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Low Dose TGF-β Attenuates IL-12 Responsiveness in Murine Th Cells

James D. Gorham,† Mehmet L. Güler,* Domenic Fenoglio,* Ueli Gubler,† and Kenneth M. Murphy**

Expression of IL-12Rs is one important checkpoint for Th1 development. BALB/c DO11.10 CD4+ T cells stimulated by Ag in neutral conditions lose expression of the IL-12R β2 subunit and become unresponsive to IL-12. In contrast, B10.D2 or F1 (BALB/c × B10.D2) DO11.10 CD4+ T cells maintain IL-12Rβ2 expression when stimulated similarly. Here we show that the loss of IL-12 responsiveness by BALB/c T cells involves the action of endogenous TGF-β. BALB/c T cells stimulated in the presence of anti-TGF-β specifically maintain IL-12 responsiveness, express IL-12Rβ2 mRNA, and can stimulate nitric oxide production in peritoneal exudate cells. Low concentrations of TGF-β added exogenously during primary activation of B10.D2 or F1 T cells significantly inhibit their development of IL-12 responsiveness. These effects of anti-TGF-β are dependent on endogenous IFN-γ and are inhibited by exogenously added IL-4. Thus, at least one effect of TGF-β on Th1/Th2 development may be the attenuation of IL-12Rβ2 expression. The Journal of Immunology, 1998, 161: 1664–1670.

The expression of functional IL-12Rs is important for early regulation of Th phenotype development (1, 2). Ag activation of naive CD4+ T cells is required for induction of the two IL-12R subunits, IL-12Rβ1 and IL-12Rβ2 (3–5), but receptor expression is also regulated by other factors such as cytokines or genetic background. Developing Th1 cells express both β1 and β2 subunits and are IL-12 responsive, whereas developing Th2 cells do not express the β2 subunit and become unresponsive to IL-12 (1, 5, 6). Genetic background can also influence the maintenance of IL-12 responsiveness (2). In vitro, under neutral conditions of activation (i.e., where no exogenous cytokines are added), Ag-stimulated naive murine CD4+ T cells in the BALB/c genetic background are initially IL-12 responsive but rapidly lose expression of the IL-12Rβ2 chain. By contrast, under these same conditions, B10.D2 CD4+ T cells maintain the expression of both IL-12R subunits and maintain IL-12 responsiveness (2, 7).

The in vivo effect of genetic background on Th1/Th2 development is illustrated by murine experimental leishmaniasis, in which resistance to Leishmania major requires a host Th1 response (8–10). In response to footpad injection with L. major promastigotes, B10.D2 mice produce Th1 cells and control infection. In contrast, in BALB/c mice, a noncurative Th2 response develops, leading to dissemination of the organism (10). In both strains, high production of IL-12 p40 mRNA does not develop until several days after infection, roughly correlating with emergence of the amastigote form of L. major (11). Administration of IL-12 to BALB/c mice at the initiation of L. major infection leads to a Th1-dominated response and resistance (12, 13), whereas administration of IL-12 1 wk after parasite inoculation does not lead to Th1 development (13). Moreover, CD4+ T cells from draining lymph nodes of BALB/c mice become IL-12 unresponsive within 48 h of L. major infection (14).

TGF-β is a pleiotropic cytokine with generally anti-inflammatory and immunosuppressive properties. TGF-β inhibits macrophage activation (15), the generation of CTL (16, 17), and the expression of MHC class II molecules (18, 19). Importantly, TGF-β also has clear bimodal effects, with low concentrations exerting distinct physiologic effects (20–22). Mice deficient in TGF-β1 develop a lethal multorgan inflammatory immune infiltrate at 3 wk of age (23–25) with increased expression of inflammatory cytokines such as IFN-γ and TNF (23) and of inflammatory mediators such as nitric oxide (26). Interestingly, in vivo neutralization of TGF-β in Leishmania amazonensis-infected BALB/c mice permits the development of cutaneous Th1 responses, demonstrating a requirement for TGF-β in susceptibility to this pathogen (27). In this report, we describe the effects of TGF-β on the expression of the IL-12R by naive BALB/c CD4+ T cells during primary activation. We find that the rapid loss of IL-12Rβ2 expression depends on endogenous TGF-β and characterize the interactions that occur with other factors known to regulate IL-12 receptor expression.

