

Regulation of TGF- β Response During T Cell Activation Is Modulated by IL-10¹

Françoise Cottrez and Hervé Groux²

TGF- β 1 is an important pleiotropic cytokine that has been described to have both stimulatory and inhibitory effects on cell growth and differentiation. For several cell types, the effect of TGF- β 1 was found to correlate with the differentiation stage of the cells and the presence of other cytokines. In this report, we address the influence of TGF- β 1 on CD4⁺ T cell activation by evaluating the effect of TGF- β 1 on the proliferative and cytokine responses of purified resting and activated human or mouse CD4⁺ T cells. TGF- β 1 inhibits proliferation and cytokine secretion on resting CD4⁺ T cells but has no inhibitory effect on activated T cells. Moreover, TGF- β 1 unresponsiveness of activated T cells was correlated with a down-regulation in the expression of the TGF- β receptor type II. Interestingly, IL-10 addition enhances TGF- β receptor type II expression and restores TGF- β responsiveness on activated T cells. These results indicated that TGF- β responsiveness is sequentially regulated on T cells by the modulation of the TGF- β receptor type II chain expression. Moreover, we have identified a novel regulatory role of IL-10 on TGF- β -dependent T cell growth that can explain the control of T cell activation on chronic vs acute inflammatory sites. *The Journal of Immunology*, 2001, 167: 773–778.

Transforming growth factor β is a member of a superfamily of growth and differentiation factors produced by a variety of cell types in a wide range of species (1). TGF- β s display multiple functions, often with opposite effects, depending on the responding cell type and the state of differentiation. This highly pleiotropic cytokine is also known to affect a number of cells of the immune system such as CD4⁺ and CD8⁺ T cells (1). Moreover, numerous studies have shown that TGF- β affects a wide array of immune processes including T cell proliferation (2), T cell apoptosis (3, 4), T cell differentiation (5–7), and APC function (8). Finally, TGF- β has proven to display varying effects on immune responses (1). One possible explanation of this fact is that TGF- β acts at different levels of lymphoid cell activation and maturation.

To better understand the role of TGF- β on T cell function, it is important to identify TGF- β target cells that express functional TGF- β Rs. TGF- β elicits its effects by binding to cell surface receptors. Three major types of TGF-binding proteins are known to be widely distributed in most TGF-responsive cells. They are referred to as TGF receptor type I (TRI),³ type II (TRII), and type III (TRIII, or glycan) receptors. TRI and TRII are glycoproteins of 53 and 75 kDa, respectively, whereas TRIII is a proteoglycan of 280–330 kDa (9). TRI and TRII possess an extracellular region, a single transmembrane portion, and a serine/threonine kinase domain in the cytoplasmic region. TRII can bind free ligand, whereas TRI can only recognize ligand that is already bound with TRII and formation of a ligand-induced heterotetramer involving both TRI

and TRII is required for signaling. In contrast, TRIII lacks a cytoplasmic protein kinase domain and appears to function mainly in the concentration and presentation of TGF- β to TRI and TRII (9). Signaling by these receptors is mediated by the recently identified Smad protein family (10, 11).

It has been shown in different biological systems that TGF- β response can be modulated by the expression of the TGF- β RII. For example, inflammation signals like LPS or IFN- γ induce a down-modulation of TGF- β R expression that is accompanied by a diminished ability of the cells to respond to TGF- β . Similar mechanisms have also been described on different tumor cell lines (12, 13).

We had previously shown that IL-10 induces anergy (14) and the differentiation of T regulatory (Tr) 1 T cell in vitro (15). Recently, Zeller et al. (16) have shown, in similar MLR experiments, that addition of TGF- β potentiates the effect of IL-10, suggesting that IL-10 and TGF- β act synergistically.

We show here that activated/memory T cells become refractory to TGF- β -mediated inhibition of proliferation through the down-regulation of the TGF- β RII. However, TGF- β R expression and TGF- β inhibitory function can be restored on activated/memory T cells by addition of IL-10. Finally, this cooperative mechanism between IL-10 and TGF- β on the regulation of T cells sheds some light on the biological function of regulatory Tr1 cells that inhibit T cell function through IL-10 and TGF- β without secreting more TGF- β than Th1 cells.

