Cutting Edge: TGF-β Inhibits Th Type 2 Development Through Inhibition of GATA-3 Expression

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TGF-β is an important immunomodulatory cytokine that can inhibit differentiation of effector T cells. In this report, we address the molecular mechanisms through which TGF-β inhibits differentiation of CD4⁺ cells into Th type 2 cells. We demonstrate that TGF-β inhibits GATA-3 expression in developing Th cells. We also show that inhibition of GATA-3 expression by TGF-β is a major mechanism of inhibition of Th2 differentiation by TGF-β as ectopic expression of GATA-3 in developing T cells overcomes the ability of TGF-β to inhibit Th2 differentiation. TGF-β likely inhibits GATA-3 expression at the transcriptional level and does so without interfering with IL-4 signaling. The Journal of Immunology, 2000, 165: 4773–4777.

Regulation of the immune response toward the cellular or humoral effector pathways is an important process responsible for the defense of the organism against different types of pathogens (1). These polarized immune responses are coordinated by two types of differentiated Th cells, each secreting a signature set of cytokines required for the most effective execution of a particular response. The differentiation of these two types of Th cells is regulated by two types of cytokines that have mutually antagonistic properties on the differentiation of the opposite Th subset, thereby ensuring the predominance of a particular response.

The molecular mechanisms by which these cytokines regulate T cell differentiation have been extensively investigated. Specifically, IL-4, which is preferentially secreted by Th2 cells and is also required for their differentiation, has been shown to up-regulate expression of the transcription factor GATA-3 (2). GATA-3, which plays a crucial role in Th2 differentiation and cytokine secretion (3, 4), in turn inhibits the expression of IL-12Rβ2 (2), which blocks the development of Th1 cells. In contrast, IL-12 inhibits GATA-3 expression in developing Th cells (2), thereby blocking differentiation of Th2 cells.

Another cytokine that plays a critical role in Th differentiation is TGF-β. Addition of TGF-β to Th1 or Th2 cultures abrogates T cell differentiation into either Th subset (5, 6), although it does not prevent the T cells from expressing activation markers (e.g., CD44highCD45RBlow). Although TGF-β has been shown to inhibit T cell proliferation (7), it inhibits Th differentiation even under conditions in which T cells proliferate normally (5). Although CD4⁺ T cells differentiated in the presence of TGF-β were not capable of secreting any of the Th1 or Th2 effector cytokines, they retained their pluripotency, as demonstrated by the ability of these cells to differentiate into Th1 or Th2 type cells upon secondary culture in the absence of TGF-β. The important role of TGF-β in CD4⁺ T cell differentiation in vivo has also been supported recently by our studies with mice that express a dominant negative TGF-β receptor under a T cell-specific promoter (8). T cells from these mice are insensitive to TGF-β signaling, and a large proportion of these cells “spontaneously” differentiates in vivo into both Th1 and Th2 subsets. The molecular mechanisms by which TGF-β prevents the differentiation of T cells presently are not known. The studies reported here were undertaken to determine the mechanism by which TGF-β controls Th2 differentiation.

Materials and Methods

Mice

Moth cytochrome c (MCC)²-specific (AND) TCR transgenic mice were obtained from Dr. K. Bottomly, and wild-type B10.BR mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and were used at 6–8 wk of age.

T cell activation and differentiation

CD4⁺ T cells from either AND TCR transgenic mice or wild-type B10.BR mice were prepared as described (8). rmIL-2 (3.5 ng/ml; Genetics Institute, Cambridge, MA) and anti-IL-4 (11B11, 10 μg/ml) were added for Th1 development; recombinant mIL-4 (1000 U/ml; Endogen, Woburn, MA) and anti-IFN-γ (XMG1.2, 10 μg/ml) were added for Th2 development. Recombinant huIL-2 (40 U/ml; Biogen, Cambridge, MA) was added to all stimulation conditions, and where indicated, recombinant huTGF-β (3 ng/ml; R&D Systems, Minneapolis, MN) was added to the culture media.

