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Mechanisms of suppression by suppressor T cells

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Mechanisms of immunosuppression by CD4$^+$CD25$^+$ suppressor T cells have been addressed using many *in vitro* and *in vivo* conditions. However, those studies have not yielded a single mode of action. This review will discuss the mechanisms of suppression, which include the local secretion of cytokines such as TGF-β and direct cell contact through binding of cell surface molecules such as CTLA-4 on suppressor T cells to CD80 and CD86 molecules on effector T cells. Suppression requires the appropriate colocalization of suppressor and effector T cells in different tissue and may involve the interference with T cell receptor signaling that triggers transcription factors important in regulating effector cell function.

The finding that suppressor activity is enriched in CD4$^+$CD25$^+$ cells has permitted further phenotypic and functional characterization of this elusive subset of T cells[1]. Although CD4$^+$CD25$^+$ cells express an αβ T cell receptor (TCR) and can be found inside and outside the thymus, their ontogeny as well as relationship to other T cell subsets has been unclear (reviewed by Fontenot and Rudensky[2] in this issue). Observations in TCR-transgenic mice that coexpress an agonist ligand indicated that this condition favors the generation of large numbers of CD4$^+$CD25$^+$ suppressor (regulatory) T cells with the transgenic TCR[3]. Subsequent studies in two independent TCR-transgenic systems showed that expression of such agonist ligands in radioresistant tissues[4,5] and specifically in thymic epithelial cells[6] results in increased numbers of CD4$^+$CD25$^+$ thymocytes. The generation of CD4$^+$CD25$^+$ thymocytes and negative selection of CD4$^+$ thymocytes with the transgenic TCR are not mutually exclusive events[5,6]. This does not mean, however, that the increase in the proportion of CD4$^+$CD25$^+$ thymocytes is simply due to the reduced numbers of CD4$^+$CD25$^-$ thymocytes. In fact, this increase is mostly due to *de novo* generation of CD4$^+$CD25$^+$ thymocytes, as their absolute number is increased by a factor of 10–30 in various transgenic models[4–6]. The selection and generation of CD4$^+$CD25$^+$ suppressor T cells by agonist ligands is not a trivial issue, given that full activation of CD4$^+$CD25$^+$ suppressor T cells in secondary lymphoid tissue can be initiated by agonist ligands, and hence the generation as well as the activation of CD4$^+$CD25$^+$ cells can be achieved by the same ligand. Thus 'organ-specific' ligands that are ectopically expressed in the thymus[7] can be involved in the intrathymic generation of CD4$^+$CD25$^+$ suppressor cells as well as their activation in the periphery[6]. This is in contrast to the selection of CD4$^+$CD25$^-$ T cells, for which the nature of intrathymic peptides involved in positive selection has little predictive value concerning the nature of peptides that will cause activation of selected cells in secondary lymphoid organs. This scenario predicts that the CD4$^+$CD25$^+$ T cell repertoire is biased to recognize 'self' agonist ligands, which is consistent with recent results analyzing the specificity of TCRs from CD4$^+$CD25$^+$ cells of normal mice[8].

Thymic selection seems not to represent the only mode by which CD4$^+$CD25$^+$ suppressor T cells can be generated because application of agonist peptides in 'subimmunogenic' conditions (low dose and/or lack of costimulation) can convert naive T cells into CD4$^+$CD25$^+$ suppressor T cells[9]. This conversion occurs in conditions in which the possibility of proliferation of preexisting rather than *de novo* production of CD4$^+$CD25$^+$ regulatory T cells can be excluded and 'tutoring' by preformed CD4$^+$CD25$^-$ cells is not required[9]. These results are consistent with those of a variety of other studies in which a similar conversion was apparently noted but expansion of preexisting suppressor populations was less rigorously excluded and/or the phenotypic and functional characterization of CD4$^+$CD25$^+$ cells was less complete[10–12]. Thus, there is evidence in at least two different TCR-transgenic mouse strains that agonist ligands can achieve the conversion of naive T cells into true CD4$^+$CD25$^+$ suppressor T cells[9,11,12]. There is also consensus that to achieve this conversion, the ligand must be presented in 'subimmunogenic' conditions[9,11,12]. Nevertheless, further work is needed to develop protocols that achieve this goal more efficiently and with a greater variety of different ligands.

