Cutting Edge: Regulatory T Cells Induce CD4+CD25−Foxp3− T Cells or Are Self-Induced to Become Th17 Cells in the Absence of Exogenous TGF-β

LiLi Xu, Atsushi Kitani, Ivan Fuss and Warren Strober

J Immunol 2007; 178:6725-6729; doi: 10.4049/jimmunol.178.11.6725
http://www.jimmunol.org/content/178/11/6725

References
This article cites 12 articles, 8 of which you can access for free at:
http://www.jimmunol.org/content/178/11/6725.full#ref-list-1

Subscription
Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Recent studies have shown that TGF-β together with IL-6 induce the differentiation of IL-17-producing T cells (Th17) T cells. We therefore examined whether CD4+CD25-Foxp3+ regulatory T cells, i.e., cells previously shown to produce TGF-β, serve as Th17 inducers. We found that upon activation purified CD25+ T cells (or sorted GFP+ T cells obtained from Foxp3-GFP knockin mice) produce high amounts of soluble TGF-β and when cultured with CD4+CD25-Foxp3+ T cells in the presence of IL-6 induce the latter to differentiate into Th17 cells. Perhaps more importantly, upon activation, CD4+CD25-Foxp3+(GFP*) T cells themselves differentiate into Th17 cells in the presence of IL-6 (and in the absence of exogenous TGF-β). These results indicate that CD4+CD25-Foxp3+ regulatory T cells can function as inducers of Th17 cells and can differentiate into Th17 cells. They thus have important implications to our understanding of regulatory T cell function and their possible therapeutic use. The Journal of Immunology, 2007, 178: 6725–6729.

In recent studies, it has been shown that TGF-β and IL-6, acting in concert, induced the differentiation of naïve T cells into Th17 cells capable of IL-17 production (1, 2). Because CD4+CD25-Foxp3+ “natural” regulatory T cells (Treg) express cell surface or secrete TGF-β, this introduces the possibility that Tregs may be playing a role in such differentiation (3–5). Indeed, in previous studies it was shown that Tregs do serve as a source of TGF-β for Th17 development in vitro, but it was implied that this role is more usually subserved by APCs in vivo. Here we show that upon activation Tregs produced high amounts of TGF-β and with the addition of IL-6 induce CD4+CD25-Foxp3− T cells to differentiate into IL-17-producing cells (in the absence of other cells). Perhaps more importantly, we show that activated Tregs themselves differentiate into IL-17-producing cells in the presence of a source of IL-6. These results reveal that CD4+CD25-Foxp3+ T cells have dual effects on the course of an immune response.
Results and Discussion

CD4⁺CD25⁺Foxp3⁺ T cells induce CD4⁺CD25⁺Foxp3⁺ cells to become IL-17-producing cells in the absence of exogenous TGF-β

In initial studies, we asked if CD4⁺CD25⁺Foxp3⁺ T cells can induce Th17 differentiation in the absence of APCs and/or exogenous TGF-β. To address this question, we employed highly purified populations of Foxp3⁺ T cells by isolating GFP⁺ cells from the spleens of Foxp3-IRES-GFP knock-in mouse that express GFP only when the Foxp3 gene is transcriptionally active. As shown in Fig. 1A, coculture of fresh GFP⁺ (CD4⁺) T cells with GFP⁺ (CD4⁺) T cells obtained by flow cytometric cell sorting led to the appearance of a low number of IL-17-positive cells at a 1:1 cell (GFP⁺/GFP⁺) ratio (1.8%) and a somewhat increased number of these cells at a 2:1 ratio (5.76%). In addition, as shown in Fig. 1C, at the latter ratio a substantial amount of IL-17 could be detected in the culture supernatant.