Retroviral constructs and retroviral transduction

GFP-RV and GATA-3-RV retroviral vectors were provided by Dr. K. Murphy (Washington University, St. Louis, MO) (2). Phoenix-Eco packaging cell line (gift of Dr. G. Nolan, Stanford University, Stanford, CA) was transfected according to the protocol of Dr. Nolan. Primary T cells were

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2 Abbreviations used in this paper: MCC, moth cytochrome c; AND MCC–specific; CTSE, 5,6-carboxyfluorescein diacetate succinimidyl ester; GFP, green fluorescence protein.

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activated with Ag as described, infected after 24 h using 1 ml of viral supernatant and 6 μg/ml of Lipofectamine (Life Technologies, Gaithersburg, MD), and incubated at 37°C for 24 h before being supplied with fresh media and expanded until day 5 after primary activation.

Western blot analysis
Total T cell lysates were prepared as described (8), resolved by 10% SDS-PAGE, transferred to a polyvinylidene difluoride membrane (Millipore, Bedford, MA), and probed with either anti-IL-4Rα, anti-IL-2Rγ, anti-GATA-3 Abs (all obtained from Santa Cruz Biotechnology, Santa Cruz, CA), or anti-phospho(Tyr641)-STAT-6 Ab (NEB, Beverly, MA), and developed using an enhanced chemiluminescence system.

FACS analyses
Staining was performed as described (8). Ten thousand events were collected, and after gating on GFP+ or GFP- cells, intracellular cytokine staining was analyzed. Gates for cytokine staining were set using isotype-matched control Ab staining. Gates for GFP (FL1)-positive cells were determined using nontransduced controls.

ELISA
Cytokine levels in tissue culture supernatants were assayed by ELISA using Ab pairs for IL-5 and IL-4 (PharMingen, San Diego, CA) according to the manufacturer’s recommendations.

RNA preparation and semiquantitative RT-PCR
RNA and cDNA were prepared as described. Subsequently, each sample was subjected to PCR with sense and antisense primers for β-actin and GATA-3. Primers were of the following sequences: for β-actin, 5'-GTGGCCGCTTACGGCACCA (sense) and 5'-CGGTTGGCCTAGGGTTCAAGGGGGG (antisense); and for GATA-3, 5'-TCTCGAGGAGGAAACGCTAATGG (sense) and 5'-GAACGTCTACGCACACTTGGAGAC (antisense). To specifically amplify mRNA, primers for both β-actin and GATA-3 were designed to span an intron. PCRs were performed at different numbers of cell cycles to ensure that comparison of PCR products for various samples is performed at the linear part of an amplification curve.

Results and Discussion
Several groups have previously shown that the addition of TGF-β to CD4+ T cells inhibits their differentiation under Th2 culture conditions (5, 6). Importantly, this inhibition occurs even in the presence of IL-2 and is independent of the inhibitory effects of TGF-β on T cell proliferation, thereby ruling out a role of the antiproliferative effect of TGF-β on the inhibition of Th2 differentiation (5). To confirm this observation and to determine conditions in which TGF-β has no effect on T cell proliferation, we stimulated AND TCR transgenic CD4+ cells with Ag in the presence or absence of rhTGF-β1 and measured [3H]thymidine incorporation. As seen in Fig. 1A, CD4+ T cell proliferation was inhibited by TGF-β at various Ag doses. Addition of 40 U/ml of exogenous IL-2 completely eliminated the inhibitory effect of TGF-β on T cell proliferation at high doses of Ag. Therefore, we conducted all
subsequent differentiation experiments under those conditions: 40 U/ml of IL-2 and 1 μg/ml of MCC peptide.

