

# The role of the combination of IL-2 and TGF- $\beta$ or IL-10 in the generation and function of CD4<sup>+</sup> CD25<sup>+</sup> and CD8<sup>+</sup> regulatory T cell subsets

David A. Horwitz,<sup>1</sup> Song Guo Zheng, and J. Dixon Gray

*Division of Rheumatology and Immunology, Department of Medicine, Keck School of Medicine of the University of Southern California, Los Angeles*

**Abstract:** Recently, considerable attention has been focused on thymus-derived CD4<sup>+</sup> regulatory T cells that constitutively express CD25 and have a contact-dependent, cytokine-independent mechanism in vitro. However, peripheral CD4<sup>+</sup> and CD8<sup>+</sup> T cells can also be induced to become regulatory T cells. Here we review our studies using the combination of IL-2 and transforming growth factor  $\beta$  (TGF- $\beta$ ) to generate regulatory T cell subsets ex vivo, and the work of others using IL-10 to induce suppressive activity. Under certain conditions, the autocrine effects of TGF- $\beta$  and IL-10 induce peripheral T cells to produce immunosuppressive levels of each of these cytokines. This effect of TGF- $\beta$  is IL-2 dependent. Under other conditions IL-2 and TGF- $\beta$  can induce CD4<sup>+</sup> cells to develop potent contact-dependent, cytokine-independent regulatory activity. At present, there is considerable confusion concerning the mechanism of action of CD4<sup>+</sup> CD25<sup>+</sup> cells because cytokine-producing regulatory T cells generated in the periphery can express CD25 and other markers displayed by naturally occurring, thymus-derived regulatory T cells. We, therefore, propose a nomenclature that identifies thymus-derived and peripheral regulatory cells, and that also differentiates T regulatory cells from T helper cells. Because T regulatory cells broadly control T helper cell reactivity, the mechanisms that control regulatory cell function are also reviewed. Finally, the potential use of regulatory T cells generated ex vivo as an adoptive immunotherapy for certain autoimmune diseases, to prevent organ graft rejection, or to prevent pathologic host responses to infectious agents is discussed. *J. Leukoc. Biol.* 74: 471–478; 2003.

**Key Words:** IL-10 · TGF-Beta · IL-2 · autoimmunity · CD4<sup>+</sup> CD25<sup>+</sup> · regulatory cells

## Origin and nomenclature of heterogeneous regulatory T cell populations

Regulatory or suppressor T cells prevent the activation of potentially harmful self-reactive cells and modulate the reac-

tivity of other T cells. They constitute a network of heterogeneous CD4<sup>+</sup> cell subsets, CD8<sup>+</sup> cells and other minor T cell populations. Although suppressor T cells were first described in the 1970s [1], their identity and the molecular basis of their mechanism of action was difficult to validate in the 1980s, and interest in these cells waned. Recently, however, the importance of these cells in autoimmunity and transplantation tolerance has been rediscovered and has led to a renaissance in their investigation [2–9]. T suppressor cells have been renamed T regulatory (Tr) cells and consist of a network of diverse populations that include CD4<sup>+</sup>, CD8<sup>+</sup>, CD4<sup>-</sup>, CD8<sup>-</sup> (double negative),  $\gamma\delta$ , and natural killer T (NKT) cell.

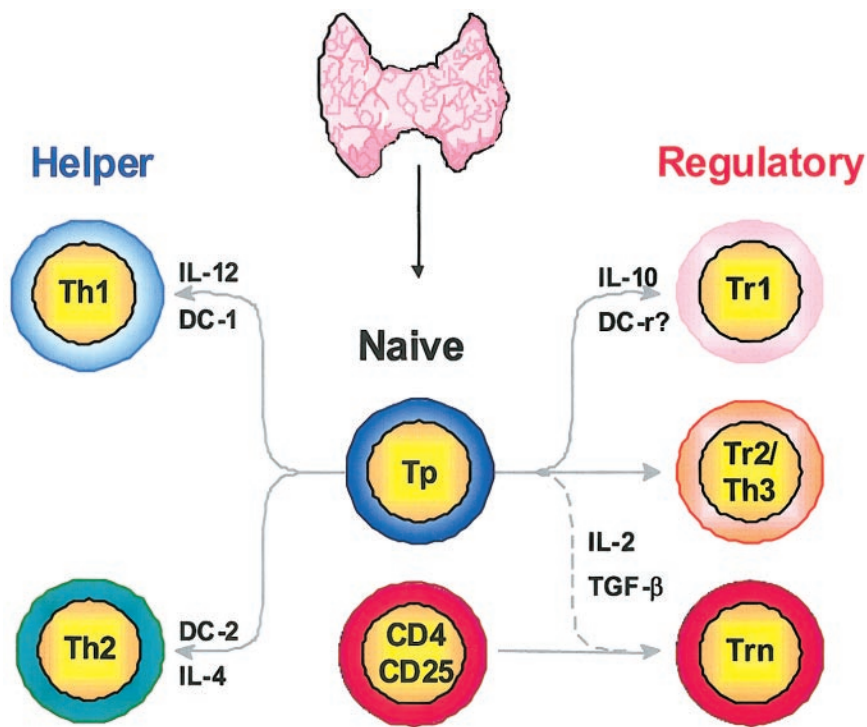
Suppressor T cell subsets can originate in the thymus or can develop from T cells activated in the periphery (**Fig. 1**). CD4<sup>+</sup> cells that constitutively express the  $\alpha$  chain of the IL-2 receptor (CD25) originate in the thymus and have a poorly understood, contact-dependent, cytokine-independent mechanism of action. These CD4<sup>+</sup> CD25<sup>+</sup> Tr cells were first demonstrated in 1995 [3] and have been referred to as “professional” or “natural” Tr cells [8, 9]. These Tr cells constitute a unique lineage, although their expression of  $\alpha\beta$  T cell receptors (TCR) does not differ from conventional T cells. CD4<sup>+</sup> CD25<sup>+</sup> Tr cells are different than thymus-derived NKT Tr cells, which have restricted usage of TCR. We will call these natural T regulatory cells “Trn” to differentiate them from other Tr cell subsets generated in the periphery.

CD4<sup>+</sup> CD25<sup>+</sup> Trn cells compose between 5–10% of CD4<sup>+</sup> cells in mice and a smaller percentage in humans. Moreover, in humans, only a small minority of CD4<sup>+</sup> cells that stain brightly for CD25 are Trn [10]. In the thymus and after birth CD4<sup>+</sup> CD25<sup>+</sup> cells have the phenotype of naïve cells. In the periphery, they display the markers of previously activated cells as a consequence of their response to self and nonself antigens. They also express cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4), glucocorticoid-induced tumor necrosis factor receptor (GITR), and high levels of I-selectin (CD62L<sup>hi</sup>) [9]. Re-

<sup>1</sup>Correspondence: University of Southern California, Keck School of Medicine, Division of Rheumatology and Immunology, 2011 Zonal Avenue, HMR 711, Los Angeles, CA 90033-1034. E-mail: dhorwitz@hsc.usc.edu

Received May 19, 2003; revised July 1, 2003; accepted July 2, 2003; doi: 10.1189/jlb.0503228

**Fig. 1.** Differentiation pathways for T helper and T regulatory cells. Naïve CD4<sup>+</sup> and CD8<sup>+</sup> T cells (not shown) differentiate in the thymus. The T cells selected include low-affinity TCR self-reactive T cells and high-affinity TCR self-reactive natural CD4<sup>+</sup> CD25<sup>+</sup> T regulatory cells (Trn). Both conventional and Trn are exported to the periphery where they respond to specific antigens. Conventional CD4<sup>+</sup> and CD8<sup>+</sup> cells become either specific T helper or T regulatory cell subsets depending upon the affinity of their TCR to antigen, the strength of the costimulatory signals provided by antigen-presenting cells, and the cytokine milieu. Specific subsets of T helper cells and T regulatory cells each produce a characteristic profile of cytokines. Th1 cells and Th2 cells produce predominantly IFN- $\gamma$  or IL-4 while Tr1 and Tr2 cells produce predominantly IL-10 or TGF- $\beta$ . Trn have a cytokine-independent, contact-dependent mechanism of action in vitro. In the periphery, IL-2 and TGF- $\beta$  can also induce CD4<sup>+</sup> cells to become Trn-like cells that have a phenotype and functional properties indistinguishable from natural, thymus-derived CD4<sup>+</sup> CD25<sup>+</sup> Trn cells. In addition to the pathways shown, it is possible that Th1 cells or Th2 cells can be induced to become Tr2 cells by stimulation with IL-2 and TGF- $\beta$ .



cently, the transcription factor Scurfin/Foxp3 has been shown to be essential in the development of Trn [10–12].