Materials and Methods

Cells and culture

PBMC were prepared by centrifugation over Ficoll-Hypaque. CD4⁺ T cells were purified by negative selection. Negative purification was performed using a mixture of Abs directed against non-CD4⁺ T cells: CD8, CD14, CD16, CD19, CD20, CD56, and HLA-DR. Cells were incubated with saturating amounts of Abs for 20 min at 4°C. After washing, Dynabeads (Dyna, Oslo, Norway) were added at a 10:1 bead-target ratio and incubated for 1 h at 4°C. Beads and contaminated cells were removed by magnetic sorting. The remaining cells were resuspended with the same amount of beads, and a second incubation period of 1 h at 4°C was performed. After removing contaminating cells, CD4⁺ T cells were analyzed by FACScan (BD Biosciences, Mountain View, CA) and revealed to be >90–95% positive.

Institut National de la Santé et de la Recherche Médicale, Unité 343, Nice, France

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² Address correspondence and reprint requests to Dr. Hervé Groux, Institut National de la Santé et de la Recherche Médicale, Unité 343, Route de St. Antoine de Gines-tière, 06200 Nice, France. E-mail address: groux@unice.fr

³ Abbreviations used in this paper: TRI, TGF receptor type I; TRII, TGF receptor type II; TRIII, TGF receptor type III; Tr, T regulatory.

Human Th1 (JDV 305) or Tr1 (JDV 15) were previously described (17). T cells clones were stimulated with cross-linked CD3 and CD28 mAb under serum-free conditions using Yssel medium as previously described (14), and supernatants were collected after 24 h. To deplete Th1 supernatants of IL-2, supernatants were incubated three times for 2 h on wells previously coated with anti-IL-2 mAb (10 μ g/ml in PBS, 17H12). To remove Abs contained in the different supernatants, Abs were incubated for 1 h with anti-mouse IgG-coated magnetic beads (Dyna), and the beads were removed by magnetic sorting.

Naive mouse CD4⁺ T cells were prepared as previously described (17). Briefly, immunomagnetic depletion of CD8⁺, B220⁺, and Mac-1⁺ splenocytes was performed using goat anti-rat Ig-coated beads (Dyna). The depleted population was then stained with anti-CD4-FITC and anti-L-selectin-PE, and CD4⁺ L-selectin^{high} cells were sorted using a FACStar SE cell sorter (BD Biosciences). The purities achieved were >98%, and staining these cells did not alter their function (data not shown).

Th1 and Th2 populations were obtained from DO11-10 mice after *in vitro* differentiation as previously described (17). Briefly, 2-ml cultures were prepared in 24-well plates with 2.5×10^5 sorted naive T cells, 5×10^6 splenic APC (RBC lysed and irradiated with 3000 rad), and 0.6 μ M OVA₃₂₃₋₃₃₉ peptide. Th2 conditions were IL-4 at 0.1 ng/ml and anti-IL-12 at 10 μ g/ml. Th1 conditions were IL-12 at 10 ng/ml and anti-IL-4 at 10 μ g/ml. After 3 days, cultures were split 1:3, and after 6 days T cells were harvested, counted, and restimulated at 2.5×10^4 per well in 200 μ l in 96-well plates for phenotype analysis by cytokine secretion or to be used for activated T cells. For cytokine analysis, wells were coated with anti-CD3 at 10 μ g/ml, and anti-CD28 was used at 1 μ g/ml in solution. Supernatants were harvested at 48 h for assessment of IL-4, IL-5, or IFN- γ as previously described (17).

Reagents

Recombinant cytokines were obtained as follow: mouse IL-4, human IL-10, and mouse IL-10 were kindly provided by R. L. Coffman and A. O'Garra (DNAX, Palo Alto, CA), mouse IL-12 was obtained from BD PharMingen (San Diego, CA), and human TGF- β 1 was obtained from Genzyme (Cambridge, MA). Monoclonal anti-cytokine Abs used in culture were anti-IL-4 (clone 11B11), anti-IL-12 (clone C17.8.20, a kind gift of A. O'Garra), anti-IL-10R (a kind gift from K. Moore, DNAX), and anti-TGF- β (Genzyme). Nonconjugated or PE- or FITC-conjugated human or mouse CD28, CD3, CD4, CD8, CD14, CD16, CD19, CD20, CD56, MHC class II, and control mAbs of the appropriate isotype were purchased from BD PharMingen. mAb used in IL-4, IL-5, and IFN- γ ELISA were as previously described (17).