Cell division has been suggested to play an important role in CD4+ T cell differentiation, whereby the more divisions the cell undergoes, the higher is the probability of the cell to differentiate into a Th2 cell (9). Therefore, we sought to determine the effect of TGF-β on the number of T cell divisions under these experimental conditions. By stimulating 5,6-carboxyfluorescein diacetate succinimidyl ester (CFSE)-labeled CD4+ T cells we determined that TGF-β had no effect on the number of divisions cells undergone over the 3-day stimulation period, in the presence of exogenous IL-2 (Fig. 1B). Although TGF-β did not affect CD4 T cell proliferation as measured by either [3H]thymidine incorporation or CFSE staining, it did completely inhibit Th2 differentiation (Fig. 1, C and D), in agreement with previously reported data (5, 6). Importantly, as seen from the results of intracellular cytokine staining (Fig. 1D), TGF-β inhibits the number of cells differentiated into Th2 phenotype rather than the amount of Th2 cytokines secreted by each differentiated T cell.

Because IL-4 signaling is crucial for Th2 differentiation, we examined how TGF-β affects elements of the IL-4 signaling pathway. As seen in Fig. 2A, stimulation of naive T cells with anti-CD3/CD28 mAbs led to the up-regulation in expression of both chains of the IL-4 receptor (IL-4Rα and the common γ-chain). This up-regulation was not affected by addition of TGF-β to the culture media. Therefore, inhibition of Th2 differentiation by TGF-β does not occur through the inhibition of IL-4 receptor expression. We considered it possible that TGF-β may interfere with signaling through the IL-4 receptor. To test this hypothesis, we examined whether TGF-β inhibits phosphorylation of STAT-6 in response to IL-4. Preincubation of naïve T cells with TGF-β did not affect the ability of IL-4 to induce phosphorylation of STAT-6 (Fig. 2B). Therefore, the inhibition of Th2 differentiation by TGF-β is downstream of IL-4 receptor-proximal events. Because it has been previously demonstrated that STAT-6 signaling is necessary for the induction of GATA-3, an essential factor for Th2 differentiation (3), we examined the effect of TGF-β on GATA-3 expression. In agreement with previously reported data (2), stimulation of CD4+ T cells with anti-CD3 and anti-CD28 led to a significant increase in the level of GATA-3 expression in 20 h, and addition of IL-4 to the culture resulted in even higher levels of GATA-3 expression (Fig. 2C). Strikingly, addition of TGF-β dramatically inhibited GATA-3 expression induced under both of these culture conditions (Fig. 2D). Results of RT-PCR analysis of the mRNA prepared from CD4+ T cells stimulated with or without TGF-β revealed that TGF-β inhibits the induction of GATA-3 expression at the mRNA level (Fig. 2D). This inhibition of GATA-3 was specific and downstream of STAT-6, because
TGF-β does not inhibit expression of IL-4Rα and IL-2Rγ, and the inhibition probably does not involve IL-4 receptor proximal signaling events because TGF-β does not inhibit STAT-6 phosphorylation. Furthermore, because under the conditions tested, TGF-β does not affect T cell proliferation (Fig. 1), it is unlikely that TGF-β inhibits GATA-3 expression by interfering with TCR/CD3 signaling.

Because GATA-3 is a critical element controlling Th2 differentiation, we thought it possible that TGF-β controls Th2 differentiation through its effect on GATA-3 expression. To test this hypothesis, we ectopically expressed GATA-3, using a retroviral vector, in differentiating CD4⁺ T cells treated with TGF-β. This retroviral vector (GATA-3-RV) enables bicistronic expression of green fluorescence protein (GFP) and GATA-3 by the use of an IRES element (2). Using this strategy, it is possible to separate GATA-3-transduced cells from nontransduced cells based on GFP fluorescence. As a control we used an “empty” vector containing only the GFP gene (GFP-RV).

To examine the effects of TGF-β on the cytokine-producing ability of cells ectopically expressing GATA-3, we sorted GFP⁺ and GFP⁻ cells from different groups, restimulated them with Ag, and measured the cytokines that were secreted after 20 h of re-stimulation. As seen in Fig. 3, the presence of TGF-β completely inhibited the ability of T cells differentiated under Th2 conditions to produce IL-4 and IL-5. In contrast, T cells ectopically expressing GATA-3 were markedly resistant to the inhibitory effects of TGF-β (Fig. 3, filled columns). CD4⁺ cells transduced with GATA-3 and differentiated under Th1 conditions could also produce significant levels of IL-4 and IL-5, and this production was also resistant to inhibition by TGF-β (Fig. 3). This TGF-β-mediated inhibition of Th2 cytokine production is likely due to a block in differentiation of T cells into Th2 effector cells by TGF-β rather than a block in cytokine production by the differentiated cells because TGF-β was removed before restimulation of the differentiated T cells. Furthermore, we found that TGF-β had no effect on IL-4 and IL-5 production by an established Th2 clone, D10 (data not shown).