An additional characteristic of CD4$^+$CD25$^+$ suppressor T cells is their high expression of the Foxp3 transcription factor, which seems to have a key function in programming CD4$^+$CD25$^+$ suppressor T cells[13–15]. Although the focus of this review is on CD4$^+$CD25$^+$ suppressor T cells with high Foxp3 expression, other CD4 (refs. 16–18) as well as CD8 (ref. 19) T cells can mediate immunosuppression. As will become apparent below, the CD4$^+$CD25$^+$ Foxp3-expressing subset of suppressor T cells holds promise for the specific prevention of and/or interference with unwanted immunity in the absence of general immunosuppression, which is often associated with undesired and

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even deadly side effects. In contrast, interference with immunosuppression may considerably enhance desired immunity against tumors or infectious organisms (reviewed by Sakaguchi[20] and Belkaid and Rouse[21] in this issue). The specific induction of and interference with immunosuppression represent goals that require a detailed knowledge of how these cells are generated and operate *in vivo*. This review represents an attempt to summarize what has been learned in recent years about mechanisms of suppression by $CD25^+$ suppressor T cells.

In vitro suppression : The function of interleukin 2

Soon after it became apparent that $CD4^+CD25^+$ T cells are endowed with suppressor function *in vivo*, an *in vitro* system was established that has been widely used to analyze possible modes of suppression[22]. Typically, this system analyzes the proliferation of nonsuppressive $CD4^+$ and $CD8^+$ effector T cells either alone or in culture together with $CD25^+$ suppressor cells.

In vitro analyses have concluded that $CD25^+$ suppressor T cells are anergic; that is, they do not proliferate in culture when stimulated with antibodies to CD3 or antigens unless supplemented with high doses of interleukin 2 (IL-2)[22]. In the absence of exogenous IL-2, stimulated $CD25^+$ T cells suppress the proliferation of $CD4^+$ as well as $CD8^+$ T cells by a reaction that is independent of IL-10 and transforming growth factor-β (TGF-β) secretion, as has been shown with suppresor T cells from IL-10-deficient and TGF-β-deficient mice, which seem to suppress effectively[22]. However, others have postulated an essential function for cell-bound TGF-β on the basis of inhibition of suppression by antibodies to TGF-β[23]. The suppression of proliferation requires direct cell contact between suppressor and suppressed cells, as suppression does not occur when cells are separated by a permeable membrane. The presence of antigen-presenting cells (APCs) is not required, as suppression occurs in APC-free cultures. In all cases, the suppression requires activation of suppressor T cells by TCR ligands or antibodies to CD3 (ref. 22).

The target of suppression seems to be transcriptional control of *Il2* in effector cells[22]. A reevaluation of the function of IL-2 has concluded that the initial production of IL-2 by the cells to be suppressed is essential for initiation of the function (and some proliferation) of the suppressor cells[24]. Those *in vitro* experiments did not definitively rule out the possibility that the observed inhibition may often be due to the competitive consumption of IL-2 by suppressor T cells[25] that have much higher expression of the IL-2 receptor and hence favorably compete for IL-2, which represents an essential growth factor for freshly stimulated T cells *in vitro*. Overall, the *in vitro* results indicate that suppression involves direct cell contact between suppressor and effector T cells, targets *Il2* and thereby inhibits T cell proliferation.

Function of CLTA-4, CD80 and CD86

Some recent studies on immunosuppression *in vitro* have addressed the function of CD80 and CD86 ligands that are present on activated $CD4^+$ T cells[26]. These molecules are essential in $CD25^+$ cell–mediated suppression *in vitro*, as their absence from effector T cells results in notably reduced susceptibility to suppression by $CD4^+CD25^+$ suppressor T cells compared with that of wild-type effector T cells. On the basis of those observations, ligation of CD80 and CD86 on T cells by the cell surface molecule CTLA-4 on suppressor cells was postulated to cause 'outside-in' signaling by CD80 and/or CD86 ligands and to result in suppression[27] (also discussed below). This hypothesis could provide an explanation for the contact between suppressors and effectors that is apparently required for immunosuppression *in vitro* (**Fig. 1**).

The postulated function of a CTLA-4–CD80 or CTLA-4–CD86 receptor-ligand pair in T cell–T cell interaction differs from that postulated for the same receptor-ligand pair between CTLA-4 on suppressor T cells and CD80 and CD86 ligands on dendritic cells resulting in the activation of indoleamine 2,3-dioxygenase (IDO). IDO is responsible for the metabolism of the essential amino acid tryptophan. Reduced amounts of free tryptophan after induction of IDO are associated with decreased activation of T cells and thus this would represent an APC-dependent mechanism of *in vitro* suppression[28] (**Fig. 1**). This mechanism of suppression seems to be not essential *in vitro*, as suppression can be found in APC-free cultures[22].