As will be discussed below, two other CD4<sup>+</sup> Tr subsets are generated in the periphery have a cytokine-dependent mechanism of action. One has been called type 1, or T regulatory-1 (Tr1) cells; these produce predominantly IL-10 [13–15] (**Table 1**). The other subset was identified following the induction of oral tolerance and produces predominantly TGF- $\beta$ . These cells were named T helper-3, or Th3 cells, to distinguish them from Th1 and Th2 cells [16, 17]. Since Th3 cells, however, function as suppressor rather than helper cells, we prefer to call these cells type 2, or T regulatory-2 (Tr2) cells, so as to group them with other T regulatory cells (Table 1).

Although Trn cells constitutively display CD25, both Tr1 and Tr2 cells can acquire this marker as well [18, 19]. A lack of appreciation of the heterogeneity of CD25<sup>+</sup> cells has led to a controversy concerning the role of TGF- $\beta$  in the mechanism of action of CD4<sup>+</sup> CD25<sup>+</sup> Tr cells. Some groups have described latent TGF- $\beta$  bound to the cell surface of CD4<sup>+</sup>

CD25<sup>+</sup> cells and consider this cytokine important in the mechanism of action [20, 21]. Others, however, deny a role for TGF- $\beta$  in the mechanism of action since the suppressive activity of CD4<sup>+</sup> CD25<sup>+</sup> cells is intact in mice that are genetically unable to produce or respond to TGF- $\beta$  [22]. Unfortunately, there are no markers that distinguish the naturally occurring, thymus-derived CD4<sup>+</sup> CD25<sup>+</sup> subset (Trn) from activated, TGF- $\beta$ -producing Tr cells generated in the periphery. Because the relative percentages of Trn cells and Tr2 cells may vary in different lymphoid organs, we suspect that the present controversy regarding the role of TGF- $\beta$  in the mechanism of action of CD4<sup>+</sup> CD25<sup>+</sup> cells is attributable to a heterogeneity of CD4<sup>+</sup> CD25<sup>+</sup> cells studied, as well as differences in the experimental methods used. As discussed below, we have also shown that CD4<sup>+</sup> CD25<sup>-</sup> cells activated in the presence of TGF- $\beta$  become CD25<sup>+</sup> Tr2 cells that have a phenotype indistinguishable from Trn cells [23].

CD8<sup>+</sup> cells can also be divided into effector and regulatory subsets. CD8<sup>+</sup> effector cells have cytotoxic activity and can

TABLE 1. General Properties of CD4<sup>+</sup> and CD8<sup>+</sup> T Regulatory and Helper Subsets

T cell subset	Predominant cytokine	Response to antigen	Response to IL-2 or IL-15
<b>Regulatory/Suppressor</b>			
CD4 <sup>+</sup> CD25 <sup>+</sup> Trn	None	Unresponsive	Proliferate
CD4 <sup>+</sup> Tr1*	IL-10	Unresponsive	Proliferate
CD4 <sup>+</sup> Tr2/Th3*	TGF- $\beta$	Unresponsive	Proliferate
CD8 <sup>+</sup> Tr1* or Tr2*	IL-10 or TGF- $\beta$	Hyporesponsive	Proliferate
<b>Helper/Cytotoxic</b>			
CD4 <sup>+</sup> Th1	IL-2, IFN- $\gamma$	Proliferate	Proliferate
CD4 <sup>+</sup> Th2	IL-4, IL-13	Proliferate	Proliferate
CD8 <sup>+</sup> Tc1	IFN- $\gamma$	Proliferate	Proliferate
CD8 <sup>+</sup> Tc2	IL-4	Proliferate	Proliferate

\* CD4<sup>+</sup> or CD8<sup>+</sup> Tr1 or Tr2 subsets can also display CD25 following activation.

produce either IFN- $\gamma$  (Tc1) or IL-4 (Tc2). CD8<sup>+</sup> Tr cells, by contrast, lack cytotoxic activity and produce either IL-10 or TGF- $\beta$  (see below). CD8<sup>+</sup> Tr cells are also hyporesponsive to secondary stimulation [24].

We consider that the various populations of Tr cells constitute an interacting network for the following reasons. First, CD4<sup>+</sup> CD25<sup>+</sup> Trn cells facilitate the generation of Tr1 cells and Tr2 cells by a phenomenon known as “infectious tolerance” [25–27]. Second, while Trn cells primarily target CD8<sup>+</sup> cells and prevent them from becoming activated and developing cytotoxic activity [28, 29], our laboratory has found that CD8<sup>+</sup> Tr cells primarily target CD4<sup>+</sup> cells and prevent their expansion following antigenic stimulation [30]. The converse was also true; CD8<sup>+</sup> Tr cells were not as potent as CD4<sup>+</sup> Tr cells in suppressing CD8<sup>+</sup> cells. Therefore, although the functional activities of all Tr subsets overlap, we suggest that each probably targets certain cell populations. Finally, our view that Tr subsets can be grouped into specific subsets must be viewed with some caution. The properties of the various Tr subsets described to date have been elucidated from *in vitro* studies. One cannot exclude the possibility that Trn cells or the Trn-like cells described herein, which have cytokine-independent mechanism of action, become cytokine-producing cells following adoptive transfer *in vivo*.

### Different properties of T helper and T regulatory cells and their differentiation pathways

Following TCR cross-linking by antigen, the T cell repertoire is selected in the thymus. T cells with high affinity receptors for self antigen are generally deleted in the thymus, but some become CD4<sup>+</sup> CD25<sup>+</sup> Trn cells for poorly understood reasons [31]. Whereas most cytokine-secreting Tr cells develop in the periphery, altered peptide (lower affinity) antigens can induce TGF- $\beta$  secreting Tr-2 cells in the thymus [32]. Thus, the generalization that all contact-dependent, cytokine-independent cells are thymus derived and that all cytokine-producing Tr cells are induced in the periphery has exceptions.

The principal difference between Th cells and Tr cells is that the former proliferate in response to antigen, whereas the latter are generally unresponsive, at least *in vitro* (Table 1). Th cells are responsible for host defense against bacteria and intracellular infections, while Tr cells act as feedback regulators of Th cells. Harmful antiself-injury triggered by persistent immune responses is, thereby, prevented. Although all Tr subsets inhibit both Th1 cells and Th2 cells, cytokines produced by the Th subsets inhibit each other by immune deviation.

