Commentary

The potential of human regulatory T cells generated *ex vivo* as a treatment for lupus and other chronic inflammatory diseases

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Abstract

Regulatory T cells prevent autoimmunity by suppressing the reactivity of potentially aggressive self-reactive T cells. Contact-dependent $CD4^+$ $CD25^+$ 'professional' suppressor cells and other cytokine-producing $CD4^+$ and $CD8^+$ T-cell subsets mediate this protective function. Evidence will be reviewed that T cells primed with transforming growth factor (TGF)-β expand rapidly following restimulation. Certain $CD4^+$ T cells become contact-dependent suppressor cells and other $CD4^+$ and $CD8^+$ cells become cytokine-producing regulatory cells. This effect is dependent upon a sufficient amount of IL-2 in the microenvironment to overcome the suppressive effects of TGF-β. The adoptive transfer of these suppressor cells generated *ex vivo* can protect mice from developing chronic graft versus host disease with a lupus-like syndrome and alter the course of established disease. These data suggest that autologous T cells primed and expanded with TGF-β have the potential to be used as a therapy for patients with systemic lupus erythematosus and other chronic inflammatory diseases. This novel adoptive immunotherapy also has the potential to prevent the rejection of allogeneic transplants.

Keywords: autoimmunity, IL-2, regulatory T cells, systemic lupus erythematosus, transforming growth factor-β

Introduction

It has become evident that self-reactive T cells with the potential to cause autoimmune disease comprise a part of the normal T-cell repertoire, but their activation is prevented by suppressor cells [1–3]. Although originally described in the 1970s [4], significant progress in characterizing suppressor T-cell subsets has been made only recently, where they have been renamed 'regulatory' T cells.

A subset of thymus-derived $CD4^+$ cells that constitutively expresses CD25, the α-chain of the IL-2 receptor, protect their host from spontaneous organ-specific autoimmune diseases. These $CD4^+$ $CD25^+$ cells have been called 'professional' suppressor cells and have a contact-dependent mechanism of action, at least *in vitro* [5]. Other subsets of $CD4^+$ and $CD8^+$ cells, natural killer T cells, and cells displaying γδ TCRs also have downregulatory (suppressor) activity. In the periphery, suppressor T cells generated in response to environmental antigens protect their hosts from immune-mediated tissue injury by producing immunosuppressive cytokines.

The mechanisms responsible for the generation of suppressor T cells were poorly understood until recently. Our group has accumulated evidence that the multifunctional cytokine transforming growth factor-β (TGF-β) plays an essential role in the expansion of thymus-derived, professional, $CD4^+$ $CD25^+$ precursors that circulate in the blood. TGF-β also plays a key role in the generation of peripherally induced $CD4^+$ and $CD8^+$ cytokine-producing suppressor cell subsets.

This article will briefly review the evidence for contact-mediated and cytokine-producing suppressor cells, especially in humans, and the role of TGF-β in the generation of these cells. This knowledge can be used to generate suppressor T cells *ex vivo* in large numbers, and raises the possibility that the transfer of these cells back to the donor

CTLA-4 = cytotoxic T-lymphocyte-associated antigen-4; TGF-β = transforming growth factor-β; IL = interleukin; MHC = major histocompatibility complex; SLE = systemic lupus erythematosus; TCR = T-cell receptor; Th = T helper cells; Tr1 = Treg 1 regulatory $CD4^+$ cells.

can serve as a therapy for autoimmune diseases such as systemic lupus erythematosus (SLE). This T-cell-based therapy could also be used to prevent graft rejection.

Thymus-dependent, 'professional', contact-dependent, regulatory T cells

The existence of thymus-derived suppressor cells was suggested by studies in mice where neonatal thymectomy on day 3 led to the development of a multiorgan autoimmune disease [6]. This disease is due to the loss of $CD4^+$ $CD25^+$ suppressor cells that do not appear until the first week after birth [7,8]. Mature $CD4^+$ $CD25^+$ cells are found in the $CD45RB^{low}$ activated/memory fraction mouse T cells. Because potentially aggressive, self-reactive T cells are found in the $CD45RB^{hi}$ naive fraction of mouse T cells, the injection of $CD45RB^{hi}$ cells from nonautoimmune, normal mice into immunodeficient mice results in generalized, multiorgan inflammatory disease. Similar to neonatal thymectomy, this disease is prevented by supplementing the injected cells with purified $CD4^+$ $CD25^+$ cells [9,10]. Because these thymus-derived $CD4^+$ $CD25^+$ T cells appear to be crucial for the prevention of spontaneous autoimmune diseases, they have been called 'professional' suppressor cells [5,8].