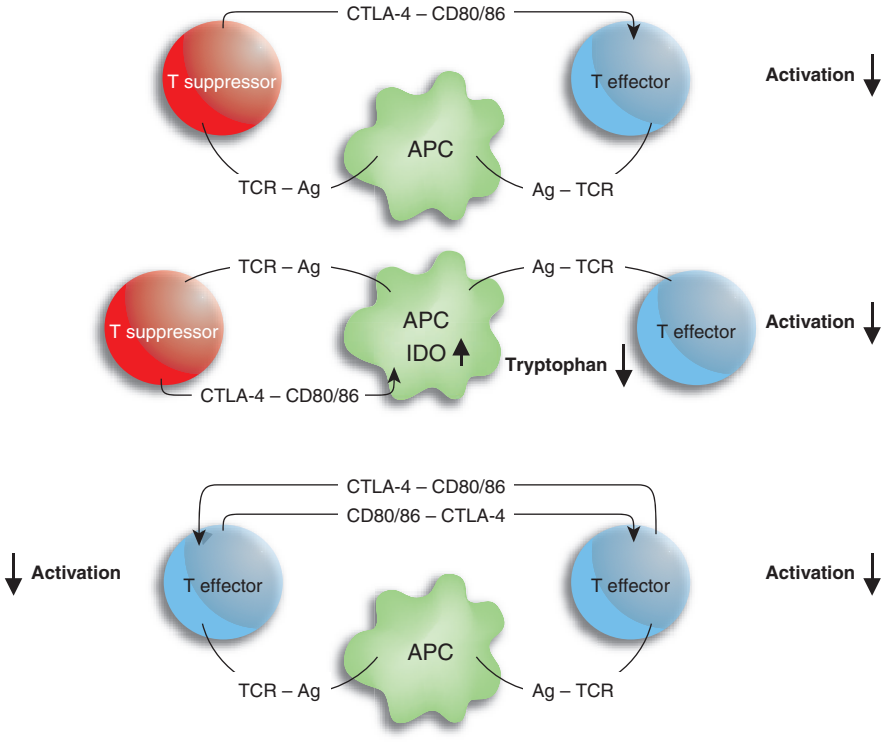

Figure 1 CTLA-4 and CD80 and/or CD86 in suppression. Top, suppressor T cells (T suppressor) and effector T cells (T effector) could meet at an APC, facilitating the binding of CTLA-4 on suppressors to CD80 and/or CD86 (CD80/86) on activated effectors. 'Outside-in' signaling by CD80 and/or CD86 would then prevent further activation of the effectors. Middle, suppressor T cells could activate IDO when CTLA-4 on suppressors bind to CD80 and/or CD86 on APCs. The IDO-dependent metabolism of tryptophan would prevent activation of effectors. Bottom, in the absence of suppresor T cells, mutual binding of CTLA-4 and CD80 and/or CD86 by on effector cells could interfere with their further activation through cell-autonomous inhibitory signals by CTLA-4. Ag, antigen.

Function of perforin and granzyme

Cytolytic activity has been invoked as a possible mechanism of suppression. Human

CD4$^+$CD25$^+$ Foxp3-expressing T cells can be activated by a combination of antibodies to CD3 and CD46 to express granzyme A and kill activated CD4$^+$ and CD8$^+$ T cells by a perforin-dependent mechanism in a reaction that does not involve Fas–Fas ligand binding[29]. Antibodies to CD18 interfere with the killing, suggesting that CD18 is involved in the interaction of suppressor T cells with their targets. Antibodies to CD3 and CD46 are superior to antibodies to CD3 and CD28 in inducing granzyme- and perforin-mediated killing[29]. It remains to be determined whether activation by antigen can have similar consequences.