Although T cell receptor engagement is not sufficient for activation, Tr cells do proliferate when TCRs are cross-linked in the presence of IL-2 [33]. Tr1 cells also proliferate in the presence of IL-15 [18]. In addition, anergic CD4<sup>+</sup> CD25<sup>+</sup> cells proliferate vigorously when transferred to lymphopenic mice [34]. Moreover, mouse Tr cells generated *ex vivo* with IL-2 and TGF- $\beta$  proliferate when transferred to nonlymphopenic syngeneic mice [35]. Trn cells and Tr2 cells can retain their suppressive properties after exposure to IL-2, whereas Tr1 cells

lose this function when anergy is broken and become Th cells (Horwitz et al., unpublished results). Thus, although Tr cells are anergic *in vitro*, they expand *in vivo*.

Whether T cells develop helper or regulatory function following antigen stimulation is multifactorial and depends upon the affinity of the antigen for the T cell receptor (TCR), the costimulating properties of the antigen-presenting cells, and the cytokines in the micro environment. High-affinity binding favors Th or Trn differentiation, while, as stated above, altered peptide ligands with decreased binding affinity favor the development of Tr-2 cells [32]. Antigen-presenting cells (APC), dendritic cells (DC) in particular, have a major role in T cell differentiation. DC1 and DC2 subsets control the generation of Th1 and Th2 cells, respectively [36]. Some workers have proposed that another dendritic cell subset (DC<sub>c</sub>) that secretes IL-10 rather than IL-12 directs naïve T cells to a Tr1 subtype [36, 37]. Finally, a self-maintaining regulatory loop has been proposed in which tolerogenic DC induce Tr cells, and these cells program the generation of new tolerogenic DC from progenitors [38].

### The role of IL-10 and antigen-presenting cells and in the generation of Tr-1 and anergic suppressor cells

Whereas antigen-activated T cells provided with strong costimulatory signals become helper cells, they become regulatory cells when activated with IL-10 or TGF- $\beta$ . These cytokines that decrease expression of costimulatory molecules on antigen-presenting cells (Fig. 1). CD4<sup>+</sup> cells become Tr1 cells when repeatedly stimulated with IL-10 or with immature dendritic cells. These IL-10-producing Tr1 cells have strong suppressive effects, both *in vitro* and *in vivo* [14, 15, 39]. CD8<sup>+</sup> cells activated by immature dendritic cells also become Tr1 cells [24, 40]. The role of IL-10 in their mechanism of action appears pivotal because neutralizing anti-IL-10 antibodies completely block the generation of Tr1 cells [24]. In all examples, the IL-10-conditioned CD4<sup>+</sup> T cells became unresponsive to antigen and lost the capacity to produce IL-2. In addition to producing high levels of IL-10, they also produced moderate amounts of TGF- $\beta$  and low levels of Th1 or Th2 cytokines [14]. Tr1 cells suppressed the activation of cocultured T cells in an antigen nonspecific fashion. CD4<sup>+</sup> Tr1 clones express CD25 and CTLA-4 such that they would be difficult to distinguish from the naturally occurring CD4<sup>+</sup> CD25<sup>+</sup> cells [18].

Tr1 cells can also be generated by stimulating mouse or human naïve CD4<sup>+</sup> cells with vitamin D3 and dexamethasone [41]. These cells produce high levels of IL-10 and some IL-4. The latter disappeared upon neutralization of Th2 cytokines in primary culture. The adoptive transfer of these Tr1 cells prevented central nervous system inflammation.

Other investigators have generated Tr1 *ex vivo* by stimulating CD4<sup>+</sup> cells with anti-CD3 and the complement regulator anti-CD46 [42]. Cross-linking CD46 with C3b also produced the same result. These IL-10-producing cells also proliferated vigorously, unlike the Tr1 induced with IL-10 and immature DC. Thus, Tr1 cells can be generated by inducing CD4<sup>+</sup> cells

to become unresponsive, or by activating them with complement fragments where they retain their ability to proliferate.

Finally, IL-10 can be used to induce anergic CD4<sup>+</sup> cells that do not produce cytokines but suppress the activation of other T cells through a contact-dependent mechanism. Following treatment of dendritic cells with IL-10, CD4<sup>+</sup> and CD8<sup>+</sup> cells become unresponsive. These anergic T cells act as suppressor cells by competing with other antigen-stimulated T cells for the membrane of APCs and for locally produced IL-2. These suppressor cells have a contact-dependent mechanism of action, do not produce cytokines, and are irradiation insensitive. Allospecific T cells rendered unresponsive in this manner block T cell proliferation in an antigen-specific manner in vitro [43]. Moreover, the adoptive transfer of anergic T cells to the recipients of allogeneic skin grafts leads to prolonged skin graft survival. Thus, anergic T cells can function as antigen-specific suppressor cells both in vitro and in vivo [44].

### The role of IL-2 and TGF- $\beta$ in the generation of CD8<sup>+</sup> and two different CD4<sup>+</sup> Tr subsets

The pleiotropic TGF- $\beta$  family of cytokines regulate T cell growth and development [45]. While TGF- $\beta$  inhibits the differentiation of Th1 and Th2 cells [46, 47], this cytokine induces at least 3 Tr subsets through positive effects on cell growth and differentiation. Our laboratory has reported that with a sufficient amount of IL-2 to counteract its suppressive effects, TGF- $\beta$  has costimulatory effects on CD8<sup>+</sup> and CD4<sup>+</sup> cells. These effects enhance the proliferation and survival of human T cells that develop potent suppressive activities [23, 29]. TGF- $\beta$  has been reported to considerably enhance the proliferation of mouse CD8<sup>+</sup> cells [48]. This cytokine enhances human CD4<sup>+</sup> cell expression of CD25, CTLA-4, CD40L, and TNFR II [29, 49]. TGF- $\beta$  increases TNF- $\alpha$  production by both CD4<sup>+</sup> and CD8<sup>+</sup> cells [49]. TGF- $\beta$  accelerates activation-induced cell death of some T cells [50, 51] but protects others from apoptosis [52, 53]. Thus, TGF- $\beta$  not only has inhibitory effects on T cell and B cell function but can induce several Tr subsets with the potential to suppress immune-induced inflammation [54].

In the mid-1990s, TGF- $\beta$  was shown to induce both mouse and human CD8<sup>+</sup> cells to become suppressor cells. Mouse CD8<sup>+</sup> cells stimulated with staphylococcal enterotoxin B and TGF- $\beta$ 1 secreted increased levels of TGF- $\beta$  and IL-10 and inhibited the proliferation of other T cells [55]. Independently, we learned that TGF- $\beta$  had an important role in the generation of human CD8<sup>+</sup> suppressor cells. Using a model in which we could induce T cell-dependent antibody production without accessory cells, we found that CD4<sup>+</sup> cells, by themselves, could not induce CD8<sup>+</sup> cells to suppress antibody production. The addition of NK cells to the cultures, however, led to potent suppressor activity. Subsequent studies revealed that NK cells produced active TGF- $\beta$  when they interact with CD8<sup>+</sup> cells, and this cytokine was needed for CD8<sup>+</sup> cells to become suppressors of antibody production [56, 57]. In other studies, a brief exposure of CD8<sup>+</sup> cells to IL-2 and TGF- $\beta$  led to potent suppressive activity. In unpublished studies, we have found that the suppressive activity of CD8<sup>+</sup> cells is cytokine

dependent and that neutralizing anti-TGF- $\beta$  monoclonal antibodies sometimes, but not always, abolishes suppressive activity. Because others have shown that CD8<sup>+</sup> suppressor cells can produce IL-10 and TGF- $\beta$  [24, 55], it is likely that both cytokines have an important role in the suppressive effects of these cells.