Materials and Methods

Cytokines and Abs

Anti-TGF-β mAb (clone 2G7) (28) and the IgG2b isotype control Ab anti-gp120 (clone 1C10) were gifts from Genentech (San Francisco, CA). Porcine TGF-β1 was purchased from R&D Systems (Minneapolis, MN). Recombinant murine IL-12 was provided by Dr. S. Wolf (Genetic Institute, Cambridge, MA). Conditioned medium of murine IL-4 gene-transfected P815 cells (29) was used as a source of IL-4 (sp. act. = 6000 U/ml). Anti-IFN-γ (H22) was provided by Dr. R. Schreiber (St. Louis, MO). LPS derived from Escherichia coli was purchased from Sigma (St. Louis, MO).
Mice

Mice homozygous for the DO11.10 TCR transgenes have been maintained in the BALB/c background, as previously described (30). BALB/c background TCR transgenic mice were generated by male mouse (Harlan Sprague-Dawley, Indianapolis, IN). TCR transgenic B10.D2/N mice were derived by successive back-crosses (n > 6) into the B10.D2/N substrain (The Jackson Laboratory, Bar Harbor, ME) background, using the DO11.10 TCR clonotypic Ab KJ1-26 to identify transgene carriers, as previously described (31). TCR transgenic F1 (BALB/c × B10.D2/N) mice were derived from matings between male TCR transgenic B10.D2/N males and TCR transgenic homozygous females in the BALB/c background. Mice were housed in a specific pathogen-free barrier facility at Washington University Medical Center (St. Louis, MO).

Tissue culture media and peptide

Cultures were maintained in Iscove’s modified DMEM (Washington University Medical Center Tissue Culture Center) supplemented with 10% FCS (HyClone, Logan, UT), 2 mM L-glutamine, 0.1 mM pyruvate, 0.1 mM sodium pyruvate, 0.1 mM MEM nonessential amino acids, 100 U/ml penicillin, 100 μg/ml streptomycin, and 50 μg/ml 2-ME (Sigma). The antigenic peptide OVA (232-238) was synthesized and purified by HPLC as previously described (32).

Transgenic T cell purification and culture

T cell culture was performed essentially as described by Gorham et al. (30). CD4+ T cells from peripheral lymph nodes of 5- to 7-wk-old TCR-heterozygous transgenic mice were purified using Dynal anti-CD4 Dynabeads (Dynal, Chantilly, VA) according to the manufacturer’s directions to yield a population of >99% pure CD4+ T cells. For the experiment shown in Figure 2, cells were further purified by FACS sorting (FACS Vantage, Becton Dickinson, San Jose, CA) for cells doubly positive for anti-CD4 (PharMingen, San Diego, CA) and anti-CD4-FTC (PharMingen) to yield a population of >99% CD4+ MEL-14 high T cells. T cells (1.25 × 10^7) were stimulated in 1-ml cultures in 48-well plates with 0.3 μM OVA peptide presented by the H-2^d-expressing cell hybridoma TA3 (10,000 rad, 2.5 × 10^5 well). Where indicated, anti-TGF-β (10 μg/ml), IL-4 (100 U/ml), or IL-12 (5 U/ml) was included. We previously observed that anti-TGF-β at 10 μg/ml completely neutralized (in a PAI/L assay; see below) 2.5 μg/ml of exogenously added TGF-β (data not shown). The IL-12 responsiveness of anti-TGF-β at 1.0 μg/ml was nearly as active as anti-TGF-β at 10 μg/ml; at and below 0.1 μg/ml, activity diminished markedly (data not shown). Cells were expanded threefold into fresh medium at 72 h. On days 7 to 10, the T cells were harvested, washed, and restimulated in a secondary stimulation (1.25 × 10^7 well) with OVA peptide and the appropriate APC (either TA3 cells or BALB/c splenocytes (2000 rad, 2.5 × 10^5 well), as indicated) without or with recombinant murine IL-12 (5 U/ml) as indicated. IFN-γ and/or IL-4 concentrations were determined in 48-h supernatants by ELISA as previously described (31).