Interference with suppression

Interference with the suppression by CD4$^+$CD25$^+$ T cells is probably required for most effective immune responses. This issue has been addressed by analysis of the involvement of cytokines and ligands on APCs in inhibiting suppression *in vitro*. Polyclonal activation of CD4$^+$CD25$^+$ suppressor T cells in the presence of APCs that have been activated through their Toll-like receptors by lipopolysaccharide or CpG dinucleotide ligands does not suppress the proliferation of naive T cells *in vitro*[30]. Moreover, supernatants from those stimulated APCs make T cells resistant to suppression. An essential component in the supernatants is IL-6, which may act in synergy with other unspecified factors to confer resistance of T cells to suppression[30] (**Fig. 2**). The fact that some immune responses in IL-6-deficient mice seem to be reduced as compared with those of wild-type mice is consistent with the proposed function of IL-6, but IL-6 may not represent the only molecule that confers resistance to suppression. In fact, in gene expression screens, CD4$^+$CD25$^+$ suppressor T cells show increased expression of the glucocorticoid-inducible TNF receptor (GITR)[31,32]. That receptor, however, not only is found on suppressor T cells but also is expressed on activated effector T cells. Initially it was concluded that ligation of GITR on CD4$^+$CD25$^+$ suppressor T cells alleviates immunosuppression mediated by these T cells, but the molecular mechanisms of this alleviation remained poorly understood[31,32]. A reevaluation with T cells from GITR-deficient mice has shown that ligation of GITR on CD4CD25$^-$ but not the CD4$^+$CD25$^+$ cells interferes with the suppression by making CD4CD25$^-$ cells less susceptible[33]. Thus, both IL-6 (ref. 30) and GITR[34] ligands may be involved in conferring resistance to suppression by CD4$^+$CD25$^+$ suppressor T cells by making effector T cells less susceptible and hence may constitute essential factors for the optimal activation of T cells (**Fig. 2**).

Requirements for *in vivo* suppression

Analysis of *in vivo* suppressor mechanisms must take into consideration the ability of CD4$^+$CD25$^+$ suppressor T cells to localize to various parts of the body such that close contact with most effector T cells with a given antigenic specificity either directly or through an APC intermediate becomes possible. Thus, unlike the situation in culture, there is ample space for T cells to evade suppression *in vivo* unless CD4$^+$CD25$^+$ suppressor T cells can localize together with them in response to antigenic stimulation that begins in antigen-draining lymph nodes. Therefore, migration and homing of suppressor T cells should closely resemble that of effector T cells to allow an effective interaction of suppressors with effectors when responding to ligands which are presented by APCs in the same microenvironment.

Homing and expansion of suppressor cell populations *in vivo*

CD4$^+$CD25$^+$ suppressor T cells that commit to suppression after antigenic stimulation inside or outside the thymus can survive for long periods of time without cell division in the absence of the antigen that induced their formation[9,35,36]. Such cells express L-selectin

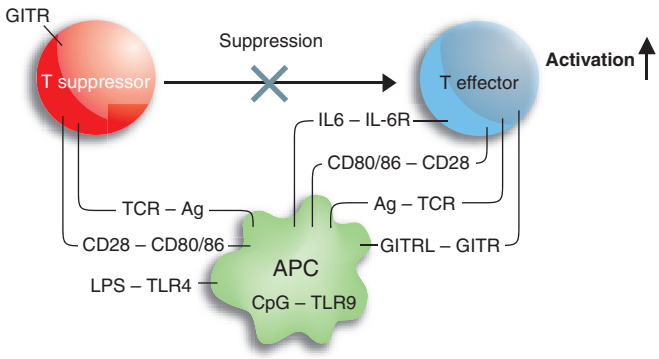

Figure 2 Making effector T cells insensitive to suppression. Activation of APCs through Toll-like receptors (TLR) after binding of lipopolysaccharide (LPS) or CpG dinucleotides results in production of IL-6 and/or GITR ligands (GITRL). Activated effector T cells expressing IL-6 receptors (IL-6R) and/or GITR will bind IL-6 and GITR ligand, which makes them insensitive to the action of suppressor T cells.

(CD62L) and apparently recirculate like naive T cells from blood to lymph, because after antigenic stimulation CD4$^+$CD25$^+$ suppressor T cells, like naive T cells, home to antigen-draining lymph nodes and can accumulate through cell division at roughly the same rate as naive T cells[5,36–38]. Thus, it seems that *in vivo*, specific homing and antigen-driven proliferation that are induced by TCR agonist ligands represent essential features that enable suppressor T cells to effectively suppress the local immune response. The importance of these features has been demonstrated by experiments in which suppressor T cells with various TCRs for antigens were analyzed for their capacity to prevent or revert diabetes in the nonobese diabetic mouse: only cells able to home to and proliferate in pancreatic lymph nodes by recognizing agonist TCR ligands were able to prevent or suppress disease after transfer into nonobese diabetic mice. In contrast, cells with similar suppressor potential *in vitro* but unable to accumulate and expand their populations in response to antigenic stimulation in draining lymph nodes were without any effect[39–42]. Thus, the specific homing to and activation of suppressor T cells in antigen-draining lymph nodes reflect features of suppressor T cells that are mandatory for effective suppression early during an immune response. In fact, early suppression in draining lymph nodes can interfere with the exit of effector T cells into inflamed tissue because of their reduced expression of the chemokine receptor CXCR3 (ref. 43).