In the late 1990s, we turned our attention from the inhibition of antibody production to the suppression of cytotoxic T lymphocyte (CTL) activity and found that the predominant effect of TGF- $\beta$  was on CD4<sup>+</sup> cells rather than CD8<sup>+</sup> cells. Surprisingly, the phenotype and functional properties of the CD4<sup>+</sup> Tr subset induced by TGF- $\beta$  were indistinguishable from CD4<sup>+</sup> CD25<sup>+</sup> Trn. In these studies we stimulated naïve CD4<sup>+</sup> cells with allogeneic irradiated non-T stimulator cells and TGF- $\beta$ . The addition of less than 10 percent of these TGF- $\beta$  conditioned cells to fresh autologous responder T cells strongly blocked the activation of CD8<sup>+</sup> cells and prevented them from developing cytotoxic activity. This suppressive activity was contact dependent and not affected by neutralizing antibodies to IL-10 and TGF- $\beta$ . When the CD25<sup>+</sup> subset was isolated and expanded 5 to 10 fold in IL-2, the addition of less than 1% of these cells to fresh responder T cells markedly suppressed the development of cytotoxicity.

The ability of TGF- $\beta$  to induce Trn-like cells was IL-2 dependent. Partial neutralization of IL-2 abolished the induction of suppressive activity (Horwitz et al., unpublished observations). While we used naïve CD4<sup>+</sup> cells (which rarely express CD25) to become suppressor cells in our initial study [29], we have recently induced allo-stimulated total CD4<sup>+</sup> CD25<sup>-</sup> cells to develop Trn-like activity. This effect required the addition of both IL-2 and TGF- $\beta$  to the cultures (Horwitz et al., unpublished observations). Finally, the combination of IL-2 and TGF- $\beta$  enhances the suppressive effects of purified CD4<sup>+</sup> CD25<sup>+</sup> mouse Tr cells [58] and the corresponding human subset (our unpublished observations).

It has become apparent that the ability of a small number of CD4<sup>+</sup> CD25<sup>+</sup> cells induced ex vivo to have remarkably potent suppressive effects is due to their capacity to recruit other T cells to develop suppressive activity. Others have shown that CD4<sup>+</sup> CD25<sup>+</sup> T cells can induce CD25<sup>-</sup> T cells to become Tr1 or Tr2 cells by infectious tolerance [26, 27]. Studies in progress indicate that Trn-like cells induced ex vivo can also induce human CD4<sup>+</sup> CD25<sup>-</sup> cells to become suppressor cells that produce IL-10 and TGF- $\beta$  (Horwitz et al., unpublished observations). The ability to generate potent suppressor cells ex vivo that also have the ability to educate other T cells to develop suppressive activity may have important therapeutic implications.

As stated above, certain thymic CD4<sup>+</sup> cells with high affinity TCRs for self-antigen become Trn rather than being deleted. It is not unlikely that these CD4<sup>+</sup> cells are recognizing self-antigens on thymic epithelial cells in the presence of TGF- $\beta$ . This cytokine has been previously reported to have a role in thymic T cell differentiation [59]. Thymic epithelial cells can express tissue-specific self-antigens, and some can produce TGF- $\beta$  [60, 61]. Thus, if self-reactive CD4<sup>+</sup> T cells respond to self-antigens in a milieu containing TGF- $\beta$ , they may become CD25<sup>+</sup> Trn rather than being negatively selected.



A role for TGF- $\beta$  in the generation of Tr2 has also been documented. Recent studies from our laboratory have revealed that stimulation of CD4<sup>+</sup> CD25<sup>-</sup> cells with low-dose staphylococcal enterotoxin B (SEB) and TGF- $\beta$  induces a CD25<sup>+</sup> Tr subset that produces TGF- $\beta$ , but not IL-2, IFN- $\gamma$ , or IL-10 [23]. The addition of 5% of these CD4<sup>+</sup> CD25<sup>+</sup> cells markedly suppressed T cell-dependent antibody production by a mechanism that was TGF- $\beta$  dependent. Anti-TGF- $\beta$  completely blocked the suppressive activity of these cells. The cytokine profile and mechanism of action of this subset, therefore, was clearly distinguishable from other Tr subsets. Moreover, TGF- $\beta$  not only was responsible for the generation of this Tr subset, but it protected them from apoptosis upon further stimulation. Interestingly, the low dose of SEB used to induce T cell activation stimulated CD4<sup>+</sup> CD45RO<sup>+</sup> cells, but not the CD45RA<sup>+</sup> naïve subset. This result suggests that we had induced CD4<sup>+</sup> previously activated effector or memory cells to develop regulatory activity. Further studies will be performed to learn whether T cells previously polarized to become Th1 or Th2 cells can become Tr2 cells. Others have described a small subset of mouse CD4<sup>+</sup> CD25<sup>-</sup> cells that express the latent form of TGF- $\beta$  on their cell surface, and these cells have suppressive activity mediated by this cytokine [62]. It is likely that the autocrine effects of TGF- $\beta$  have induced these lymphocytes to become Tr2 cells.

Although both Tr cells generated by IL-10 and TGF- $\beta$  can proliferate in response to IL-2, the consequences of this response on the regulatory effects of these cells is strikingly different. IL-2 releases Tr1 from their anergic state, and they regain the ability to respond to antigen stimulation. By contrast, TGF- $\beta$  induced Tr cells retain their suppressive activity after treatment with IL-2 (Horwitz et al., unpublished observations).

The relative contribution of IL-2 and TGF- $\beta$  in the generation of Tr cells remains to be elucidated, but both are important. We agree with Papiernik and co-workers who have suggested that IL-2 has an essential role in the generation of suppressor T cells [63]. Although IL-2 was originally described as a T cell growth factor, mice with a deficiency of this cytokine or a functional IL-2 receptor develop a lethal lymphoproliferative and autoimmune syndrome [64–67]. This syndrome can be corrected directly by the adoptive transfer of CD4<sup>+</sup> CD25<sup>+</sup> cells, or indirectly by the transfer of CD4<sup>+</sup> CD25<sup>-</sup> cells. Presumably, the latter provide the IL-2 that is needed for the generation of the suppressor cells. An aggressive lethal lymphoproliferative and autoimmune syndrome also occurs in TGF- $\beta$ 1 knockout mice [68]. Nonetheless, CD4<sup>+</sup> suppressor cell precursors apparently develop in both IL-2 and TGF- $\beta$ 1-deficient mice. IL-2 signaling enables CD4<sup>+</sup> CD25<sup>+</sup> from IL-2-deficient mice to develop suppressive activity [69]. Similarly, CD4<sup>+</sup> CD25<sup>+</sup> cells from TGF- $\beta$ 1-deficient mice can suppress wild-type T cells [22]. In this case the wild-type antigen-presenting cells could serve as a source of TGF- $\beta$ . Thus, both IL-2 and TGF- $\beta$ 1 could be maturation factors for suppressor cell precursors. While IL-2 would promote the growth of these cells, TGF- $\beta$  would protect them from activation-induced apoptosis.

In addition to a role in the generation of Tr cells, IL-2 has an important role in their functional activity. Trn cells and Trn-

like cells generated ex vivo express CD122 (IL-2R $\beta$  chain) and signaling through IL-2R  $\beta$  is critical for suppressive activity. Mice with a genetic deletion of CD122 spontaneously develop autoimmune disease [70]. In our laboratory, neutralizing anti-CD122 antibodies completely abolished the control of endogenous Tr cells on allo-CTL activity (S. G. Zheng and D. A. Horwitz unpublished observation).