In general, the properties of rodent and human $CD4^+$ $CD25^+$ T cells appear to be very similar. In humans, 6–18% of $CD4^+$ T cells constitutively express CD25 [11–17]. Purified $CD4^+$ $CD25^+$ cells do not proliferate in response to cross-linking of their TCRs. They inhibit the activation of other T cells by a contact-dependent mechanism [5–17]. A large percentage constitutively express intracellular cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4 or CD152), the IL-2 receptor β-chain (CD122), transferrin receptors (CD71) and class II MHC markers [17].

Almost all the $CD4^+$ $CD25^+$ are in the 'activated' state ($CD45RB^{low}$ in the mouse, $CD45RA^-$ RO^+ in the human). This suggests they may be continuously stimulated by their internal environment. Although activation of $CD4^+$ $CD25^+$ cells is antigen specific, once these cells are activated they not only suppress T cells stimulated by the same antigen, but they also inhibit T cells stimulated by other antigens; so-called bystander effects [18]. Although $CD4^+$ $CD25^+$ cells are nonresponsive to cross-linking their TCRs, they do proliferate when costimulated with IL-2 or anti-CD28.

Cytokine production by $CD4^+$ $CD25^+$ cells is controversial. While some groups claim that these cells do not produce cytokines [8,17], other groups have found that they can produce IL-10 [12,13,19], TGF-β [15,19], IL-4 [12] and low amounts of interferon-γ [15]. All groups agree that these cells do not produce IL-2 and that they have a contact-dependent mechanism of action. Their suppressive activities are not abolished by neutralizing antibodies to IL-10, and all groups agree that suppression is not abolished by anti-TGF-β, but for one exception [19]. Nakamura *et al*. reported that immunosuppression by $CD4^+$ $CD25^+$ regulatory T cells is mediated by cell surface-bound TGF-β [19]. Many of these differences can possibly be explained by the heterogeneity of $CD4^+$ $CD25^+$ T cells. One group separated human $CD4^+$ $CD25^+$ cells into high- and low-intensity fractions by cell sorting, and they found that the suppressive effects were only displayed by the high-intensity fraction [17]. This subset did not produce cytokines.

Cytokine-dependent regulatory T cells

$CD8^+$ and $CD4^+$ T cells that produce immunosuppressive cytokines have been described. Those that produce predominantly TGF-β and variable amounts of IL-10 and IL-4 have been called Th3-type cells, and they have been generated *in vivo* by immunization through an oral or other mucosal route [2,20]. This route of antigen administration, however, does not only result in Th3 cells. Both Th2 cells and $CD4^+$ $CD25^+$ cells can also be generated by this procedure [20–22]. The conditions needed for the generation of Th3 cells are poorly understood.

Other workers have produced regulatory $CD4^+$ cells by repeatedly stimulating with the antigen in the presence of IL-10 [23–26], or using immature antigen-presenting cells that lack potent costimulatory activity [27]. These regulatory $CD4^+$ cells have been called Treg 1 (Tr1) cells and they produce significant quantities of IL-10. They do not proliferate in response to antigen and do not produce IL-2. Therefore, they are anergic.

Th3 and Tr1-like cells have been described in humans. One group has reported the appearance of Th3 cells in patients with multiple sclerosis following oral administration of myelin basic protein [28]. Human Tr1 cells suppressed an alloantigen-induced proliferative response [29]. Th3 or Tr1 cells mediate antigen-specific cellular hyporesponsiveness in patients with chronic helminth infections [30].

The fact that some regulatory T cells produce predominantly TGF-β and others IL-10 is not fortuitous. The combination of TGF-β and IL-10 is more immunosuppressive than either of the cytokines by themselves [31]. Significantly, shortly after antigen activation, T cells downregulate their signal transducing type II receptor (TGF-βRII) and become refractory to the effects of TGF-β [32]. These cells then become mature effector cells. IL-10 appears as a feedback regulator later in the response and induces the re-expression of TGF-βRII. The synergistic inhibitory effects of TGF-β and IL-10 then terminate the response.