Synthetic peptide encoding chicken OVA₃₂₃₋₃₃₉ was kindly provided by H. Gras Masse (Institut de Biologie de Lille, Lille, France). Tissue culture medium used was Yssel medium with 2-ME (0.05 mM; Sigma, St. Louis, MO) supplemented or not with 10% FCS (heat-inactivated for 1 h at 56°C; Boehringer Mannheim, Mannheim, Germany).

Proliferation and activation assays

In all proliferation and activation assays, cells were cultured in Yssel medium (18) supplemented or not with 10% FCS. For cross-linked anti-CD3 mAb activation, 500 ng/ml anti-CD3 mAb diluted in 0.1 M Tris buffer, pH 9.5, was incubated for 1 wk at 4°C in flat-bottom 96-well plates. After washing the plates three times, CD4⁺ T cells were added at 5×10^4 cells/well with or without 10 μ g/ml anti-CD28 mAb.

cDNA synthesis and RT-PCR analysis

Total RNA was isolated and purified using RNeasy (Quantum Biotechnologies, Durham, NC) according to the manufacturer's instructions and quantitated by OD readings. Reverse transcription was performed as described previously (19). Briefly, 2 μ g of total RNA was resuspended in 13 μ l of H₂O, and 1 μ l of oligo(dT) at a concentration of 1 mg/ml was added. The samples were heated at 70°C for 10 min and cooled at room temperature, after which 11 μ l of the enzyme mixture (5 μ l buffer; 5 $\times$ superscript buffer (Life Technologies, Grand Island, NY), 4 μ l of 10 mM dNTP, 1 μ l of 0.1 M DTT, 0.1 μ l of 40 U/ μ l RNasin (Promega, Madison, WI), and 1 μ l of Superscript II (Life Technologies)) was added. The samples were spun for 2 min at 1000 rpm in a microfuge, incubated at 42°C for 1 h, and heat-denatured at 95°C for 3 min; after rapid cooling on ice, 200 μ l of H₂O was added to each sample, which was then frozen until use for RT-PCR amplification. RT-PCR were performed using the following primers: G3PDH 5', ACC ACA GTC CAT GCC ATC AC-5'; G3PDH 3', TCC ACC ACC CTG TTG CTG TA (452-bp fragment); TR1 5', TGA CCC CAA GCT CCC CTA CCA TGA; TR1 3', TGA TGT CAG AGC GGT CAT CTT CCA (376 bp); TR2 5', TAT CAC CAA CAG CAT GTG TAT AGC TG; and TR2 3', AGC CAG AAC CTG ACG TTG TCA TAT CAT

(376 bp). PCR cycles were 30 s at 94°C, 30 s at 60°C, and 30 s at 72°C, with 35 cycles for glucose 3 phosphodehydrogenase TGF- β RI, and TGF- β RII. PCR products were analyzed on agarose gels stained with ethidium bromide, and the picture obtained was scanned and analyzed on a computer using the Kodak 1D software (Kodak, Rochester, NY).

Results

TGF- β 1 has inhibitory effects on resting but not activated T cells

We analyzed the effect of TGF- β 1 on the proliferative response of resting vs activated human purified T cells. As shown in Fig. 1A, addition of TGF- β 1 to resting T cells led to a decrease in cell proliferation measured at 72 h after activation of culture. However, addition of TGF- β 1 (even at high concentrations; data not shown) did not lead to any significant inhibition of the proliferative response of activated T cells.

