1/7C3C12A | 10 | 76,063 ± 50,216 | 4/4 | 3.63 ± 0.31 |
| | 14 | 105,775 ± 62,404 | 3/4 | 5.67 ± 1.42 |
| | 30 | 186,368 ± 59,785 | 3/4 | 9.33 ± 1.92 |
| Untreated control mice | 30 | 25,374 ± 2,710 | 0/4 | 0 |

BALB/c nu/nu mice were used; injections, tumor measurements, and determinations of serum TGF-β levels were performed as indicated in Materials and Methods.

* Represents serum levels of activated TGF-β ± SEM obtained after acid-activation for tumor-bearing animals. All nonacid-activated serum contains less than detectable levels of TGF-β (detection limit 20 pg/ml).

† Average size ± SEM includes only tumor-bearing animals.
pression was obtained when 0.1 μg rHuTGF-β1 was administered (data not shown). Administration of 2 μg of activated rHuTGF-β1 daily by either the intraperitoneal, intravenous, or subcutaneous route reduced NK activity to a similar degree (Fig. 3 B). The i.p. administration of rHuTGF-β1 proved consistently the most effective in suppressing NK activity. As our previous data demonstrated that predominantly latent rHuTGF-β1 was produced in vivo by CHO/TGF-β1 cells, we questioned whether mice bearing these CHO-TGF-β1 tumors exhibited changes in NK activity. The results demonstrated a significant reduction in NK activity in the tumor-bearing mice (Fig. 3 C). NK activity for nu/nu mice bearing CHO dhfr+ cells was similar to that of untreated control animals (data not shown). The spleen cells from mice administered 1 μg rHuTGF-β1 intraperitoneally which showed reduced NK activity also demonstrated a suppressed responsiveness to Con A in comparison with untreated control animals (data not shown). However, it is interesting that in certain studies, mice treated with rHuTGF-β1 demonstrated reduced NK levels without the concomitant suppression of Con A responses (data not shown). The findings support that latent TGF-β1 activated in vivo can suppress NK activity and the ability to respond to mitogenic stimuli.

**Figure 3.** TGF-β1-mediated inhibition of murine NK activity. NK activities are shown for: (A) C3H/HeJ mice injected intraperitoneally with 1 μg dosage of acid-activated rHuTGF-β1 given daily (days 1–7) (○), on alternative days (days 1, 3, 5, 7) (▲), day 1 only (■), or control (□); (B) C3H/HeJ mice given 2 μg once daily (days 1–7) dosage of rHuTGF-β1 as a function of route of administration, or (C) untreated control (○), or experimental nu/nu mice injected subcutaneously 30 d before with CHO/TGF-β1 cells (●). Each data point represents the mean NK activity ± SEM determined from five animals.

Discussion

We have examined the immunoregulatory effects of TGF-β using CHO lines that synthesize and secrete latent rHuTGF-β1. Latent TGF-β was chosen as substrate to define the conditions under which immune cells process latent TGF-β to a functional polypeptide. The latency of rHuTGF-β1 was determined by inactivity in biological growth inhibition and radioreceptor assays. While it is generally true that most cells synthesize latent TGF-β (3), some cell lines such as the human breast cancer cell lines MCF-7 and T-47D, and human glioblastoma cells have been reported to secrete TGF-β that is biologically active without activation by acid, pH, or urea (39–41). The different mechanisms regulating the decision whether to release TGF-β as active or latent product is currently unclear. Since only active TGF-β can bind to cellular receptors (10), elucidation of those cells that can either acti-

### Table 3. Effect of rHuTGF-β1 on an Heterotopic Murine Heart Graft Survival

| rHuTGF-β1 treatment | n  | Schedule | Mean graft survival time* | p Value vs. control |
|---------------------|----|----------|--------------------------|-------------------|
| None               | 10 | 1–13     | 10.6 ± 0.2               | —                 |
| 1 μg                | 10 | 1–13     | 12.9 ± 1.5               | <0.00006          |
| 1 μg                | 4  | 4–13     | 12.5 ± 1.6               | 0.002             |
| 5 μg/μl            | 3  | 1–6/     | 16.0 ± 4.3               | <0.0003           |
| 2.5 μg/μl          | 7, 9, 11, 13 |         |                          |                   |

BALB/c neonatal hearts were transplanted into C3H/km mice as described in Materials and Methods. 1 or 4 d after surgery, mice were injected intraperitoneally with indicated amounts of rHuTGF-β1 or saline control. * Results are reported as mean ± the 95% confidence limit.
vate TGF-β directly, or are in proximity to those cells that can, is important for the understanding of autocrine or para-
crine regulation by TGF-β. However, at least for some cells, the
masking of active TGF-β by association with “latency-
associated peptide” (LAP) appears an important means to pro-
tect themselves from the potent regulatory effects of TGF-β
they synthesize (1, 8, 20-22, 42).

In keeping with the protective role of LAP, we found that
CHO/TGF-β1 cells injected into nu/nu mice secrete rHuTGF-β1
into the animal’s circulation as latent protein. Since activated
TGF-β1 was not found systemically at detectable levels in
CHO/TGF-β1 recipient mu/mu mice, but is required to
decrease NK activity, the results suggest that
TGF-β1 activation is a localized event in vivo, safeguarding
the release of large amounts of this growth regulator. The
localized activation of latent TGF-β may provide a means for
cells to regulate TGF-β activity only in specific tissues.