The ELISA results provide a quantitative measure of total cytokine producing ability of Th2-differentiated cells. To determine the number of cells that have differentiated into Th2 cells, intracellular cytokine staining was performed (Fig. 4). As previously reported (2), ectopic expression of GATA-3 in CD4⁺ T cells cultured under Th1 culture conditions leads to the appearance of IL-4 and IL-5-producing cells, and ectopic expression of GATA-3 in Th2-differentiating cells leads to the augmentation of Th2 differentiation. As expected, TGF-β dramatically inhibited Th2 differentiation of both GFP⁻ and GFP⁺ cells transduced with the control GFP-RV, essentially eliminating the development of Th2 cells. Likewise, T cells that did not express exogenous GATA-3 (i.e., uninfected GFP-negative cells of the GATA-3-RV transduced group) were also sensitive to TGF-β-mediated inhibition of Th2 differentiation. In sharp contrast, T cells that ectopically expressed...
GATA-3 (i.e., GFP^+ cells of the GATA-3-RV-transduced group) displayed a marked resistance to TGF-β-mediated inhibition of Th2 differentiation. Specifically, in the absence of ectopic GATA-3, TGF-β completely inhibited the development of IL-4 and IL-5-producing T cells even under Th2 culture conditions, whereas in the presence of ectopic GATA-3, TGF-β was only able to reduce the number of IL-4- and IL-5-secreting cells by only 2- to 3-fold.

Altogether, these data demonstrate that TGF-β inhibits Th2 differentiation through the inhibition of GATA-3 expression. The inability of ectopic GATA-3 to fully restore the numbers of T cells differentiated into Th2 cells in the presence of TGF-β could be explained by insufficient GATA-3 present in these cells; specifically, TGF-β likely blocks the retroviral GATA-3-driven autoregulation of the endogenous GATA-3 gene reported previously (10). Alternatively, TGF-β may inhibit some other factor(s) necessary for efficient Th2 differentiation. Additional experiments will be needed to definitively distinguish between these possibilities. Nevertheless, the ability of exogenous GATA-3 to restore, at least partially, TGF-β-mediated specific inhibition of GATA-3 expression demonstrates the importance of this pathway in TGF-β-mediated inhibition of Th2 differentiation.

The ability of TGF-β to inhibit Th differentiation through inhibition of GATA-3 expression may provide a general mechanism to redirect or switch differentiation of many different cell types. Studies using a RAG2 knockout complementation system in conjunction with embryonic stem cells with a homozygous GATA-3-null mutation (GATA-3^−/−) revealed an essential role for GATA-3 in thymocyte differentiation, as development of GATA-3-null thymocytes was blocked at the very early double negative stage (11). Recently GATA-3 has been shown to be the key regulator of CD4^+ Th differentiation (2, 3). Timely induction and repression of GATA-3 expression seem to be critical coordinators of differentiation in different tissues. In this report we demonstrate that TGF-β is an important regulator of GATA-3 expression in differentiating mature CD4^+ T cells. Although we do not yet know whether TGF-β is responsible for the regulation of GATA-3 in cells other than CD4^+ T cells, it has been recently demonstrated that BMP (a TGF-β family member) is required for Xenopus embryonic patterning and achieves this, at least in part, through the inhibition of GATA-3 expression (12). Given the important role of TGF-β and its other family members in embryonic development (13–15), it is conceivable that regulation of GATA-3 by TGF-β superfamily members is an important process required at multiple stages during embryonic development and tissue differentiation.

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References