To analyze whether TGF- β 1 has similar inhibitory effects on the proliferation and differentiation of T cells undergoing Th1 or Th2

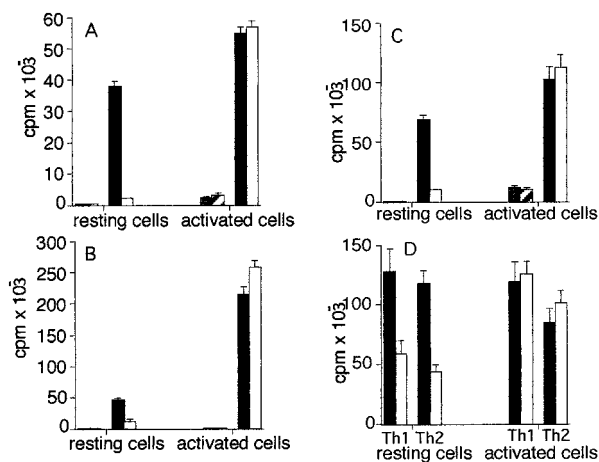


FIGURE 1. TGF- β suppressed proliferation of resting but not activated T cells. **A**, Resting human-purified CD4⁺ T cells were stimulated with cross-linked CD3 mAb (500 ng/ml) and soluble CD28 mAb (10 μ g/ml) in the presence or absence of TGF- β 1 (1 ng/ml). For activated cells, resting human-purified CD4⁺ T cells were stimulated with cross-linked CD3 plus CD28 mAb for 3 days. Then, after Ficoll gradient purification, cells were rested in fresh medium containing IL-2 (10 U/ml) for 48 h and restimulated with CD3 plus CD28 in the presence or absence of TGF- β . Proliferation was measured by [³H]thymidine incorporation during the last 16 h of incubation. Results are expressed as the mean $\pm$ SD from three independent experiments. **B–C**, Naive OVA TCR-transgenic CD4⁺ T cells were stimulated under Th1 (**B**) or Th2 (**C**) priming conditions with OVA peptide (OVAp; 0.3 μ M) in the presence of irradiated splenocytes and the corresponding cytokines and anti-cytokines in the presence or absence of TGF- β 1. For activated cells, naive CD4⁺ OVA TCR-transgenic T cells were primed in the presence of OVAp (0.3 μ M) and irradiated APC for 6 days under Th1 or Th2 priming induction; after 6 days, cells were collected and restimulated with CD3 plus CD28 mAb in the presence of TGF- β 1 (1 ng/ml), and proliferation was measured by [³H]thymidine incorporation during the last 16 h of incubation. Results are expressed as the mean $\pm$ SD from four experiments each performed in triplicate. $\square$, Unstimulated cells; $\blacksquare$, unstimulated cells plus TGF- β 1; $\bullet$, CD3 plus CD28 mAb or OVAp-stimulated cells; $\square$, CD3 plus CD28 mAb or OVAp-stimulated cells in the presence of TGF- β 1. **D**, One Th1 (A-7-3) and one Th2 (D4-6) T cell clone obtained from OVA TCR-transgenic DO11-10 mice as described previously (15) were stimulated with OVAp (0.5 μ M) and irradiated splenocytes in the presence ($\square$) or absence ($\bullet$) of TGF- β 1 (1 ng/ml). Resting cells were obtained by collecting T cells clones 2 wk after their last stimulation with OVAp and splenic APCs, washing them, and resting them in complete medium in the absence of cytokines for 12 h. Activated T cells have been stimulated with OVAp and splenic APCs and collected 5 days after activation.

development, naive TCR-transgenic CD4⁺ DO11-10 T cells were cultured with splenic APC and OVA peptide in Th1 (IL-12, anti-IL-4) or Th2 (IL-4 and anti-IL-12) conditions with or without the addition of TGF- β 1. As shown in Fig. 1, B and C, TGF- β inhibited cell proliferation of cells stimulated under Th1 or Th2 conditions. This inhibition in the proliferative response of Th1 or Th2 cell populations was also correlated with inhibition of cytokine secretion. For Th2 differentiation, cytokine analysis was performed on IL-5 secretion as IL-4 is added to primary cultures to achieve Th2 differentiation. In another set of experiments, CD4⁺ T cells cultured under Th1 or Th2 conditions were collected after 4 days and restimulated with CD3 plus CD28 mAb in the presence or absence of TGF- β 1. In contrast to its effect on resting T cells, TGF- β had no inhibitory effect on proliferative response (Fig. 1, B and C) or cytokine secretion of activated Th1 or Th2 T cells.