Exit into inflamed tissue

During the course of antigenic stimulation, suppressor T cells, much like effector T cells, can change their migratory activity and exit into inflamed tissues. It would seem that this property is of crucial importance in dealing with activated T cells that no longer recirculate but are present in nonlymphoid tissue, such as effector T cells that are involved in the destruction of β-cells in pancreatic islets. CD4$^+$CD25$^+$ suppressor T cells that have been continually activated can change their homing receptors and, in the mouse, become positive for the $\alpha_E\beta_7$ integrin, which permits these cells to exit into inflamed tissue and suppress activated T cells[44]. Notably, $\alpha_E\beta_7$-positive, L-selectin-negative CD4$^+$CD25$^+$ suppressor T cells are more effective in suppressing a local inflammatory reaction than are $\alpha_E\beta_7$-negative, L-selectin-positive CD4$^+$CD25$^+$ suppressor T cells with the same antigenic specificity[44]. Thus, the capacity of suppressor T cells to suppress other T cells is not restricted to inhibition of their early

proliferation but also involves the suppression of effector function of activated T cells in inflamed tissues. It is evident, therefore, that the ability of suppressor T cell populations to expand *in vivo* and to home to distinct sites during an ongoing immune response after stimulation by TCR agonist ligands represents an essential feature that permits the regulation of immunity at various stages of the immune response.

Reversible suppression

Although *in vitro* studies have not consistently addressed the fate of suppressed T cells, a chief function for direct cytotoxicity by $CD4^+CD25^+$ Foxp3-expressing cells in the elimination of T cells *in vivo* seems unlikely, at least in murine species. Early experiments in mice coexpressing a major histocompatibility complex class II–restricted TCR and its agonist ligand intra- and extra-thymically showed that $CD4^+CD25^+$ suppressor and $CD4^+CD25^-$ suppressed cells can coexist for long periods of time[3]. Furthermore, various studies that have analyzed $CD4^+CD25^+$ suppressor and $CD4^+CD25^-$ suppressed cells in antigen-draining lymph nodes have not provided any evidence for increased apoptosis of suppressed cells[35]. Thus, direct cytotoxicity as an explanation for suppression of $CD4^+$ and $CD8^+$ T cells *in vivo* seems unlikely, at least in mice. In contrast, *ex vivo* analysis of suppressed cells has indicated that the *in vivo* expression is fully reversible in that separation of suppressor and suppressed cells demonstrates an intact functional potential of the latter, as shown by the fact that isolated $CD4^+CD25^-$ cells can proliferate normally in the absence of suppressors and produce IL-2 after antigenic stimulation[3,35]. Therefore, the preceding studies are not compatible with the hypothesis that suppressor cells generally either kill effector cells or irreversibly inactivate genes whose expression is required during immune responses. The idea of reversible suppression extends not only to the production of IL-2 but also to the secretion of cytokines such as interferon-γ (IFN-γ). When $CD4^+CD25^+$ suppressor T cells and $CD4^+CD25^-$ naive T cells with the same antigenic specificity are injected together and cytokine secretion of both populations is monitored during the course of the immune response in antigen-draining lymph nodes, commitment to IFN-γ secretion is not irreversibly suppressed. By bypassing TCR signaling through stimulation with phorbol 12-myristate 13-acetate and ionomycin, the same fraction of IFN-γ-producing cells can be shown among $CD25^-$ T cells on a per-cell basis whether or not these cells were injected together with suppressor cells[35]. These results emphasize that suppression can occur late during an immune response when T cells are already committed to IFN-γ production and that suppression can interfere with TCR signaling that results in cytokine secretion but not with the already established commitment of cells to secrete certain cytokines, at least during the time period of observation. The late interference may explain why suppressor T cells are able to not only prevent but also revert diabetes early after the onset of disease[40,41]. Reversion of autoimmune disease has also been noted after injection of $CD4^+CD25^+$ cells into mice suffering from colitis[45]. Thus, $CD4^+CD25^+$ suppressor T cells can interfere with an immune response at different stages that are characterized by the secretion of distinct cytokines, and the effect of suppression on $CD25^-$ cells is mostly reversible, at least for some time after suppression has set in. However, this scenario does not exclude the possibility that $CD4^+CD25^+$ suppressor T cells can generate a milieu in which some effector T cells are converted into suppressor cells, perhaps much in the way as described for the conversion of naive T cells[9], a phenomenon known as 'infectious tolerance'. Alternatively, some $CD4^+CD25^-$ cells may be induced by $CD4^+CD25^+$ T cells to secrete TGF-β and/or IL-10 as proposed in some models of 'infectious tolerance'[46].