Nitrite production assays

Lymph node CD4+ T cells were isolated from BALB/c DO11.10 mice and TA3/OVA-stimulated in 1-ml cultures for 1 wk as described above. Cytokines or Abs were included as in Figure 4. One week later, peripheral exudate cells (PEC) were harvested from the peritoneum of BALB/c mice that had been injected with thioglycolate i.p. 4 days previously. PEC were allowed to adhere (100,000/well) for several hours at 37°C in 96-well tissue culture dishes. Nonadherent cells were removed by two successive washes. The DO11.10 T cells (stimulated for 1 wk) were then extensively washed and added to the PEC at 25,000, 12,500, or 6,250 cells/ml, and OVA peptide was added to a final concentration of 0.3 μM. After an additional 36 h of incubation, 100-μl supernatants were collected, and nitrite production was measured by addition of 100 μl of the Griess reagent (33) followed by spectrophotometric reading of the A_540.

Measurement of active TGF-β in T cell cultures

Active (mature) TGF-β was measured using a bioassay described by Abe et al. (34). The PAI-L cell line used in this assay is a mink lung epithelial cell line harboring a stable construct composed of a luciferase reporter driven by a plasmogen activator inhibitor-1 (PAI-1) promoter (35). The induction of luciferase in these cells is sensitive to picogram quantities of, and specific for, TGF-β (34). T cells were stimulated with OVA presented by TA3 in 10% FCS-containing medium in primary stimulations for between 16 and 72 h and in secondary stimulations for 48 h, and supernatants were collected and frozen at -80°C. For measurement of active TGF-β, PAI-L cells were seeded in 96-well tissue culture dishes (16,000 cells/well) and allowed to adhere overnight in a 37°C, 5% CO2 incubator. Adhered PAI-L cells were incubated overnight in a 37°C, 5% CO2 incubator and washed several times with cold PBS. Luciferase activity was determined from 50 μl of cell extract, using the luciferase assay substrate (Promega, Madison, WI) with an Opticom II automated luminometer (MGM Instruments, Hamden, CT). This assay is specific for the active form of TGF-β and does not detect latent TGF-β, which is abundant in FCS-containing medium (36, 37). For measurement of latent TGF-β, T cell supernatants were acidified by adding 1 N HCl to a final pH of 2.0, incubated for 1 min at room temperature, neutralized by adding 1 N NaOH to a final pH of 8.0, and then used in the PAI-L assay. As indicated in Figure 5B, anti-TGF-β Ab was included in some PAI-L assay wells.

Northern blot analysis

Total cellular RNA was isolated from T cells 5 days after secondary stimulation. Twenty micrograms of total RNA was loaded in each lane, electrophoresed, and transferred, and membranes were sequentially probed with full-length murine probes specific for IL-12β2 (3) and IL-12β1 (4).

Results

We previously reported that BALB/c CD4+ T cells activated under in vitro conditions where cytokines were not manipulated (termed the neutral condition) lost IL-12 responsiveness due to extinction of the IL-12 signaling subunit (2, 7). Because TGF-β is required for BALB/c susceptibility to L. amazonensis, we asked whether TGF-β is required for the loss of IL-12 responsiveness by BALB/c CD4+ T cells. CD4+ DO11.10 BALB/c T cells were activated for 1 wk in the presence or the absence of a neutralizing TGF-β-specific mAb or control Ab, harvested, washed, and restimulated in the presence or the absence of IL-12, and IFN-γ production was measured (Fig. 1). Control T cells activated in neutral conditions were unresponsive to IL-12. However, T cells activated in the presence of a neutralizing TGF-β Ab showed clear responsiveness to IL-12, producing 70 U/ml IFN-γ with added IL-12, but only 5 U/ml IFN-γ without IL-12 (Fig. 1A). The isotype-matched control Ab did not cause this effect and led to the expected loss of IL-12 responsiveness. The effect of anti-TGF-β Ab was not due to LPS contamination, since boiling the anti-TGF-β Ab eliminated its effect, whereas boiling LPS did not (Fig. 1B). The effect of anti-TGF-β treatment was evident with Mel-14 high DO11.10 CD4+ T cells T cells as well (Fig. 2). Neutralizing TGF-β in primary Mel-14high T cell activation also led to maintenance of IL-12 responsiveness, whereas control conditions led to loss of IL-12 responsiveness. Thus, endogenous TGF-β participates in loss of IL-12 responsiveness during activation of naive T cells.