We also tested the inhibitory effect of TGF- β on OVA-specific mouse Th1 and Th2 T cell clones. As shown in Fig. 1D, similarly to T cell populations, TGF- β inhibits the proliferative response of "resting" T cell clones but has no effects on activated T cell clones irrespective of their Th1 or Th2 differentiation profile.

TRII is down-regulated after T cell stimulation

To analyze whether TGF- β 1 function could be regulated by the expression of its receptor as previously reported in different systems, we analyzed by RT-PCR the level of expression of TGF-

β RII transcripts on resting or day 1- and day 5-activated human-purified CD4⁺ T cells. Analysis of TGF- β RII transcripts revealed that TGF- β RII was constitutively expressed on resting T cells but was down-regulated 24 h after activation of T cells with CD3 plus CD28 mAb (Fig. 2A). In contrast to the expression of TGF- β RII transcripts, the expression of the TGF- β RI was constitutive and not modified by T cell activation (Fig. 2A). This result suggests that the absence of TGF- β inhibitory effects on activated T cells is due to the down-regulation of TGF- β RII transcripts induced by T cell activation.

IL-10 enhances TGF- β RII expression and restored TGF- β response on activated T cells

Preliminary experiments had shown that activated T cell proliferation was inhibited by supernatants of Tr1 secreting IL-10 through a mechanism involving TGF- β as proliferative response and could be partially restored by addition of blocking anti-TGF- β Abs. We thus analyzed the effect of IL-10 on TGF- β function and TGF- β RII expression on resting and activated human CD4⁺ T cells. Proliferative response of resting CD4⁺-purified T cells stimulated with cross-linked CD3 plus CD28 mAb was inhibited by addition of IL-10 (50%) or TGF- β (40%), and addition of both cytokines further enhanced these inhibitions (Fig. 2B). On activated human CD4⁺ T cells stimulated by cross-linked CD3 plus CD28 mAb, addition of IL-10 alone inhibited proliferative response by 30%,