Function of IL-2 *in vivo*

It was apparent from the very beginning, when IL-2 production by T cells was invoked as a target of immune suppression *in vitro*, that this could not represent the only mechanism by which suppressor cells interfere with immunity *in vivo*. Analysis of IL-2 or IL-2 receptor (IL-2R)–deficient mice has shown that IL-2 does not represent an essential growth factor for T cells *in vivo* and that immune responses proceed very effectively in the absence of IL-2 (ref. 47). In fact, IL-2- or IL-2R-defective mice suffer not from immunodeficiency but from severe autoimmunity that develops several weeks after birth[48,49]. Although the latter was initially attributed to defective Fas-mediated apoptosis of activated T cells[50], which could be demonstrated *in vitro*, this seems to represent a less likely scenario of the autoimmune phenotype *in vivo*, which can be 'cured' by the transfer of $CD4^+CD25^+$ suppressor T cells[49,51]. In fact, IL-2- and IL-2R-deficient mice are also deficient in $CD4^+CD25^+$ suppressor T cells in secondary lymphoid organs, as IL-2 represents an essential factor required to maintain the number and functional integrity of $CD4^+CD25^+$ suppressor T cells[52–54].

The fact that $CD4^+CD25^+$ suppressor T cells can suppress the autoimmune response of IL-2R-deficient T cells[49] excludes the possibility that competition for IL-2 or suppression of *Il2* transcription[22] represent an essential mechanism of suppression *in vivo*. Thus, *in vivo* there must be targets other than *Il2* that are affected by $CD4^+CD25^+$ suppressor T cells.

Function of CTLA-4, CD80 and CD86 *in vivo*

Involvement of CTLA-4, CD80 and CD86 in T cell–T cell interaction *in vivo* has been postulated on the basis of experiments involving a graft-versus-host reaction of $CD4^+CD25^-$ cells when injected into lymphopenic recipients deficient in recombination-activating gene 2. Disease occurs when wild-type and CD80-deficient as well as CD86-deficient $CD25^-$ T cells are injected. Injection of $CD4^+CD25^+$ T cells together with $CD4^+CD25^-$ cells prevents disease caused by wild-type T cells but not T cells deficient in CD80 and/or CD86. The accumulation of $CD4^+CD25^-$ cells deficient in CD80 and/or CD86 after transfer together with $CD4^+CD25^+$ cells is almost completely prevented when the these cells are transduced with intact CD80 and CD86 and much less so when cells are transduced with cDNA encoding CD80 and CD86 with an altered cytoplasmic tail that results in surface expression in a glycosylphosphatidyl inositol–linked form. Those results are explained by an 'outside-in' signaling model of CD80 and CD86 that results in suppression of T cell activation when these ligands on activated T cells are bound by CTLA-4 on suppressor T cells[27] (**Fig. 1**). Those results are consistent with earlier studies of chimeras composed of CTLA-4-deficient and CTLA-4-competent hematopoietic cells. The extensive lymphoproliferation of CTLA-4-deficient cells was not noted in the presence of CTLA-4-competent cells; that is, it was not cell autonomous[55,56]. The phenotype of such chimeric mice could also be explained, however, by arguing that CTLA-4-competent suppressor T cells can affect APCs (for example, by inducing IDO) and thus prevent the activation of CTLA-4-deficient T cells[28] (**Fig. 1**). Both scenarios (that is, a CTLA-4-dependent interaction of regulatory T cells with other T cells or APCs) cannot be used to explain the finding that CD80- and CD86-deficient T cells are more potent than wild-type T cells in inducing graft-versus-host disease after depletion of regulatory T cells[26]. Thus, in the last scenario, a cell-autonomous effect of CTLA-4 signaling on T cells may contribute to the reduced potential of wild-type T cells to cause graft-versus-host disease (**Fig. 1**).