Previously, we found that IL-12 responsiveness in CD4+ T lymphocytes was inhibited by IL-4 and stimulated by IFN-γ (7). We thus asked whether these cytokines exerted similar effects in the absence of endogenous TGF-β. Freshly isolated lymph node CD4+ T cells from BALB/c DO11.10 mice that were stimulated by Ag in the presence of anti-TGF-β and anti-IFN-γ Abs subsequently lost IL-12 responsiveness, consistent with the previously observed effect of IFN-γ induction of IL-12 responsiveness (Fig. 3). Thus, IFN-γ participates in maintaining IL-12 responsiveness that occurs after neutralizing endogenous TGF-β. This suggests that endogenous TGF-β could be acting to inhibit IFN-γ production or IFN-γ signaling to attenuate the IFN-γ-induced IL-12β2 expression. Also, treatment with anti-TGF-β together with IL-4...
led to loss of IL-12 responsiveness, consistent with IL-4 inhibiting IL-12Rβ2 expression (Fig. 3A). We also examined the effect of anti-TGF-β treatment on development of Th1 or Th2 populations (Fig. 3B). CD4+ DO11.10 BALB/c T cells were activated as before, except that BALB/c splenocytes were used as APC in the secondary stimulation to provide endogenous IL-12 (2), and IFN-γ and IL-4 were measured after 48 h. Control DO11.10 BALB/c T cells produced 12 U/ml IFN-γ and 38 U/ml IL-4 at secondary stimulation (Fig. 3B). Anti-TGF-β-treated T cells produced greater IFN-γ (160 U/ml), but showed no change in IL-4 production (Fig. 3B). As in Figure 3A, the effect of anti-TGF-β treatment was dependent on endogenous IFN-γ (Fig. 3B), since neutralizing IFN-γ blocked the effects of anti-TGF-β. Addition of IL-4 to the primary stimulation resulted in Th2 development. Anti-TGF-β treatment did not block the effect of IL-4; in contrast, IL-4 completely blocked the Th1-promoting activity of anti-TGF-β treatment.

We verified the effects of anti-TGF-β treatment on IL-12 responsiveness in T cells by using another functional parameter of T cell activity. Th1 cells, through the production of IFN-γ, promote nitric oxide (NO) production in macrophages by inducing NO synthase (iNOS) (38, 39). To assess the ability of primed T cells to induce iNOS in PECs, CD4+ DO11.10 BALB/c T cells were stimulated with Ag for 1 wk under various conditions (Fig. 4), then harvested, extensively washed, and replated with OVA peptide and
freshly plated BALB/c PECs as APC, and NO production was measured 36 h later (see Materials and Methods). T cells harvested from neutral primary stimulations did not induce NO production from PECs during PEC/OVA restimulation. In contrast, T cells harvested from primary stimulations containing anti-TGF-β did induce NO production and were approximately as effective in this activity as T cells harvested from primary stimulations containing IL-12 (Fig. 4). Again, the NO-inducing activity of the anti-TGF-β-derived T cells required the presence of IFN-γ in the primary stimulation and was inhibited by the inclusion of IL-4 in the primary stimulation. Thus, endogenous TGF-β present during naive T cell activation inhibits both subsequent IFN-γ production and the ability to subsequently induce iNOS in PEC cocultures.

Next, we attempted to measure the level of active TGF-β that may be present during primary T cell activation, using a TGF-β-sensitive cell line, Mv1Lu, stably transinfected with the TGF-β-responsive PAI-1 promoter/luciferase reporter construct (34, 35) (PAI/L assay; Fig. 5A). The PAI/L assay could detect exogenous active porcine TGF-β1 with an analytical sensitivity of 30 pg/ml (Fig. 5A), which is, however, above the level of TGF-β that can exert some physiologic effects (20–22). Conditioned media (CM) from unmanipulated primary BALB/c T cell stimulations (16–72 h after activation) induced no luciferase activity above background in PAI/L assay cells. In contrast, CM from secondary T cell stimulations induced luciferase activity (Fig. 5A; calculated level = 95 pg/ml TGF-β), demonstrating that the PAI/L assay cells are able to respond to murine TGF-β. Thus, the level of endogenous active TGF-β present during primary T cell activation appears to be <30 pg/ml.