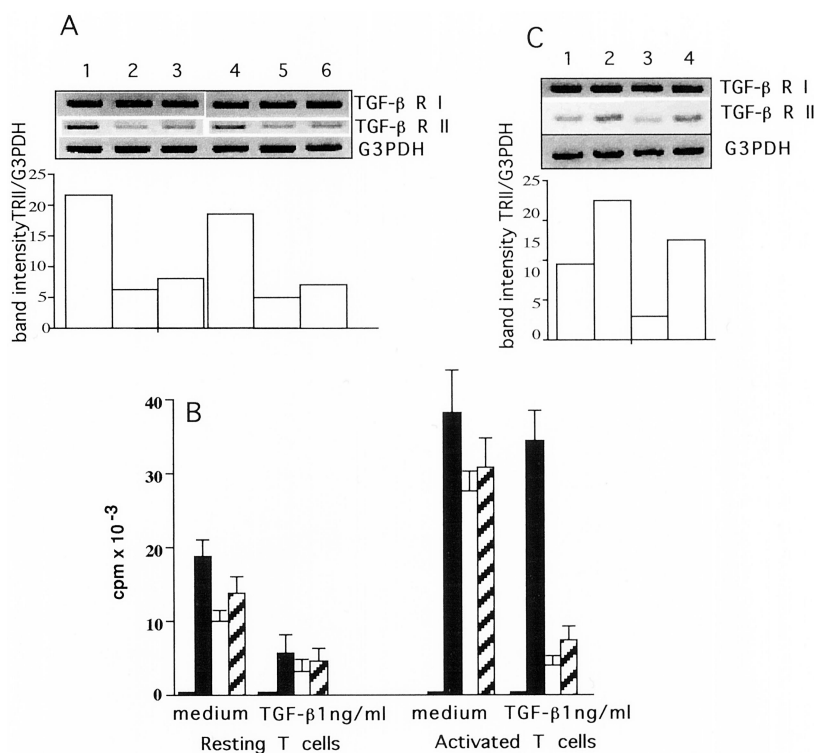


FIGURE 2. TGF- β RII is down-regulated after stimulation of T cells and IL-10 enhances TGF- β RII expression and restores TGF- β function on activated T cells. **A**, cDNA were prepared from human resting purified CD4⁺ T cells isolated from two different donors (lanes 1 and 4, respectively) or from the same cells stimulated with CD3 plus CD28 mAb for 24 h (lanes 2 and 5, respectively) or for 3 days and then expanded with IL-2 for 48 h (lanes 3 and 6, respectively). cDNAs were adjusted to yield comparable amplification by RT-PCR with primers specific for G3PDH and then reanalyzed by RT-PCR with primers specific for G3PDH, TGF- β RII, and TGF- β RI as indicated. PCR products were loaded on an agarose gel and stained with ethidium bromide, and band intensity was calculated using Kodak 1D software. Histograms represent the ratios in arbitrary units between TGF- β RII bands and G3PDH. Results represent one representative experiment of three. **B**, Resting or activated human-purified CD4⁺ T cells were stimulated with anti-CD3 and anti-CD28 mAb (■) in the presence of IL-10 (□, 100 U/ml; ▨, 1 U/ml) and TGF- β 1 (1 ng/ml) as indicated. ▢ (barely seen), Nonstimulated cells. Proliferation was measured in the last 16 h of 3-day cultures. Results represent the mean $\pm$ SD of triplicate culture for one experiment of three. **C**, Resting human-purified CD4⁺ T cells were cultured for 24 h in medium alone (lane 1) or in the presence of IL-10 (100 U/ml) (lane 2) or were activated with CD3 plus CD28 mAb in the absence (lane 3) or presence (lane 4) of IL-10 (100 U/ml). After normalization of the cDNA with the G3PDH transcript, TGF- β RII and G3PDH transcripts were analyzed by RT-PCR, and band intensity was quantified with Kodak 1D software.

whereas addition of TGF- β 1 had no significant effect (Fig. 2B). However, addition of even low doses of IL-10 on activated T cells revealed TGF- β inhibitory effects as addition of both cytokines inhibited cell proliferation by >80% (Fig. 2B). This inhibition was mediated by the binding of TGF- β to its receptor as it was blocked by Abs directed against TGF- β 1 (data not shown). These data obtained on the cooperative effect of IL-10 on TGF- β to inhibit cell proliferation shed some lights on the apparent paradox of the results obtained with Tr1 cells that do not produce more TGF- β than Th1 clones but inhibit cell proliferation through TGF- β function (15). To further analyze this cooperative effect between IL-10 and TGF- β , we used serum-free supernatants of Tr1 T cell clones and Th1 T cell clones depleted of IL-2. As shown in Fig. 3, Tr1 supernatants were equally potent in inhibiting naive and activated T cells proliferation performed in serum-free conditions, and this inhibitory effect was reverted by anti-IL-10 and anti-TGF- β mAb. In contrast to Tr1 cell supernatants, IL-2-depleted Th1 supernatants only prevented the proliferative response of resting T cells by a TGF- β -dependent mechanism. However, when IL-10 was added the proliferative response of activated T cells was inhibited by a mechanism involving both IL-10 and TGF- β as it was inhibited by anti-IL-10 and/or anti-TGF- β mAbs (Fig. 3).

IL-10 induces the expression of TGF- β RII

We then analyzed whether IL-10 cooperative effect on TGF- β function was mediated by up-regulation of the expression of the TGF- β RII transcripts. Resting and activated human CD4⁺ T cells