## Both TGF- $\beta$ and IL-10 are required for optimal immunosuppression

In addition to the individual effects of TGF- $\beta$  and IL-10 on lymphocytes and antigen-presenting cells, we believe that both cytokines work together to terminate an immune response. Blazar and co-workers have shown that the suppressive effects of TGF- $\beta$  and IL-10 added together is greater than each acting alone [71, 72]. We have confirmed these observations in unpublished studies. Groux and co-workers have reported one mechanism that involves this synergy. They found that newly activated T cells avoid the inhibitory effects of TGF- $\beta$  by down-regulating the signal transducing TGF- $\beta$  receptor (TGF- $\beta$ RII) [73]; this would enable T cells to respond optimally to microbial antigens. This finding is consistent with our observation that the addition of TGF- $\beta$  to T cells more than 24 h after they are activated markedly diminishes their ability to become suppressor cells [56, 57]. Remarkably, Groux et al. reported that IL-10 is responsible for the re-appearance of TGF- $\beta$ RII [73]. Thus, the late appearance of IL-10 after the peak of T cell activation restores the sensitivity of these cells to TGF- $\beta$ . In this manner, the combined inhibitory effects of IL-10 and TGF- $\beta$  act together to terminate T cell activation.

Another example of the cooperation of IL-10 and TGF- $\beta$  is the regulation of T cell responses to mucosal allergens. Specific immunotherapy directed to two major allergens induces CD4<sup>+</sup> CD25<sup>+</sup> suppressor cells that produce IL-10 and TGF- $\beta$ . Neutralization of either cytokine abolishes suppressive activity, a result suggesting the cooperative effects of both are needed for optimal inhibition [74]. Other examples of interdependent effects of IL-10 on TGF- $\beta$  have also been observed [75, 76].

In addition to synergistic effects, there is evidence that production of IL-10 and TGF- $\beta$  is controlled by negative feedback regulatory effects that each of these cytokines has on the other [77, 78]. Thus, a defect in the production of IL-10 or TGF- $\beta$  can have deleterious consequences. For example, there is a marked imbalance of IL-10 and TGF- $\beta$  in patients with systemic lupus erythematosus. Levels of IL-10 are high, while lymphocyte production of TGF- $\beta$  is decreased [79, 80]. This imbalance may strongly contribute to dysregulated polyclonal B cell activation characteristic of SLE. In autoimmune pancreatitis, a loss of TGF- $\beta$  signaling accelerates the progression of this disease [81]. In turn, TGF- $\beta$  mediated fibrosis in chronic pancreatitis is enhanced in animals that are unable to produce IL-10 [82]. For these reasons, we believe that adequate numbers of both Tr1 and Tr2 cells are needed to control immune-induced chronic inflammation.

## Mechanisms to control Tr cell activity

Since Tr cells control the reactivity of both self-reactive and non-self-reactive T cells to antigens, mechanisms must be in place to prevent them from inhibiting protective immune responses to microbial antigens. Two such regulatory mechanisms have been described. The first concerns the strength of the activating signal. Baecher-Allan and co-workers have shown that strongly activated T cells are refractory to the inhibitory effects of CD4<sup>+</sup> CD25<sup>+</sup> Tr cells [83]. Thus, Tr cells could not block T cells from responding to potent microbial antigens but could inhibit a weaker response to self-antigens. The second mechanism involves the innate immune system. Both antigen-presenting cells and CD4<sup>+</sup> CD25<sup>+</sup> cells express Toll-like receptors (TLR-4). These pattern receptors are triggered by microbial products. The resulting signals induce antigen-presenting cells to produce IL-6 and other cytokines, which block the suppressive effects of CD4<sup>+</sup> CD25<sup>+</sup> Tr cells, thereby, allowing T cells to respond maximally to the infectious agent [84]. Triggering TLR-4 expressed by CD4<sup>+</sup> CD25<sup>+</sup> cells markedly enhances the proliferation and suppressive activity of these regulatory T cells [85]. Thus, when levels of IL-6 fall, an expanded population of Tr cells are ready to terminate the response.

Finally, the availability of IL-2 regulates Tr activity. As discussed above, both the generation and functional activity of Tr cells is dependent upon IL-2 [64–70]. Since one principal effect of Tr cells is to block IL-2 synthesis, shutting down the production of this cytokine will result in feedback inhibition of Tr function.

## The therapeutic potential of Tr cells generated ex vivo

Evidence has been reviewed that the combination of IL-2 and TGF- $\beta$  or IL-10 can induce CD4<sup>+</sup> cells ex vivo to become either Trn-like, Tr1, or Tr2 subsets, and induce CD8<sup>+</sup> cells to become Tr1 or Tr2 subsets. The CD4<sup>+</sup> Trn-like cells generated in the periphery have in vitro functional properties identical with thymus-derived Trn cells. Here TGF- $\beta$  has an important role in the generation, but not in mechanism of action. The ability of IL-2 and TGF- $\beta$  to generate specific Tr subsets ex vivo raises the possibility that one's own T cells can be harnessed for therapeutic purposes.

Tr1 cells generated ex vivo can prevent an immune-mediated colitis in lymphopenic mice [10]. We have been able to induce both CD4<sup>+</sup> cells and CD8<sup>+</sup> cells to develop suppressive activity by activating mouse T cells with alloantigens plus the combination of IL-2 and TGF- $\beta$ . These T cells prevented a lupus-like disease and doubled the survival of mice that had already developed antidouble-stranded DNA antibodies [35]. In addition, these Tr cells have enabled heterotopic heart allografts in mice to beat for 100 days (Horwitz et al., unpublished observation). Blazar's group has recently used TGF- $\beta$  and IL-10 to induce CD4<sup>+</sup> CD25<sup>-</sup> T cells ex vivo to suppress acute graft vs. host disease in vivo [86].

During the next few years, we will have the opportunity to learn whether the adoptive transfer of various autologous Tr subsets induced ex vivo from human blood lymphocytes obtained by pheresis can prevent graft rejection or alter the course of certain autoimmune diseases. The prime candidates are diseases characterized by spontaneous exacerbations and remissions such as systemic lupus and inflammatory bowel disease. Here, Tr cells induced with polyclonal T cell activators can be tested. For diseases such as rheumatoid arthritis, antigen-specific Tr would have a much better chance of success. Previously, collagen-specific Tr cells were induced by oral tolerance in anticipation that they would migrate to the rheumatoid joint, where they would be activated by endogenous type II collagen and inhibit joint inflammation via bystander suppression. Oral tolerance, however, had only modest beneficial effects in rheumatoid arthritis [87], presumably because of an inadequate number of Tr cells generated. It is possible that collagen-specific Tr cells induced *ex-vivo* may have greater clinical effects than those induced by oral tolerance. In addition to autoimmune diseases, Tr cells generated ex vivo to block IL-4 production deserve consideration in the treatment of patients with severe steroid-dependent allergic asthma. Another possible use deserves comment. The host immune response against viral infections can result in severe tissue injury, as recently revealed by the SARS virus. Because adoptively transferred Tr cells traffic through the lungs, it is perhaps possible that Tr cells induced ex vivo can blunt the pathologic host response to the SARS virus by blocking T cell activation at that site.

Because of the various physiologic mechanisms to control Tr activity described above, we do not anticipate that the adoptive transfer of Tr cells will impair host defense against pathogenic infectious agents. This therapy, therefore, should be much safer than the corticosteroids and immunosuppressive drugs currently used for the treatment of autoimmune diseases. Finally, since tumor-associated Tr cells may block the various immunologic strategies designed to kill malignant cells, novel approaches to antagonize Tr cells may lead to advances in the treatment of cancer.

## ACKNOWLEDGMENTS

This research was supported in part by National Institutes of Health grant AI 41768, The Nora Eccles Treadwell Foundation, and the Arthritis Foundation, Southern California Chapter.