TGF-β circulates in a latent form as a noncovalent complex with the TGF-β propeptide homodimer, termed latency-associated peptide. Activation is associated with the release of active TGF-β from latency-associated peptide and is a major regulatory step controlling the effects of TGF-β. The release of active TGF-β is mediated by a variety of mechanisms, many of which occur at the cell surface (40). Active TGF-β may then be rapidly cleared by specific serum binding proteins or cell surface receptors. These various regulatory processes can preclude the detection of very low levels of active TGF-β (40). To demonstrate the presence of the latent form of TGF-β in the T cell cultures, CM was collected from primary T cell stimulations at 24 and 48 h. A portion of the CM was briefly treated with acid to convert latent TGF-β to the active form (see Materials and Methods) and then tested in the PAI/L cell assay to quantitate TGF-β. Untreated T cell CM did not induce luciferase activity above background in PAI/L cells. By contrast, acid-treated CM induced high luciferase activity (Fig. 5B; the calculated concentration of TGF-β is shown) that was largely inhibited by the inclusion of anti-TGF-β, indicating that the activity is specific for TGF-β. Similar results were observed with nonconditioned media (Fig. 5B), as expected, since latent TGF-β is abundant in FCS-containing medium (36, 37). These results directly demonstrate the presence of TGF-β in T cell primary stimulations.

Because TGF-β attenuates IL-12 responsiveness in BALB/c T cells, we asked whether TGF-β can also inhibit IL-12 responsiveness in B10.D2 or F1 (BALB/c × B10.D2) T cells. B10.D2 or BALB/c D011.10 CD4+ T cells were activated under neutral conditions in the presence of either the absence of either anti-TGF-β or various concentrations of TGF-β (Fig. 6A). For B10.D2 cells, IL-12 responsiveness was maintained under neutral conditions of development, but subsequent IFN-γ production was quantitatively greater (140 U/ml) when anti-TGF-β was present in the primary stimulation compared with the neutral control (95 U/ml). Addition of low doses of TGF-β during primary activation inhibited IL-12 responsiveness in B10.D2 T cell cultures. Maximal inhibition was seen with 10 pg/ml TGF-β (Fig. 6A; p = 0.03 and 0.0009 vs neutral and anti-TGF-β, respectively, by Student’s t test). For BALB/c T cells, anti-TGF-β treatment induced the maintenance of
IL-12 responsiveness, whereas T cell populations derived under neutral conditions or with low concentration TGF-β (0.1–10 pg/ml) were uniformly IL-12 unresponsive (Fig. 6A). The addition of low dose TGF-β inhibited IL-12 responsiveness in F1 (BALB/c × B10.D2) T cells as well (Table I). Addition of TGF-β at 10 pg/ml resulted in inhibition of IL-12-dependent IFN-γ production at secondary stimulation by 54 to 71% (compared with the neutral point), and addition of TGF-β at 100 pg/ml inhibited IL-12 responsiveness by 72 to 90%. Interestingly, addition of TGF-β at higher doses (10,000 pg/ml) augmented IL-12 responsiveness independently of the T cell genetic background (Table I). This result suggests that the effects of TGF-β on IL-12 responsiveness in CD4+ T cells are bimodal, as described for other activities of TGF-β (20–22). Together, these results show that exogenously added, low dose TGF-β in primary stimulations of B10.D2 or F1 CD4+ T cells significantly inhibits subsequent IL-12 responsiveness, similar to the inhibitory effects of low dose (endogenous) TGF-β in BALB/c T cell stimulations.

Consistent with published data (41), BALB/c T cells developing under neutral conditions made significantly more IL-4 at secondary stimulation than did B10.D2 T cells (Fig. 6B). IL-4 production during secondary stimulation was unaffected by primary neutralization or addition (0.1–100 pg/ml) of TGF-β or by the presence or the absence of IL-12 in the secondary stimulation (Fig. 6B). Thus, in either BALB/c or B10.D2 T cells, TGF-β regulates IL-12 responsiveness and IFN-γ production, but has little effect on subsequent IL-4 production (5, 7).