In previous studies, we found that activation of CD4⁺CD25⁺Foxp3⁺ T cells was associated with increased membrane TGF-β expression and TGF-β secretion; we therefore determined the ability of pre-activated CD4⁺CD25⁺Foxp3⁺ (GFP⁺) T cells to induce Th17 differentiation. In these studies, we stimulated sorted GFP⁺ or GFP⁻ T cells with anti-CD3/anti-CD28 for 48 h and then rested the stimulated cells in IL-2 for 24 h to minimize activation-induced cell death; we then cocultured the activated GFP⁺ or GFP⁻ T cells with fresh GFP⁻ T cells for 4 days in the presence of IL-6. As shown in Fig. 1A, coculture of activated CD4⁺Foxp3⁺ T cells with fresh CD4⁺Foxp3⁻ T cells led to a cell population containing ~21% IL-17-producing cells, whereas coculture of activated CD4⁺Foxp3⁻ T cells with CD4⁺Foxp3⁻ T cells led to a cell population containing virtually no IL-17-producing cells. CD4⁺Foxp3⁻ T cells stimulated with rTGF-β (3 ng/ml) and IL-6, 12% of the cell population were IL-17-producing cells indicating that the Tregs were even more efficient than exogenous TGF-β in inducing IL-17-producing cells.
Finally, to rule out the possibility that the IL-17-producing cells came from the CD4+Foxp3+ T cell population rather than from the CD4+Foxp3+ population that lost Foxp3 expression after stimulation with IL-6 (1) we labeled purified CD4+CD25+ T cells with CFSE before coculture with CD4+CD25+ T cells activated as in the experiment above. As shown in Fig. 1B, we found that 26.8% of CFSE+ T cells were IL-17-producing cells after coculture of activated CD4+CD25+ T cells whereas only 2.41% of CFSE+ cells were IL-17-producing cells after coculture of activated CD4+CD25+ T cells.

The induction of IL-17-producing cells by activated CD4+CD25+Foxp3+ T cells is TGF-β dependent

To determine whether the differentiation of CD4+CD25+Foxp3+ T cells into IL-17-producing cells induced by activated CD4+CD25+ Foxp3+ T cells was TGF-β dependent, we next cultured cells in the presence or absence of a TGF-βRI inhibitor, i.e., an inhibitor of the kinase activity of TGF-βRI (ALK5) (4). Accordingly, we cultured activated CD4+CD25+ T cells with CD4+CD25+ T cells labeled with CFSE as described above but in this case in the presence or absence of ALK5 inhibitor (2.5 μM/ml). As shown in Fig. 2A, the addition of ALK5 inhibitor dramatically decreased the number of CFSE-positive IL-17-positive cells (from 26.93% to 3.04%) whereas it increased the number of IFN-γ-producing cells (from 10.1% to 24.8%). Thus, the differentiation of IL-17-producing cells induced by activated CD4+CD25+ T cells does in fact require TGF-β signaling.

To determine whether the activated CD4+CD25+Foxp3+ T cells inducing IL-17 T cell differentiation are themselves producing a sufficient amount of TGF-β to affect such differentiation, we then measured both the active and total TGF-β in the culture medium of the above described cocultures. As shown in Fig. 2B, substantial amounts of TGF-β (600 pg/ml) was detected in the culture supernatant obtained from cocultured activated CD4+CD25+Foxp3+ and CD4+CD25−Foxp3− T cells but not from the culture supernatant of cocultured activated CD4+CD25−Foxp3− and CD4+CD25+Foxp3+ T cells. This TGF-β is not coming from CD4+CD25−Foxp3− T cells after coculture with activated CD4+CD25+Foxp3+ T cells since activated CD4+CD25+Foxp3+ T cells produce a similar amount of TGF-β when cultured alone. These results provide strong evidence that the induction of IL-17-producing cells by activated CD4+CD25+Foxp3+ T cells was TGF-β dependent.

CD4+CD25+Foxp3+ T cells themselves differentiate into IL-17-producing cells in the presence of IL-6

In further studies, we determined if CD4+CD25+Foxp3+ T cells themselves differentiate into IL-17-producing cells. Accordingly, we stimulated sorted CD4+CD25+ T cells or CD4+CD25− T cells alone with anti-CD3/anti-CD28 in the presence of IL-6 and then, after 6 days determined IL-17 expression. As shown in Fig. 3A, we found that the cultured CD4+CD25+ T cell population contained 10% IL-17-producing cells whereas the cultured CD4+CD25− T cells contained only 1% IL-17-producing cells. To exclude the possibility that the IL-17-producing cells arose from Foxp3+ cells in the CD25+ cell population we next conducted studies of FACS-sorted CD4+GFP+ cells from Foxp3 knock-in mice.
since in this case we could be certain that the cells were in fact Foxp3 positive at the initiation of the study. As shown in Fig. 3B, we found that IL-17-producing T cells were detected as early as day 3 (3%), and were optimally expressed at day 4 or day 6 when 17% and 20% of the cells were IL-17-producing cells, respectively. Of interest, the percentages of both IL-17 single-positive and GFP/IL-17 double-positive cells increase during the culture period, suggesting that Foxp3-positive cells can coexpress IL-17 and that IL-17 single-positive cells pass through an Foxp3/IL-17 double-positive stage. Finally, we subjected sorted GFP+ and GFP− cells to polarization under Th1 conditions and, as shown in Fig. 3B, found that whereas GFP− cells could not be induced to become IFN-γ producing cells, GFP+ cells developed into IFN-γ-producing cells at a high rate. Thus, CD4+Foxp3+ cells can undergo self-induced Th1 differentiation, but resist Th1 differentiation.