## REFERENCES

1. Gershon, R. K. (1975) A disquisition on suppressor T cells. *Transplant. Rev.* **26**, 170–185.
2. Sakaguchi, S., Fukuma, K., Kuribayashi, K., Masuda, T. (1985) Organ-specific autoimmune diseases induced in mice by elimination of T cell subset. I. Evidence for the active participation of T cells in natural self-tolerance; deficit of a T cell subset as a possible cause of autoimmune disease. *J. Exp. Med.* **161**, 72–87.
3. Sakaguchi, S., Sakaguchi, N., Asano, M., Itoh, M., Toda, M. (1995) Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor  $\alpha$ -chains (CD25). Breakdown of a single mechanism of

- self-tolerance causes various autoimmune diseases. *J. Immunol.* **155**, 1151–1164.
4. Wood, K. J., Sakaguchi, S. (2003) Regulatory T cells in transplantation tolerance. *Nat. Rev. Immunol.* **3**, 199–210.
  5. Mason, D. (2001) Some quantitative aspects of T-cell repertoire selection: the requirement for regulatory T cells. *Immunol. Rev.* **182**, 80–88.
  6. Maloy, K. J., Powrie, F. (2001) Regulatory T cells in the control of immune pathology. *Nat. Immunol.* **2**, 816–822.
  7. Annacker, O., Powrie, F. (2002) Homeostasis of intestinal immune regulation. *Microbes Infect.* **4**, 567–574.
  8. Shevach, E. M. (2001) Certified professionals: CD4+ CD25+ suppressor T cells. *J. Exp. Med.* **193**, 41–46.
  9. Shevach, E. M. (2002) CD4+ CD25+ suppressor T cells: more questions than answers. *Nat. Rev. Immunol.* **2**, 389–400.
  10. Khattri, R., Cox, T., Yasayko, S. A., Ramsdell, F. (2003) An essential role for Scurfin in CD4+CD25+ T regulatory cells. *Nat. Immunol.* **4**, 337–342.
  11. Fontenot, J. D., Gavin, M. A., Rudensky, A. Y. (2003) Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat. Immunol.* **4**, 330–336.
  12. Hori, S., Nomura, T., Sakaguchi, S. (2003) Control of regulatory T cell development by the transcription factor Foxp3. *Science* **299**, 1057–1061.
  13. Baecher-Allan, C., Brown, J. A., Freeman, G. J., Hafler, D. A. (2001) CD4+CD25high regulatory cells in human peripheral blood. *J. Immunol.* **167**, 1245–1253.
  14. Groux, H., O'Garra, A., Bigler, M., Rouleau, M., Antonenko, S., de Vries, J. E., Roncarolo, M. G. (1997) A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* **389**, 737–742.
  15. Levings, M. K., Roncarolo, M. G. (2000) T-regulatory 1 cells: a novel subset of CD4 T cells with immunoregulatory properties. *J. Allergy Clin. Immunol.* **106**, S109–S112.
  16. Weiner, H. L. (2001) Oral tolerance: immune mechanisms and the generation of Th3-type TGF-beta-secreting regulatory cells. *Microbes Infect.* **3**, 947–954.
  17. Weiner, H. L. (2001) Induction and mechanism of action of transforming growth factor-beta-secreting Th3 regulatory cells. *Immunol. Rev.* **182**, 207–214.
  18. Bacchetta, R., Sartirana, C., Levings, M. K., Bordignon, C., Narula, S., Roncarolo, M. G. (2002) Growth and expansion of human T regulatory type 1 cells are independent from TCR activation but require exogenous cytokines. *Eur. J. Immunol.* **32**, 2237–2245.
  19. Zhang, X., Izikson, L., Liu, L., Weiner, H. L. (2001) Activation of CD25+ CD4+ regulatory T cells by oral antigen administration. *J. Immunol.* **167**, 4245–4253.
  20. Nakamura, K., Kitani, A., Strober, W. (2001) Cell contact-dependent immunosuppression by CD4+CD25+ regulatory T cells is mediated by cell surface-bound transforming growth factor beta. *J. Exp. Med.* **194**, 629–644.
  21. Chen, W., Wahl, S. M. (2003) TGF-beta: the missing link in CD4+CD25+ regulatory T cell-mediated immunosuppression. *Cytokine Growth Factor Rev.* **14**, 85–89.
  22. Piccirillo, C. A., Letterio, J. J., Thornton, A. M., McHugh, R. S., Mamura, M., Mizuhara, H., Shevach, E. M. (2002) CD4+CD25+ regulatory T cells can mediate suppressor function in the absence of transforming growth factor beta1 production and responsiveness. *J. Exp. Med.* **196**, 237–246.
  23. Zheng, S. G., Gray, J. D., Ohtsuka, K., Yamagiwa, S., Horwitz, D. A. (2002) Generation ex vivo of TGF-beta-producing regulatory T cells from CD4+CD25- precursors. *J. Immunol.* **169**, 4183–4189.
  24. Gilliet, M., Liu, Y. J. (2002) Generation of human CD8 T regulatory cells by CD40 ligand-activated plasmacytoid dendritic cells. *J. Exp. Med.* **195**, 695–704.
  25. Cobbold, S. P., Graca, L., Lin, C. Y., Adams, E., Waldmann, H. (2003) Regulatory T cells in the induction and maintenance of peripheral transplantation tolerance. *Transpl. Int.* **16**, 66–75.
  26. Jonuleit, H., Schmitt, E., Kackmar, H., Stassen, M., Knop, J., Enk, A. H. (2002) Infectious tolerance: human CD25+ regulatory T cells convey suppressor activity to conventional CD4+ T helper cells. *J. Exp. Med.* **196**, 255–260.
  27. Dieckmann, D., Bruett, C. H., Ploettner, H., Lutz, M. B., Schuler, G. (2002) Human CD4+CD25+ regulatory, contact-dependent T cells induce interleukin 10-producing, contact-independent type 1-like regulatory T cells. *J. Exp. Med.* **196**, 247–253.
  28. Piccirillo, C. A., Shevach, E. M. (2001) Cutting edge: control of CD8+ T cell activation by CD4+CD25+ immunoregulatory cells. *J. Immunol.* **167**, 1137–1140.