To determine the effect of endogenous or low dose TGF-β on expression of IL-12R, BALB/c and B10.D2 T cells were stimulated for 7 days under neutral conditions or with addition of anti-TGF-β or TGF-β (10 pg/ml), washed, restimulated for 5 days without addition of IL-12, and harvested for total cellular RNA. For BALB/c T cells, development under neutral conditions or with 10 pg/ml TGF-β led to loss of IL-12Rβ2 expression, whereas neutralization of TGF-β led to sustained IL-12Rβ2 expression. For B10.D2 T cells, the addition of TGF-β inhibited IL-12Rβ2 expression, whereas neutralization of TGF-β slightly increased expression. By comparison, expression of the IL-12Rβ1 subunit showed essentially no regulation by TGF-β (Fig. 7). Thus, the mode of regulation exerted by TGF-β appears to be through effects on IL-12Rβ2, which is also a target of regulation by other cytokines as described previously (7).

**Discussion**

Recent studies have demonstrated that murine *L. major* susceptibility is a polygenic process (42, 43), and that several cellular factors participate in regulating Th1/Th2 responses to the pathogen (10). Early IL-4 production in the susceptible BALB/c strain is associated with the loss of T cell responsiveness to IL-12 (14), helping to promote eventual Th2 responses despite IL-12 production in subsequent stages of the infection (11). Notably, TGF-β contributes to susceptibility to another *Leishmania* species (27). Endogenous TGF-β is required for BALB/c susceptibility to *L. amazonensis*, since in vivo neutralization of this cytokine is sufficient to allow the development of a protective Th1 response (27).

The effects of TGF-β on Th1/Th2 development are complex (32, 44–49). TGF-β does not directly induce Th1 or Th2 differentiation (32) and does not signal through STAT4 or STAT6 (50). Rather, TGF-β is likely to regulate Th1/Th2 responses indirectly, through, for example, effects on the production of other cytokines or on T cell sensitivity to cytokines. Because BALB/c T cells lose IL-12 responsiveness when activated either in vitro, under neutral conditions, whereas T cell populations derived under neutral conditions or with low concentration TGF-β (0.1–10 pg/ml) were uniformly IL-12 unresponsive (Fig. 6A). The addition of low dose TGF-β inhibited IL-12 responsiveness in F1 (BALB/c × B10.D2) T cells as well (Table I). Addition of TGF-β at 10 pg/ml resulted in inhibition of IL-12-dependent IFN-γ production at secondary stimulation by 54 to 71% (compared with the neutral point), and addition of TGF-β at 100 pg/ml inhibited IL-12 responsiveness by 72 to 90%. Interestingly, addition of TGF-β at higher doses (10,000 pg/ml) augmented IL-12 responsiveness independently of the T cell genetic background (Table I). This result suggests that the effects of TGF-β on IL-12 responsiveness in CD4+ T cells are bimodal, as described for other activities of TGF-β (20–22). Together, these results show that exogenously added, low dose TGF-β in primary stimulations of B10.D2 or F1 CD4+ T cells significantly inhibits subsequent IL-12 responsiveness, similar to the inhibitory effects of low dose (endogenous) TGF-β in BALB/c T cell stimulations.

**Table I. TGF-β inhibits the maintenance of IL-12 responsiveness in F1, CD4+ cells**

<table>
<thead>
<tr>
<th>First stimulation condition</th>
<th>IFN-γ Production (U/ml) at Second Stimulation (with IL-12)</th>
<th>IL-12 Responsiveness, vs Neutral Point (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BALB/c</td>
<td>F1</td>
</tr>
<tr>
<td>Anti-TGF-β</td>
<td>144.0</td>
<td>22.5</td>
</tr>
<tr>
<td>Neutral</td>
<td>14.5</td>
<td>&lt;2.0</td>
</tr>
<tr>
<td>TGF-β (0.1)α</td>
<td>8.4</td>
<td>ND</td>
</tr>
<tr>
<td>TGF-β (1.0)</td>
<td>8.9</td>
<td>ND</td>
</tr>
<tr>
<td>TGF-β (10)</td>
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<tr>
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<tr>
<td>TGF-β (1,000)</td>
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<td>4.4</td>
</tr>
<tr>
<td>TGF-β (10,000)</td>
<td>151.3</td>
<td>137.2</td>
</tr>
</tbody>
</table>

α A total of 2.0 U/ml constitutes the limit of detection of the IFN-γ ELISA.