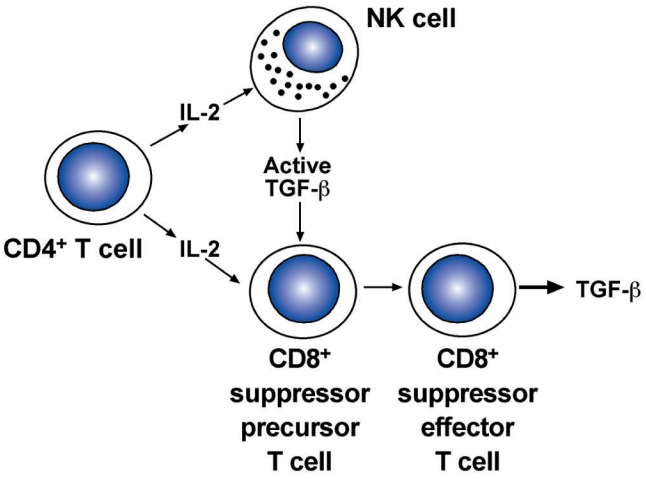
Whether Th3 cells and Tr1 cells come from similar precursors or comprise different subsets of regulatory T cells is not known. Many variables determine the differentiation

pathway that a naive T cell will take following activation. These include the antigen concentration and route of administration, the cytokine milieu, and the pattern of co-stimulatory signals. Self-MHC-reactive T cells in humans can either provide B-cell helper function or suppress antibody production, depending on how they are activated. In each case, regulatory function depends on the cytokines produced [33]. In determining the T-cell response to myelin basic protein, another group found that TCR usage was similar whether the T cells became Th1 encephalitogenic cells or regulatory Th3 cells [34]. These studies suggest common precursors for T cells that take different differentiation pathways.

TGF-β induces CD4+ and CD8+ T cells to become suppressor cells

While TGF-β has well-known inhibitory effects on lymphocyte cytokine production and functional properties [35], our laboratory has accumulated data that these effects can be overcome by IL-2 and can be superceded by co-stimulatory activities. The net effect is that TGF-β induces IL-2-activated CD8+ and CD4+ T cells to develop potent suppressive activities. In parallel, other groups have observed that TGF-β inhibits the differentiation of T cells to Th1 or Th2 subsets [36,37].

Figure 1



The role of transforming growth factor-β (TGF-β) in the differentiation pathway of CD8+ regulatory T cells. In response to antigen stimulation, the combination of IL-2 produced by CD4+ cells and the active form of TGF-β produced by natural killer (NK) cells or macrophages (not shown) induce CD8+ cells to lose their cytotoxic potential and become regulatory, TGF-β-producing, Th3-like cells. IL-2 also enhances the extracellular conversion of TGF-β from the latent to the biologically active form.

The initial observation that TGF-β is an IL-2-dependent differentiation factor for regulatory T cells was made in a study designed to determine the conditions required for human CD8+ T cells to become suppressors of antibody production. Using a model where we could induce T-cell-dependent antibody production without accessory cells, we found that CD4+ T cells, by themselves, lacked suppressor-inducing activity. The CD4+ cells produced IL-2 but, notwithstanding previous reports [38,39], this cytokine could not induce suppressor cells by itself. We learned that the interaction of IL-2-activated natural killer cells with CD8+ cells leads to the production of active TGF-β, and that the presence of this cytokine was critical for CD8+ cells to suppress antibody production (Figure 1) [40,41]. Moreover, the suppression was cytokine dependent and was abolished by a neutralizing anti-TGF-β monoclonal antibody (JD Gray and DA Horwitz, unpublished observation, 2000). Both IL-2 and TGF-β were thus critical for CD8+ cells to become Th3-like regulatory cells.