We further verified Th17 differentiation by determination of IL-17 released into the culture medium by sorted GFP+ and GFP− cells. As shown in Fig. 3G, GFP+ cells did secrete substantial amounts of IL-17, albeit less than that secreted by GFP− cells stimulated with TGF-β and IL-6; this was probably due to the low proliferation and expansion rate of GFP+ cells compared with GFP− cells after stimulation. These converted IL-17-producing cells exhibited a stable phenotype after culture for 14 days or after a second round of stimulation in the absence of exogenous IL-6 (data not shown).

Differentiation of IL-17-producing cells from CD4+CD25+Foxp3+ T cells in the coculture of DCs in response to TLR ligation

To determine whether Tregs can differentiate into IL-17-producing T cells after the interaction with stimulated DCs rather than exogenous IL-6 we cocultured sorted CD4+GFP+ (Foxp3+) cells with bone marrow-derived DCs in the presence of LPS a TLR4 ligand. The proliferation and expansion of CD4+ GFP+ (Foxp3+) cells in such cultures was poor, probably due to the lack of IL-2 (6). Nevertheless, as shown in Fig. 4A, a small number of IL-17-producing cells were generated under these conditions.

In further studies along these lines, we determined the cellular source of TGF-β in these cultures by intracellular staining with anti-TGF-β. As shown in Fig. 4B, the GFP+ regulatory T cells contained a subpopulation of TGF-β-containing cells that was larger and more intensely stained than that in GFP− (CD4+) T cells. In addition, the DC population also contained a TGF-β-positive subpopulation when cocultured with GFP− T cells but this was greatly augmented when these cells were cocultured with GFP+ T cells. Thus, regulatory T cells augment the induction of IL-17-producing cells in two ways: they produce TGF-β themselves and they induce DCs to produce increased amounts of TGF-β.

The data reported above indicate that CD4+CD25+Foxp3+ regulatory T cells can either induce other cells to differentiate into Th17 cells or to induce themselves to undergo such differentiation. However, they provide little information about the quantitative importance of this differentiation pathway and it remains possible (or even probable) that other cells, such as activated APCs are the main source of TGF-β necessary for Th17 differentiation and that most Th17 cells do not arise from regulatory T cells. Recently, it has been shown that in a graft-vs-host disease model in which IL-17 is the predominant cytokine, the provision of regulatory T cells favors the development of an increased percentage of IL-17-producing cells given early (7). In addition, it has been shown that Foxp3+ cells are increased in human inflammatory lesions of the gastrointestinal tract including Crohn’s disease (8). These findings are compatible with the data reported here and suggest that Tregs do in fact induce the differentiation of Th17 cells under in vivo conditions. However, additional studies are necessary to prove this point.

Regardless of its quantitative significance, the fact that this differentiation pathway exists at all has certain implications. One implication is that the repertoire of the regulatory T cell may need to be expanded to include its conversion into IL-17-producing cells and the production of IL-17, particularly in settings where IL-17 mediates certain homeostatic functions rather than proinflammatory effect functions (9). A possible example of this is the recent finding that in the gastrointestinal tract, IL-17 has been shown to promote epithelial barrier function by stimulating tight junction protein and antimicrobial peptides expression and mucin secretion (10, 11). Another and perhaps more important implication is that treatment of autoimmune states by administration of regulatory T cells can, through conversion to IL-17-producing cells, lead to increased rather than decreased inflammation. This did not seem to be the case in a recent study in which it was found that provision of regulatory T cells ameliorated established cell transfer colitis (12). On the other hand, we found in preliminary studies that mice develop a more severe trinitrobenzene sulfonic acid-colitis
accompanied by increased IL-17 production when existing colitis is accompanied by administration of regulatory T cells (data not shown). Thus it is obvious that additional work will be necessary to clarify this issue.

Acknowledgments
We thank Carol Henry from Flow Cytometry Section, NIAID, National Institutes of Health for excellent technical supporting of FACS sorting. We thank Dr. Yasmine Belkaid (NIAID, National Institutes of Health) and Dr. Mohamed Oukka (Harvard Medical School) for providing Foxp3-IRES-GFP knock-in breeding mice.

Disclosures
The authors have no financial conflict of interest.

References