β Numbers in parenthesis indicate concentration (pg/ml).

γ Not available.
Indeed, we found that the effects of TGF-β partly attributable to the variability in experimental conditions, or during early L. major infection, we examined the role of TGF-β during neutral Th cell development. Here, we show that endogenous TGF-β inhibits expression of the IL-12R signaling subunit in BALB/c T cells and that neutralization of TGF-β permits maintenance of IL-12Rβ2 and IL-12Rβ1. Equal loading of lanes is indicated by a negative image of the 18S portion of the ethidium bromide-stained gel before transfer (bottom).

The effects of TGF-β on Th1/Th2 development in vitro are controversial (32, 44–49). The lack of consistency of results may be partly attributable to the variability in experimental conditions used. Indeed, we found that the effects of TGF-β were dependent on at least three experimentally controlled parameters: dose, T cell genetic background, and source of the APC. First, the dose of TGF-β added was important, in that low concentrations (10–100 pg/ml) of TGF-β inhibited IL-12 responsiveness in T cells, whereas high concentrations (10,000 pg/ml) of TGF-β significantly augmented IL-12 responsiveness. Interestingly, TGF-β exhibits a similar bimodal dose effect on the proliferation of smooth muscle cells (21), apparently due to TGF-β dose-dependent effects on the expression of a smooth muscle cell growth factor (platelet-derived growth factor) and its receptor. Second, the genetic background of the T cells influenced responses to TGF-β, since at doses up to 100 pg/ml, TGF-β was completely inhibitory for BALB/c T cells and was only partially inhibitory for B10.D2 T cells. Hoehn et al. have previously reported a genetic effect on T cell responses to TGF-β (46). Third, the source of APC was important, since the effect of anti-TGF-β was clearly evident in the homogenous priming conditions used in the present study in which TA3 cells were used as APCs, but was weaker when heterogeneous populations of APCs were used in primary T cell activation (data not shown).

In vivo, TGF-β exhibits potent anti-inflammatory activity. The inflammatory wasting syndrome that develops in 3 wk postnatally in TGF-β1-deficient mice is ameliorated in TGF-β1+/+SCID mice (51), and in TGF-β1−/−class II MHC−/−double knockout mice (52). Thus, CD4+ T cells appear to mediate much of the disease manifested by the TGF-β1 knockout mouse. Supporting this idea, administration of anti-CD4 mAb improves survival in TGF-β1−/−mice (52). Since neutralizing TGF-β permits continued expression of the IL-12R by BALB/c T cells in vitro, it is possible that the inflammatory disease in TGF-β1−/−mice is at least in part Th1 mediated. Consistent with this is the observation that TGF-β1−/−mice exhibit enhanced expression of IFN-γ, TNF-α (23), and NO (26).

In several other mouse models, TGF-β has been shown to antagonize Th1-type immune responses. For example, inflammatory bowel disease can be initiated in SCID mice by the adoptive transfer of CD45RBhigh T cells that induce elevations in IFN-γ and TNF-α mRNA (53, 54). Protection from disease is mediated by CD45RBlow T cells and requires TGF-β but not IL-4 (54). In 2,4,6-trinitrobenzene sulfonic acid-induced murine chronic colitis, also a Th1-mediated disease, the development of oral tolerance is abrogated by treatment with anti-TGF-β (55). In murine experimental autoimmune encephalomyelitis (EAE), in which Th1 cells are disease promoting (56, 57), administration of anti-TGF-β exacerbates disease manifestations, suggesting a protective role for endogenous TGF-β (58, 59), whereas administration of TGF-β ameliorates the disease (60). Moreover, TGF-β-producing clones specific for myelin basic protein suppress experimental autoimmune encephalomyelitis disease upon adoptive transfer (61). Finally, BALB/c mice infected with L. amazonensis generate a noncuring Th2 response, but when treated with anti-TGF-β during the first week of infection generate a curative Th1 response (27). It will be important to determine in these various experimental disease models whether any relevant in vivo immunobiologic effects of TGF-β are mediated through modulation of IL-12R expression and Th1 development potential.

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