We have also induced CD4+ T cells to become Th3 cells. We used the superantigen, staphylococcal enterotoxin B, as the T-cell activating agent. Low-dose staphylococcal enterotoxin B can induce T-cell-dependent antibody production without additional accessory cells [42]. Briefly exposing CD4+ cells to TGF-β downregulated B-cell helper activity and induced certain CD4+ cells to develop suppressive activity that was neutralized by anti-TGF-β. Activating both CD4+ and CD8+ cells in the presence of TGF-β thus induced them to develop cytokine-dependent suppressive activity [43]. Other workers have also reported similar effects of TGF-β on CD8+ T cells [44]. One group found that IL-4 and TGF-β are involved in the differentiation of naive CD4+ cells to cytokine-producing Th3-type cells [45]. Another group reported that *in vitro* differentiation of Th3-type cells from Th0 precursors from TCR transgenic mice is enhanced by culture with TGF-β [20].

We next focused our attention on the induction of naive (CD45RA+ RO−) CD4+ T cells to become suppressor cells. Using the alloantigens as the T-cell activating agent, we found that TGF-β induced naive CD4+ T cells to develop extremely potent suppressive activity. These CD4+ cells had the phenotype and functional characteristics of 'professional' regulatory T cells. Using the generation cytotoxic T-cell activity and T-cell proliferation to assess suppressive activity, we learned that the suppressor cells were CD25+, and that a large percentage expressed CTLA-4. Their suppressive effects were contact dependent and were not neutralized by anti-TGF-β or IL-10. Adding less than 1% of these cells to T cells strongly inhibited the generation of cytotoxic T-lymphocyte activity by preventing the activation of CD8+ cells [14]. Other workers have also reported that CD4+ CD25+ cells have potent suppressive effects on CD8+ cells. Rodent CD4+ CD25+ regulatory cells cause CD8+ cells to enter cycle arrest [46].

The precursors of the human CD4+ CD25+ T cells induced by IL-2 and TGF-β appear to be the small number of CD25+

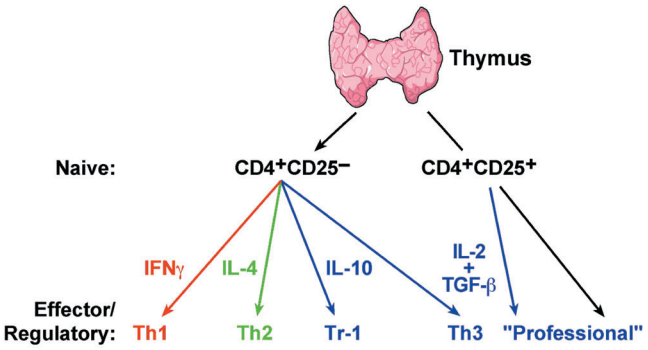
cells in the naive fraction. Although <1% of these cells express CD25, depletion of these cells abrogated the generation of suppressive activity in some experiments [14]. The principal difference between the cytokine-induced $CD4^+$ $CD25^+$ cells and the murine and human positively selected $CD4^+$ $CD25^+$ cells that are predominantly found in the $CD45RO^+$ 'memory' fraction is their capacity for expansion. The positively selected cells are anergic while the $CD4^+$ $CD25^+$ cells generated from naive cells can be expanded in IL-2 and retain their suppressive activity [14].

Studies on the mechanism of action of TGF-β have revealed that this cytokine has potent costimulatory effects on IL-2-activated T cells. These effects include upregulation of CD25, CTLA-4 and CD40 ligand expression on $CD4^+$ cells [14,47], and increased tumor necrosis factor-α production by both $CD4^+$ and $CD8^+$ cells [47]. The TGF-β costimulated human $CD4^+$ T cells are resistant to activation-induced apoptosis. They took up less annexin and expanded fivefold greater in primary cultures than control, alloactivated $CD4^+$ T cells [14] (SG Zheng and DA Horwitz, unpublished observations, 2001). Some workers have reported that TGF-β can accelerate activation-induced cell death of some T cells [48,49], while others observed that this cytokine protected T cells from apoptosis [50,51]. We favor the hypothesis that TGF-β promotes the death of mature Th1 and Th2 cells while protecting newly generated regulatory T cells from undergoing apoptosis. This view is consistent with a report indicating positive effects of TGF-β on naive T cells [52].