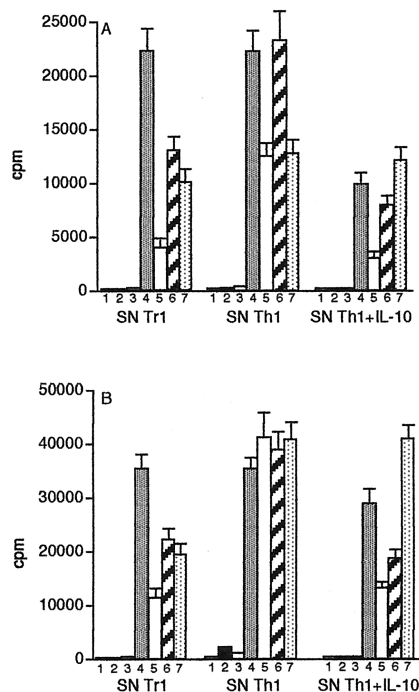


FIGURE 3. IL-10 addition can reveal TGF- β function on activated T cells in Th1 cell supernatants. Serum-free Tr1 T cell clone supernatants (JDV 15) and IL-2-depleted Th1 T cell clone (JDV 305) supernatants were added (10%) to resting (A) or activated human-purified CD4⁺ T cells (B). T cells were cultured in serum-free conditions in medium alone (lane 1) or with 10% T cell clone supernatant in the absence (lane 2) or presence (lane 3) of anti-TGF- β mAb. T cells were also stimulated with CD3 plus CD28 mAb alone (lane 4) or with 10% T cell clone supernatant in the absence (lane 5) or presence (lane 6) of anti-TGF- β mAb or (lane 7) anti-IL-10R mAb. As indicated, 50 U/ml IL-10 was added with Th1 supernatants. Proliferation was measured in the last 16 h of 3-day culture. Result represent mean $\pm$ SD of triplicate cultures for one experiment of three.

were stimulated for 24 h with CD3 plus CD28 mAb in the presence or absence of IL-10, and the level of TGF- β RII transcripts was analyzed by RT-PCR. Fig. 2C shows that addition of IL-10 enhances the expression of TGF- β RII on both activated and resting T cells, suggesting that IL-10-regulated TGF- β response through the modulation of the expression of TGF- β RII.

Discussion

The results presented in this paper demonstrate that TGF- β response is controlled in T cells by a modulation in the expression the TGF- β RII. Activation of resting T cells expressing high levels of TGF- β RII is inhibited by TGF- β , whereas no effect of TGF- β is observed on activated T cells in which the TGF- β RII expression is down-regulated. These data provide a molecular basis for the previously described TGF- β unresponsiveness of memory Th2 cells (7) and help explain several discrepancies that become apparent when comparing resting and activated/memory T cells. Moreover, using well-defined resting and activated T cell clones we showed that although TGF- β inhibits the proliferative response of resting T cell clones of both Th1 and Th2 profiles, it has no inhibitory effect on recently activated T cell clones. We have also demonstrated that addition of IL-10 enhances the expression of TGF- β R expression on resting and activated T cells and restores the inhibitory effect of TGF- β on activated T cells.

Differential expression of cytokine receptors accounts for important regulatory mechanism in T cells. It was reported by Pernis et al. that murine Th1 clones, in contrast to Th2 clones, do not express transcripts for the β -chain of IFN- γ R (20), which is primarily involved in signaling and not in binding of IFN- γ (21, 22). These results provided a molecular basis for the long-standing observation that IFN- γ inhibits the proliferation of murine Th2, but not of Th1 cells (23). Similarly, polarized Th2 cells, in contrast to Th1 cells, no longer respond to the IFN- γ -inducing effects of IL-12. This commitment to the Th2 lineage is due to the extinction of signaling through IL-12R (24, 25) and is the result of loss of the β_2 -chain of IL-12R on the surface of mouse (24, 25) as well as human (26) T cells. Besides, regulation of the TGF- β response by modulation of TGF- β R has already been described for different cell types. For example, down-regulation of TGF- β R is induced on human monocytes by treatment with IFN- γ or LPS (27). Moreover, alterations in TGF- β RII have been implicated in the pathogenesis of a variety of human cancers and animal tumor models (12, 13).

There is growing evidence for an essential role of TGF- β in immune regulation of both normal and autoimmune states (1). In the absence of TGF- β 1, mice develop a massive multifocal inflammatory disease, and further analysis has shown that the disease consists of an initial inflammatory response followed by an autoimmune manifestation (28) mainly mediated by activated CD4⁺ T cells (29). Moreover, it has been shown recently that addition of TGF- β to Th1 or Th2 cultures abrogates T cell differentiation into either Th subsets (6, 7). However, TGF- β is one of the most widely distributed cytokines that acts on virtually all cell types. All three isoforms of TGF- β in mammals share a high level of homology and use the same receptor complex for signaling to exert a seemingly redundant set of functions in vivo (30, 31). Thus one of the major difficulties in defining a specific role for TGF- β in immune regulation comes from the widespread expression of TGF- β and its receptors (32), which makes it difficult to envision the control of the regulation of TGF- β on T cells. The first level of regulation is the proteolytic conversion between the secreted latent form to the active form of TGF- β . This transformation is mediated by hematopoietic cells extracellularly. The serum contains high amounts of TGF- β bound to the serum proteins, whereas only minute amounts