Function of IL-10 *in vivo*

Even though IL-10 is not essential for suppression *in vitro*[22], IL-10 is considered to be one of the key molecules involved in immunosuppression, and thus the involvement of IL-10 in immunosuppression *in vivo* merits careful consideration. Initially it was reported that chronic antigenic stimulation *in vivo* could result in the generation of $CD4^+$ T cells that were anergic in terms of proliferation *in vitro* and secreted IL-10 (ref. 16), a result well in line with observations that IL-10 was important in unresponsiveness that was induced *in vivo*[57–59]. Subsequently, it was shown that $CD25^-$ cells without high expression of Foxp3 but nevertheless able to secrete IL-10 could be obtained through chronic antigenic stimulation[18,60]. Such cells, which are known as T_R1 cells, were also reported to arise in certain culture conditions[61,62]. Those findings do not preclude the possibility, however, that $CD4^+CD25^+$ cells can also produce IL-10. In fact, $CD4^+CD25^+$ T cells produce IL-10 *in vivo*[35,63], and it has been established that certain forms of immunity such as colitis can be suppressed by $CD4^+CD25^+$ T cells and require their secretion of IL-10 (refs. 64,65), whereas others such as autoimmune gastritis[22,65] can be suppressed independently of IL-10. Those observations suggest that $CD4^+CD25^+$ T cells have several modes of suppressive action at their disposal that may depend on the microenvironment in which the suppressor cells are activated and that may be differentially used to suppress different forms of immunopathology.

Function of TGF-β *in vivo*

Although using *in vitro* 'readouts' the function of TGF-β in suppression of T cell proliferation is controversial[23], there is some consensus that *in vivo*, the suppression of $CD8^+$ T cells that mediate autoimmunity[66] or tumor rejection[67] by $CD4^+CD25^+$ T cells requires an intact TGF-β receptor II on the $CD8^+$ T cells. Similar to $CD4^+$ T cells, $CD8^+$ T cells can be suppressed at different stages of their response such that population expansion, cytokine secretion and cytolytic activity are differentially affected. Studies using a model of $CD8^+$ T cell–mediated autoimmune diabetes have shown that $CD4^+CD25^+$ T cells can suppress disease caused by wild-type effector T cells but do not interefere with T cells with a dominant negative TGF-β receptor[66]. It is not clear whether in those studies[66] the defective TGF-β signaling was required for suppression of proliferation, cytokine secretion or cytolytic activity, and thus it is possible that suppression of all three or only one requires TGF-β. Some studies have shown that $CD4^+CD25^+$ suppressor T cells allow extensive proliferation of $CD8^+$ T cells before suppression of their cytokine secretion and cytolytic activity[68]. However, other studies have shown that $CD4^+CD25^+$ regulatory T cells do not interfere with proliferation or IFN-γ secretion but specifically abolish cytolytic activity and tumor rejection[67]. In the last model, suppression of both cytolytic activity of $CD8^+$ T cells and tumor rejection required an intact TGF-β receptor II on $CD8^+$ T cells[67]. In that scenario, $CD8^+$ T cells still form conjugates with their targets but are unable to lyse them, indicating a specific TGF-β-dependent inhibition of cytolytic activity by $CD4^+CD25^+$ T cells rather than diminished cytolytic activity because of interference with the formation of the receptor synapse[67]. Thus, in contrast to the inhibition of $CD4^+$ T cell–dependent immunity in which no essential involvement of the TGF-β receptor on $CD4^+$ T cells was reported, in *in vivo* studies, $CD4^+CD25^+$ cell–mediated inhibition of $CD8^+$ T cells seems to have an obligatory requirement for intact TGF-β signaling in the latter, at least as far as suppression of cytolytic activity is concerned. It is not apparent whether this inhibition requires TGF-β production by the $CD4^+CD25^+$ suppressor T cells themselves or by other cells such as APCs. In some studies, $CD4^+CD25^+$ suppressor T cells stain with antibodies to TGF-β, whereas increased expression of TGF-β RNA has not been noted in $CD4^+CD25^+$ suppressor T cells[66] (**Fig. 3**). Because $CD4^+CD25^+$ suppressor cells have the ability to interfere with $CD8^+$ T cell proliferation[69], with cytokine secretion by $CD8^+$ T cells[68] and with cytolytic activity of $CD8^+$ T cells[67,68], which may depend on the local microenvironment as well as the number of $CD4^+CD25^+$ suppressor cells, the possibility that TGF-β is generally essential for the suppression of different effector functions of $CD8^+$ T cells cannot be excluded. TGF-β may be not only involved in suppression of $CD8^+$ effector T cells but also may contribute to generation and/or proliferation of $CD4^+CD25^+$ suppressor cells, as mice defective in TGF-β receptor II have reduced numbers of such cells[70]. Thus TGF-β could have a variety of functions in the immune system that generally favor immunosuppression.