In addition to being sensitive to the inhibitory effects of
TGF-β1, most lymphoid cells produce latent TGF-β. The
presumed conversion of latent or endogenous rHuTGF-β1
to an active form that can inhibit immune functions was
shown to occur both in vitro and in vivo. Kehrli et al. have recently
reported that polyclonal antibodies to active TGF-β can
enhance B-cell proliferation and Ig synthesis (22). In addition,
Lucas and co-workers have shown that mAbs to active TGF-β
can enhance the proliferation of PBMC to IL-2 and PHA/PM
as well as the generation of lymphokine-activated killer cells.
These findings suggest that endogenously produced latent
TGF-β can be activated by normal physiological mechanisms
which can then downregulate in an autocrine fashion these
immune functions (43). The studies presented here further
demonstrate that endogenous latent TGF-β can be activated
and suppress both CTL generation in vitro and NK activity
in vivo.

A key towards understanding the target specificity of TGF-β
action is elucidation of the processes by which latent TGF-β2
becomes biologically active. Recent studies have suggested that
activated macrophages have the potential to both secrete and
activate latent TGF-β, although the mechanisms involved
in this conversion are not known. Acidic conditions can
convert latent TGF-β to active (11), suggesting that acidic cel-
lar environments may provide a physiologic milieu in which
activation may occur (44, 45). From microelectrode studies
it has been shown that macrophages, as well as osteoclasts,
contain such acidic microenvironments (46) that may be
sufficiently low to activate TGF-β. Moreover, the observa-
tion that activated macrophages express sialidase activity (47),
coupled with the finding that removal of sialic-acid-containing
carbohydrate structures in the TGF-β precursor remnant can
activate TGF-β (14), further substantiates the possible role
of the macrophage as an activator of latent TGF-β. We have
found that LPS-stimulated human peripheral blood-derived
adherent macrophages did not significantly activate ex-
ogenously added purified latent TGF-β1 (data not shown).
This result does not preclude the possibility that antigen-
presenting macrophages can activate latent TGF-β, but may
indicate the need for cognate help by other cell types during
activation. In support of this hypothesis, Antonelli-Orlidge
et al. report that the conditioned media of endothelial cells
and pericytes each contained latent TGF-β, but only during
their co-culture can activated TGF-β be produced (48). At
the current time we have no information to support or ex-
clude the possibility that CHO or CHO-TGF-β1 cells them-
selves can activate latent TGF-β. Our results showing that
activation of latent TGF-β occurs during the generation of
immune cytolytic cells may indicate that de novo induction
of lymphoid maturation proteins or cell surface differen-
tiation markers are pre-requisites for TGF-β activation.

Once activated, TGF-β1 displays potent immunosuppression
in vivo of NK cytolytic activity, the ability of spleno-
cytes to respond to mitogenic stimuli, and CTL generation
in vitro, which are shown in this report and previously (26-28).
As suggested by Rook et al., TGF-β may affect the continuous
recruitment and activation of “pre-NK” cells by blunting their
ability to respond to IFN-α, an important growth factor for
NK activation and function (26). The blunting of IFN-α
responsiveness may reflect downregulation of IFN-α receptor
numbers expressed on NK cells by TGF-β. In addition, TGF-β
may affect the differentiation of lymphoid cells as shown for
Ig-secreting B cells (21, 22), CTLs (27), and LAK cells (30),
possibly by lowering lymphocyte cell receptors for IL-2 (20).
Alternatively, TGF-β may inhibit the generation of CTLs,
and also LAK cells, by dampening TNF-α production (25),
an important modulator of both CTL and LAK cell develop-
ment (27, 30). Interestingly, TGF-β does not affect the ability
of either lymphoid cells (27) or tumor cells (data presented
here) to serve as targets for lysis by mature CTLs. This finding
suggests that TGF-β1 might not affect MHC class I expres-
sion on CHO cells, in contrast to its ability to modulate class
II HLA-DR surface antigen expression on human cells
(34, 35).

Within the framework of the complex immune cell net-
work, TGF-β is also shown to downregulate both NK ac-
tivity and primary CTL responses in vivo (49). Tumor cells
that produce TGF-β may therefore potentially reduce the
numbers or generation of circulating cytolytic CTL and NK
cells and thus promote escape of the tumor from immune
surveillance. Clinically, patients with glioblastomas, which
secrete TGF-β2 (50), demonstrate a generalized immune sup-
pression; manifested especially by an inability of normal T
cells to respond to mitogens (51). Moreover, TGF-β-producing
tumors can grow progressively in transiently immunosup-
pressed mice while retaining expression for class I MHC (49).
Thus, TGF-β by inhibition of host immune responses may
provide a mechanism to suppress allograft rejection and may
possibly accelerate processes of carcinogenesis.

We thank Nancy Mori and Michael Bombara for their technical assistance and Mary Chestnut for tissue
harvests. We credit Mark Jackson for production and characterization of CHO/TGF-β1 clone 61.
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