  29. Yamagiwa, S., Gray, J. D., Hashimoto, S., Horwitz, D. A. (2001) A role for TGF-beta in the generation and expansion of CD4+CD25+ regulatory T cells from human peripheral blood. *J. Immunol.* **166**, 7282–7289.
  30. Horwitz, D. A., Gray, J. D., Ohtsuka, K. (1999) Role of NK cells and TGF-beta in the regulation of T-cell-dependent antibody production in health and autoimmune disease. *Microbes Infect.* **1**, 1305–1311.
  31. Jordan, M. S., Boesteanu, A., Reed, A. J., Petrone, A. L., Hohenbeck, A. E., Lerman, M. A., Naji, A., Caton, A. J. (2001) Thymic selection of CD4+CD25+ regulatory T cells induced by an agonist self-peptide. *Nat. Immunol.* **2**, 301–306.
  32. Yamashiro, H., Hozumi, N., Nakano, N. (2002) Development of CD25(+) T cells secreting transforming growth factor-beta1 by altered peptide ligands expressed as self-antigens. *Int. Immunol.* **14**, 85–95.
  33. Thornton, A. M., Shevach, E. M. (2000) Suppressor effector function of CD4+CD25+ immunoregulatory T cells is antigen nonspecific. *J. Immunol.* **164**, 183–190.
  34. Gavin, M. A., Clarke, S. R., Negrou, E., Gallegos, A., Rudensky, A. (2002) Homeostasis and energy of CD4+CD25+ suppressor T cells in vivo. *Nat. Immunol.* **3**, 33–41.
  35. Zheng, S. G., Koss, M. N., Quismorio, F. P., Wang, J. H., Gray, J. D., Horwitz, D. A. (2003) CD4+ and CD8+ regulatory T cells generated ex-vivo with IL-2 and TGF-beta suppress a stimulatory graft-versus-host-disease with a lupus-like syndrome. Submitted to the Journal of Immunology.
  36. McGuirk, P., Mills, K. H. (2002) Pathogen-specific regulatory T cells provoke a shift in the Th1/Th2 paradigm in immunity to infectious diseases. *Trends Immunol.* **23**, 450–455.
  37. McGuirk, P., McCann, C., Mills, K. H. (2002) Pathogen-specific T regulatory 1 cells induced in the respiratory tract by a bacterial molecule that stimulates interleukin 10 production by dendritic cells: a novel strategy for evasion of protective T helper type 1 responses by Bordetella pertussis. *J. Exp. Med.* **195**, 221–231.
  38. Min, W. P., Zhou, D., Ichim, T. E., Strejan, G. H., Xia, X., Yang, J., Huang, X., Garcia, B., White, D., Dutartre, P., et al. (2003) Inhibitory feedback loop between tolerogenic dendritic cells and regulatory T cells in transplant tolerance. *J. Immunol.* **170**, 1304–1312.
  39. Jonuleit, H., Schmitt, E., Schuler, G., Knop, J., Enk, A. H. (2000) Induction of interleukin 10-producing, nonproliferating CD4+ T cells with regulatory properties by repetitive stimulation with allogeneic immature human dendritic cells. *J. Exp. Med.* **192**, 1213–1222.
  40. Dhodapkar, M. V., Steinman, R. M. (2002) Antigen-bearing immature dendritic cells induce peptide-specific CD8(+) regulatory T cells in vivo in humans. *Blood* **100**, 174–177.
  41. Barrat, F. J., Cua, D. J., Boonstra, A., Richards, D. F., Crain, C., Savelkoul, H. F., de Waal-Malefyt, R., Coffman, R. L., Hawrylowicz, C. M., O'Garra, A. (2002) In vitro generation of interleukin 10-producing regulatory CD4+ T cells is induced by immunosuppressive drugs and inhibited by T helper type 1 (Th1)- and Th2-inducing cytokines. *J. Exp. Med.* **195**, 603–616.
  42. Kemper, C., Chan, A. C., Green, J. M., Brett, K. A., Murphy, K. M., Atkinson, J. P. (2003) Activation of human CD4+ cells with CD3 and CD46 induces a T-regulatory cell 1 phenotype. *Nature* **421**, 388–392.
  43. Vendetti, S., Chai, J. G., Dyson, J., Simpson, E., Lombardi, G., Lechler, R. (2000) Anergic T cells inhibit the antigen-presenting function of dendritic cells. *J. Immunol.* **165**, 1175–1181.
  44. Chai, J. G., Bartok, I., Chandler, P., Vendetti, S., Antoniou, A., Dyson, J., Lechler, R. (1999) Anergic T cells act as suppressor cells in vitro and in vivo. *Eur. J. Immunol.* **29**, 686–692.
  45. Letterio, J. J., Roberts, A. B. (1998) Regulation of immune responses by TGF-beta. *Annu. Rev. Immunol.* **16**, 137–161.
  46. Heath, V. L., Murphy, E. E., Crain, C., Tomlinson, M. G., O'Garra, A. (2000) TGF-beta1 down-regulates Th2 development and results in decreased IL-4-induced STAT6 activation and GATA-3 expression. *Eur. J. Immunol.* **30**, 2639–2649.
  47. Ludviksson, B. R., Seegers, D., Resnick, A. S., Strober, W. (2000) The effect of TGF-beta1 on immune responses of naive versus memory CD4+ Th1/Th2 T cells. *Eur. J. Immunol.* **30**, 2101–2111.
  48. Lee, H. M., Rich, S. (1993) Differential activation of CD8+ T cells by transforming growth factor-beta 1. *J. Immunol.* **151**, 668–677.
  49. Gray, J. D., Liu, T., Huynh, N., Horwitz, D. A. (2001) Transforming growth factor beta enhances the expression of CD154 (CD40L) and production of tumor necrosis factor alpha by human T lymphocytes. *Immunol. Lett.* **78**, 83–88.
  50. Chung, E. J., Choi, S. H., Shim, Y. H., Bang, Y. J., Hur, K. C., Kim, C. W. (2000) Transforming growth factor-beta induces apoptosis in activated murine T cells through the activation of caspase 1-like protease. *Cell. Immunol.* **204**, 46–54.
  51. Sillett, H. K., Cruickshank, S. M., Southgate, J., Trejdosiewicz, L. K. (2001) Transforming growth factor-beta promotes 'death by neglect' in post-activated human T cells. *Immunology* **102**, 310–316.