In summary, using several different stimuli to activate T cells, we have found that the combination of IL-2 and TGF-β can induce $CD4^+$ and $CD8^+$ T cells to become either cytokine-producing Th3-like or contact-dependent professional suppressor cells. In our studies with $CD8^+$ cells, the cultures were always supplemented with IL-2. When human $CD4^+$ cells are activated in the presence of TGF-β by irradiated allogeneic stimulator cells or with superantigens, however, sufficient IL-2 is produced for the costimulatory effects of TGF-β and suppressor cell differentiation. By contrast, cultures with mouse lymphocytes must generally be supplemented with IL-2.

As shown in Figure 2, we propose that TGF-β induces thymic-derived CD25 precursors in the naive fraction of $CD4^+$ cells to expand and to become contact-dependent 'professional' regulatory T cells. TGF-β also induces $CD4^+$ and $CD8^+$ cells that are $CD25^-$ to become Th3-like cells. Although almost all naive $CD4^+$ cells are $CD25^-$, why the predominant TGF-β effect on T cells in this fraction is the generation of 'professional' regulatory T cells remains to be determined. Our finding that both IL-2 and TGF-β are critical in the generation of regulatory T cells is of particular importance in patients with SLE since production of IL-2 and the active form of TGF-β is decreased [53].

Figure 2



The role of transforming growth factor-β (TGF-β) in the differentiation pathway of $CD4^+$ regulatory T cells. Following T-cell activation where a sufficient amount of IL-2 is produced to overcome the inhibitory effects of TGF-β, the costimulatory effects of this cytokine induce the precursors of $CD4^+$ $CD25^+$ T cells to become contact-dependent 'professional' suppressor cells or induces $CD4^+$ $CD25^-$ cells to produce immunosuppressive quantities of TGF-β. IFN, interferon; Tr-1, Treg 1 regulatory $CD4^+$ cells.

In vivo effects of Treg

Cloned Th3 cells protect mice from several autoimmune diseases that include experimental allergic encephalitis, diabetes mellitus, colitis, and uveitis [20,29,54–56]. Cytokine-producing $CD8^+$ cells were described initially [55], but reports of $CD4^+$ cells with this characteristic have become predominant. Cloned Tr1 cells protect rodents from an experimental colitis [29]. Small numbers of adoptively transferred noncloned $CD4^+$ $CD25^+$ cells protect lymphopenic mice from developing spontaneous organ-specific autoimmune diseases and also protect animals from developing graft-versus-host disease [8–10,57].

We have begun to learn whether regulatory T cells generated *ex vivo* with TGF-β can have protective effects *in vivo*. For this purpose, we selected a mouse model of SLE that has a rapid onset. The transfer of parental T cells to F1 mice can result in acute or chronic graft-versus-host disease depending on the precursor frequency of $CD8^+$ parental cells reactive against the allogeneic MHC antigens [58,59]. The transfer of DBA/2 T cells into DBA/2 x C57BL/6 F1 mice results in a lupus-like syndrome with high titers of anti-DNA antibodies and an immune complex glomerulonephritis. While alloactivated DBA/2 T cells accelerated the disease, alloactivation of splenic T cells or $CD4^+$ cells in the presence of TGF-β markedly suppressed and even prevented the development of the lupus-like syndrome. Both anti-DNA antibody production and proteinuria were significantly suppressed [60]. Recent studies have revealed that these suppressor T cells can also alter the course of established disease. A single transfer of 5 million T cells conditioned with TGF-β markedly improved survival of these mice (SG Zheng and DA Horwitz, unpublished observations, 2001).

Since it has been possible to significantly expand regulatory T cells generated with TGF-β, it should be possible to generate sufficient numbers in humans for clinical trials. Although this will be carried out initially with mitogens as the T-cell activating agent, the ultimate goal is to induce autoantigen-specific regulatory T cells. This should be possible based on the progress being made in characterizing the pathogenic peptides that trigger autoimmune diseases. It may even be possible to induce potentially aggressive naive self-reactive cells to become protective suppressor cells by activating them with TGF-β. An adoptive immunotherapy using the patients own T cells that have regained a protective function they had lost should lack the serious toxic effects associated with the agents now in use. This treatment is especially promising in autoimmune diseases characterized by a relapsing and remitting course such as SLE, inflammatory bowel disease or certain forms of multiple sclerosis. The adoptive transfer of regulatory T cells generated *ex vivo* also has the potential to prevent the rejection of allogeneic organ transplants.

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