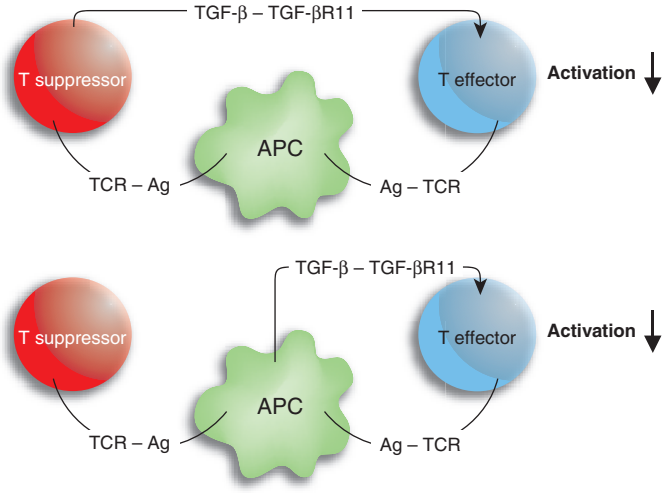

Figure 3 TGF-β in the suppression of CD8 T cells. Top, suppressor T cells bearing TGF-β interact directly with the TGF-β receptor II (TGF-βRII) on effector T cells, which results in inhibition of activation. Bottom, suppressor T cells induce TGF-β production by APCs, which binds to TGF-βRII on effector T cells, resulting in inhibiting of activation.

Interference with suppression *in vivo*

Various immune responses, including those specific for viruses[71] and tumors[72], are enhanced when $CD25^+$ cells are depleted *in vivo*, when antigen is presented on continuously activated dendritic cells[73] or when effector T cells are made resistant to suppression by ligands of GITR[33]. It remains unresolved whether in some of those scenarios, antibodies to CD25 eliminate $CD25^+$ suppressor cells that are induced *de novo* from naive T cells in peripheral lymphoid tissues or suppressor cell populations that are activated and/or expanded from the pool of intrathymically generated suppressors. In the last case, foreign antigens would mimic self antigens, which are involved in the generation of suppressors. Thus, antigenic mimicry may activate not only autoimmune effector T cells but also suppressor cells that have been generated through recognition of self epitopes in the thymus. Alternatively, there may always be some activated $CD4^+CD25^+$ T cells that suppress neighboring T cells. Eliminating these activated $CD4^+CD25^+$ T cells, which may be specific for self antigen, using antibodies to CD25 may eliminate suppressive 'noise' and thereby enhance immune responses. Which pathway is involved in the enhancement of specific responses remains to be determined in most scenarios that have been studied so far.

Conclusion

Several *in vitro* and *in vivo* models of immunosuppression have been developed in recent years, and it is likely that $CD4^+CD25^+$ suppressor T cells suppress immunity by several distinct mechanisms. The fact that $CD4^+CD25^+$ suppressor T cell populations can localize and expand together with effector T cell populations in antigen-draining lymph nodes and inflamed tissue facilitates 'specific' suppression that is initiated by antigenic stimulation and local recruitment of suppressor together with effector T cells. Suppression may then be mediated over a short range by cytokines, by direct cell contact of suppressors with effector T cells or by mechanisms that 'instruct' APCs to increase tryptophan metabolism and/or secrete suppressive cytokines such as TGF-β or IL-10 to interfere with T cell activation. Models that invoke direct cell contact are based on the observation that CD80- and CD86-deficient effector T cells are resistant to suppression that may require ligation of CD80 and/or CD86 on effector T cells by CTLA-4 on suppressor cells. Observations that have shown essential involvement of TGF-β receptor signaling in suppression of $CD8^+$ T cells are compatible with models that involve direct cell contact between TGF-β-bearing suppressors and $CD8^+$ T cells or models that involve binding of free or APC-bound TGF-β by $CD8^+$ T cells. It is notable that $CD4^+CD25^+$ suppressor T cells can selectively affect distinct effector functions of T cells such as cytokine secretion, chemokine receptor expression and cytolytic activity. The commonality of all these functions is that they require TCR signaling, perhaps of different intensity, at different stages of T cell differentiation and it is thus tempting to argue that immunosuppression mediated by $CD4^+CD25^+$ cells reversibly interferes with TCR signaling proximally of different transcription factors that induce the diverse effector functions.

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