52. Genestier, L., Kasibhatla, S., Brunner, T., Green, D. R. (1999) Transforming growth factor beta1 inhibits Fas ligand expression and subsequent activation-induced cell death in T cells via downregulation of c-myc. *J. Exp. Med.* **189**, 231–239.
53. Chen, W., Jin, W., Tian, H., Sicurello, P., Frank, M., Orenstein, J. M., Wahl, S. M. (2001) Requirement for transforming growth factor beta1 in controlling T cell apoptosis. *J. Exp. Med.* **194**, 439–453.
54. Horwitz, D. A., Gray, J. D., Zheng, S. G. (2002) The potential of human regulatory T cells generated ex vivo as a treatment for lupus and other chronic inflammatory diseases. *Arthritis Res.* **4**, 241–246.
55. Rich, S., Seelig, M., Lee, H. M., Lin, J. (1995) Transforming growth factor beta 1 costimulated growth and regulatory function of staphylococcal enterotoxin B-responsive CD8+ T cells. *J. Immunol.* **155**, 609–618.
56. Gray, J. D., Hirokawa, M., Horwitz, D. A. (1994) The role of transforming growth factor beta in the generation of suppression: an interaction between CD8+ T and NK cells. *J. Exp. Med.* **180**, 1937–1942.
57. Gray, J. D., Hirokawa, M., Ohtsuka, K., Horwitz, D. A. (1998) Generation of an inhibitory circuit involving CD8+ T cells, IL-2, and NK cell-derived TGF-beta: contrasting effects of anti-CD2 and anti-CD3. *J. Immunol.* **160**, 2248–2254.
58. Taylor, P. A., Lees, C. J., Blazar, B. R. (2002) The infusion of ex vivo activated and expanded CD4(+)CD25(+) immune regulatory cells inhibits graft-versus-host disease lethality. *Blood* **99**, 3493–3499.
59. Suda, T., Zlotnik, A. (1992) In vitro induction of CD8 expression on thymic pre-T cells. I. Transforming growth factor-beta and tumor necrosis factor-alpha induce CD8 expression on CD8- thymic subsets including the CD25+CD3-CD4-CD8- pre-T cell subset. *J. Immunol.* **148**, 1737–1745.
60. Farr, A. G., Dooley, J. L., Erickson, M. (2002) Organization of thymic medullary epithelial heterogeneity: implications for mechanisms of epithelial differentiation. *Immunol. Rev.* **189**, 20–27.
61. Schluns, K. S., Grutkoski, P. S., Cook, J. E., Engelmann, G. L., Le, P. T. (1995) Human thymic epithelial cells produce TGF-beta 3 and express TGF-beta receptors. *Int. Immunol.* **7**, 1681–1690.
62. Oida, T., Zhang, X., Goto, M., Hachimura, S., Totsuka, M., Kaminogawa, S., Weiner, H. L. (2003) CD4+CD25- T Cells That Express Latency-Associated Peptide on the Surface Suppress CD4+CD45RBhigh-Induced Colitis by a TGF-beta-Dependent Mechanism. *J. Immunol.* **170**, 2516–2522.
63. Papiernik, M., de Moraes, M. L., Pontoux, C., Vasseur, F., Penit, C. (1998) Regulatory CD4 T cells: expression of IL-2R alpha chain, resistance to clonal deletion and IL-2 dependency. *Int. Immunol.* **10**, 371–378.
64. Sacklack, B., Lohler, J., Schorle, H., Klebb, G., Haber, H., Sichel, E., Noelle, R. J., Horak, I. (1995) Generalized autoimmune disease in interleukin-2-deficient mice is triggered by an uncontrolled activation and proliferation of CD4+ T cells. *Eur. J. Immunol.* **25**, 3053–3059.
65. Willerford, D. M., Chen, J., Ferry, J. A., Davidson, L., Ma, A., Alt, F. W. (1995) Interleukin-2 receptor alpha chain regulates the size and content of the peripheral lymphoid compartment. *Immunity* **3**, 521–530.
66. Suzuki, H., Kundig, T. M., Furlonger, C., Wakeham, A., Timms, E., Matsuyama, T., Schmits, R., Simard, J. J., Ohashi, P. S., Griesser, H., et al. (1995) Deregulated T cell activation and autoimmunity in mice lacking interleukin-2 receptor beta. *Science* **268**, 1472–1476.
67. Wolf, M., Schimpl, A., Hunig, T. (2001) Control of T cell hyperactivation in IL-2-deficient mice by CD4(+)CD25(-) and CD4(+)CD25(+) T cells: evidence for two distinct regulatory mechanisms. *Eur. J. Immunol.* **31**, 1637–1645.
68. Letterio, J. J., Geiser, A. G., Kulkarni, A. B., Dang, H., Kong, L., Nakabayashi, T., Mackall, C. L., Gress, R. E., Roberts, A. B. (1996) Autoimmunity associated with TGF-beta1-deficiency in mice is dependent on MHC class II antigen expression. *J. Clin. Invest.* **98**, 2109–2119.
69. Furtado, G. C., Curotto de Lafaille, M. A., Kutchukhidze, N., Lafaille, J. J. (2002) Interleukin 2 signaling is required for CD4(+) regulatory T cell function. *J. Exp. Med.* **196**, 851–857.
70. Malek, T. R., Yu, A., Vincek, V., Scibelli, P., Kong, L. (2002) CD4 regulatory T cells prevent lethal autoimmunity in IL-2Rbeta-deficient mice. Implications for the nonredundant function of IL-2. *Immunity* **17**, 167–178.
71. Zeller, J. C., Panoskaltis-Mortari, A., Murphy, W. J., Ruscetti, F. W., Narula, S., Roncarolo, M. G., Blazar, B. R. (1999) Induction of CD4+ T cell alloantigen-specific hyporesponsiveness by IL-10 and TGF-beta. *J. Immunol.* **163**, 3684–3691.
72. Chen, Z. M., O'Shaughnessy, M. J., Gramaglia, I., Panoskaltis-Mortari, A., Murphy, W. J., Narula, S., Roncarolo, M. G., Blazar, B. R. (2003) IL-10 and TGF-beta induce alloreactive CD4+CD25- T cells to acquire regulatory cell function. *Blood* **100**, 5076–5083.
73. Cottrez, F., Groux, H. (2001) Regulation of TGF-beta response during T cell activation is modulated by IL-10. *J. Immunol.* **167**, 773–778.
74. Jutel, M., Akdis, M., Budak, F., Aebischer-Casaulta, C., Wrzyszczyk, M., Blaser, K., Akdis, C. A. (2003) IL-10 and TGF-beta cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. *Eur. J. Immunol.* **33**, 1205–1214.
75. Terui, T., Sano, K., Shirota, H., Kunikata, N., Ozawa, M., Okada, M., Honda, M., Tamura, G., Tagami, H. (2001) TGF-beta-producing CD4+ mediastinal lymph node cells obtained from mice tracheally tolerized to ovalbumin (OVA) suppress both Th1- and Th2-induced cutaneous inflammatory responses to OVA by different mechanisms. *J. Immunol.* **167**, 3661–3667.
76. Oswald, I. P., Gazzinelli, R. T., Sher, A., James, S. L. (1992) IL-10 synergizes with IL-4 and transforming growth factor-beta to inhibit macrophage cytotoxic activity. *J. Immunol.* **148**, 3578–3582.
77. Suberville, S., Belloq, A., Peguillet, I., Lantz, O., Stordeur, P., Fouqueray, B., Baud, L. (2001) Transforming growth factor-beta inhibits interleukin-10 synthesis by human monocytic cells. *Eur. Cytokine Netw.* **12**, 141–146.
78. Van Vlasselaer, P., Borremans, B., van Gorp, U., Dasch, J. R., De Waal-Malefyt, R. (1994) Interleukin 10 inhibits transforming growth factor-beta (TGF-beta) synthesis required for osteogenic commitment of mouse bone marrow cells. *J. Cell Biol.* **124**, 569–577.
79. Ohtsuka, K., Gray, J. D., Stimmler, M. M., Toro, B., Horwitz, D. A. (1998) Decreased production of TGF-beta by lymphocytes from patients with systemic lupus erythematosus. *J. Immunol.* **160**, 2539–2545.
80. Llorente, L., Richaud-Patin, Y., Wijdenes, J., Alcocer-Varela, J., Maillot, M. C., Durand-Gasselien, I., Fourrier, B. M., Galanaud, P., Emilie, D. (1993) Spontaneous production of interleukin-10 by B lymphocytes and monocytes in systemic lupus erythematosus. *Eur. Cytokine Netw.* **4**, 421–427.
81. Hahm, K. B., Im, Y. H., Lee, C., Parks, W. T., Bang, Y. J., Green, J. E., Kim, S. J. (2000) Loss of TGF-beta signaling contributes to autoimmune pancreatitis. *J. Clin. Invest.* **105**, 1057–1065.
82. Demols, A., Van Laethem, J. L., Quertinmont, E., Degraef, C., Delhaye, M., Geerts, A., Deviere, J. (2002) Endogenous interleukin-10 modulates fibrosis and regeneration in experimental chronic pancreatitis. *Am. J. Physiol. Gastrointest. Liver Physiol.* **282**, G1105–G1112.
83. Baecher-Allan, C., Viglietta, V., Hafler, D. A. (2002) Inhibition of human CD4+CD25+high regulatory T cell function. *J. Immunol.* **169**, 6210–6217.
84. Pasare, C., Medzhitov, R. (2003) Toll pathway-dependent blockade of CD4+CD25+ T cell-mediated suppression by dendritic cells. *Science* **299**, 1033–1036.
85. Caramalho, I., Lopes-Carvalho, T., Ostler, D., Zelenay, S., Haury, M., Demengeot, J. (2003) Regulatory T cells selectively express toll-like receptors and are activated by lipopolysaccharide. *J. Exp. Med.* **197**, 403–411.
86. Chen, Z. M., O'Shaughnessy, M. J., Gramaglia, I., Panoskaltis-Mortari, A., Murphy, W. J., Narula, S., Roncarolo, M. G., Blazar, B. R. (2003) IL-10 and TGF-beta induce alloreactive CD4+CD25- T cells to acquire regulatory cell function. *Blood* **101**, 5076–5083.
87. Trentham, D. E., Dynesius-Trentham, R. A., Orav, E. J., Combitchi, D., Lorenzo, C., Sewell, K. L., Hafler, D. A., Weiner, H. L. (1993) Effects of oral administration of type II collagen on rheumatoid arthritis. *Science* **261**, 1727–1730.