of this protein are required to inhibit T cell proliferation; this is presumably the reason why 1 ng/ml TGF- β is required in our culture conditions to observe potent effects because most of the cytokines became bound by serum proteins. However, besides this general level of regulation, one has to keep in mind that T cell activation and differentiation during the establishment of an immune response is a sequential event that requires successive encounters between the T cells and APCs. The first step is the activation of naive T cells by mature dendritic cells in the lymph nodes. After activation with the appropriate Ag, the effector T cells leave the lymph nodes and migrate to the peripheral organs. There, upon recognition of the Ag and based on the cytokine milieu, T cells will further differentiate into effector or regulatory T cells. Our results suggest a model where naive T cells that constitutively express TGF- β R can be regulated by TGF- β to prevent nonspecific stimulation mediated by nonprofessional APCs. However, after activation in the draining lymph nodes or spleen by specific dendritic cells, activated T cells become refractory to TGF- β inhibition, by the modulation of their TGF- β RII, and thus can safely journey from the lymph nodes to the target organs without being influenced by the TGF- β widely expressed in different tissues. There, depending on the presence or absence of IL-10, which up-regulates TGF- β RII expression, the primed T cell can either further differentiate into effector Th1 or Th2 cells or be negatively regulated by IL-10 and TGF- β (Fig. 4).

This cooperative function of IL-10 and TGF- β is also evident when analyzing the function of Tr cells in vitro and in vivo. Indeed, in vitro, the regulatory function of Tr1 T cell clones is only partially inhibited by the addition of either anti-TGF- β or anti-IL-10 blocking Abs. However, when used in vivo, anti-IL-10 blocking Abs were shown to completely prevent the regulatory function of Tr1 T cells (33). This discrepancy can be explained by the absence of up-regulation of TGF- β RII on activated T cells in the absence of IL-10, preventing the regulation of T cell growth induced by TGF- β . In this case, the addition of anti-IL-10 Abs blocks both IL-10- and TGF- β -mediated regulatory mechanisms.

The results of this study identify an important regulatory mechanism for the control of the TGF- β effect on activated T cells

where, after specific activation, T cells use receptor modification to prevent the activation of Smad proteins and achieve a TGF- β -resistant state. The finding that IL-10 plays a nonredundant role in the control of TGF- β R expression on T cells will open up new avenues for the development of therapeutic strategies that seek to control inflammatory responses and autoimmunity and provides enlightenment on the cooperative role of these two cytokines for the control of T cell response.

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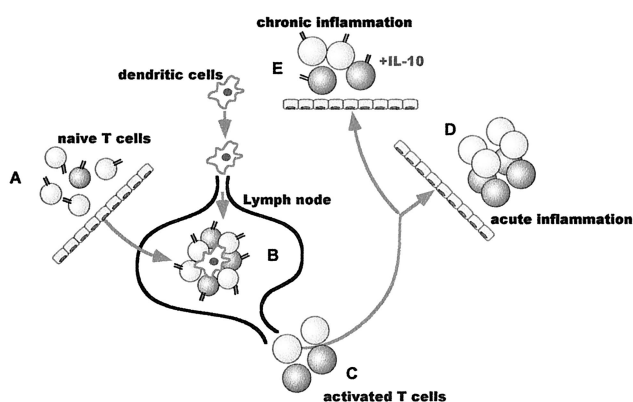


FIGURE 4. Model of the regulation of TGF- β response on T cells during activation. According to our model, naive resting T cells express TGF- β RII (A) and thus can be negatively regulated by TGF- β to prevent inappropriate activation by Ags presented by nonprofessional APCs. Upon stimulation by dendritic cells in lymph nodes (B), T cells down-regulate their expression of TGF- β R and can migrate to peripheral tissues without being negatively regulated by TGF- β widely expressed in different tissues (C). In acute inflammatory sites (D), in the absence of IL-10, upon restimulation with their specific Ag, activated T cells can further expand and differentiate into effector T cells. In contrast, in chronic inflammatory sites (E) the presence of IL-10 induces the expression of TGF- β R on activated T cells, which can then be negatively regulated by both IL-10 and TGF- β .

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