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TGF-beta and $CD4^+CD25^+$ Regulatory T cells

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1. ABSTRACT

TGF-beta and $CD4^+CD25^+$ regulatory T cells (Treg) both play an important role in the control of immune responses and the maintenance of immune homeostasis. The mechanism of suppression induced by Treg and the factors which regulate Treg function and number, have only begun to be elucidated. TGF-beta seems to act as an effector cytokine involved in the immunosuppressive function of Treg *in vitro* and *in vivo*, although its origin and mechanism of action remains to be defined. In addition, TGF-beta signaling in peripheral Treg seems to be essential for the regulation of peripheral Treg numbers and for their immunosuppressive function *in vivo*. This review will focus on the role of TGF-beta for the generation and expansion of $CD4^+CD25^+$ Treg, as well as for their immunosuppressive function *in vitro* and *in vivo*.

2. INTRODUCTION

Over the past 10 years, suppressive or regulatory T cells have reemerged as one of the central mechanisms of peripheral tolerance. Sakaguchi *et al.* first described a subpopulation of regulatory T cells characterized by the constitutive expression of the IL-2 receptor α-chain (CD25) (1). So far, $CD4^+CD25^+$ regulatory T cells (Treg) represent the best studied subpopulation of several regulatory T-cell populations described (2-4). Treg were initially identified to prevent autoimmune disease (1) but are now known to have a more general immunosuppressive role in the face of chronic inflammation as well as in tumor and transplantation immunology (5-9).

Treg were originally identified in the mouse, but a similar population of $CD4^+CD25^+$ T cells has been

identified in human peripheral blood (10-17) and thymus (16), constituting about 5-10 % of peripheral $CD4^+$ T cells. Several markers, including CTLA-4 and GITR, have been used to identify and characterize Treg (18). Among these, Foxp3 has emerged as a transcription factor essential for the generation and function of $CD4^+CD25^+$ Treg (19-21). However, with the exception of Foxp3, these markers are upregulated also in conventional T cells upon activation, restricting their use for the analysis of Treg in inflammation and disease (for review see (18)).

Treg were first described to be generated within the thymus (22). However, it has become increasingly clear that Treg numbers are regulated in peripheral lymphoid organs both, by expansion of pre-existing Treg or by de novo generation. Thymic and peripheral Treg might therefore be regarded as separate entities of $CD4^+CD25^+$ Treg, a matter which will be discussed in further detail below.

TGF-β is a pleiotropic cytokine with a number of context dependent effects on immune cells, including inhibition of T-cell proliferation and differentiation, of macrophage activation and of DC maturation (for review, see (23)). In addition to the well described suppressive effects on T-cell function (24-28), TGF-β may also have stimulatory effects, predominantly on naïve T cells (29).

Several mouse models have been established in order to investigate the role of TGF-β in immune regulation. A prominent role for TGF-β1 in the homeostatic regulation of the immune system was suggested by the phenotype of TGF-$\beta1^{-/-}$ mice (30, 31). These mice develop a rapid wasting syndrome, characterized by the infiltration of lymphocytes and macrophages in multiple organs, leading to death at the age of 3-4 weeks (30, 31). The autoimmune phenotype of TGF-$\beta1^{-/-}$ mice has been largely ascribed to the presence of activated lymphocytes (32). However, the precise mechanism of spontaneous T-cell activation in the absence of TGF-β1 remains to be elucidated.

TGF-β1 binds to the TβRII, which then recruits TβRI to form a heterodimeric receptor complex. TβRII phosphorylates and activates RI via its intracellular serine-threonine kinase domain. RI then signals via activation of the Smad signaling pathway (23). In order to analyse the specific effects of TGF-β on T cells, we and others have previously generated transgenic mice with an impaired TGF-β signaling pathway in T cells, by overexpressing a dominant negative form of the TβRII (dnTβRII) under control of T-cell specific promoters (33-35). In these mice, the transgenic TβRII has a truncated intracellular kinase domain and therefore retains cytokine binding activity, but fails to trigger downstream signaling events. In one of these models, development of colitis was observed, although these mice survived at least 3-4 months without developing overt disease (34). The decreased severity of disease observed in these mice as compared to TGF-β-/- mice could be explained by the fact that TGF-β acts on multiple cell types, whereas in dnTβRII mice, the effects on T cells are impaired specifically. The transgenic mice generated in our group did not develop any spontaneous autoimmune disease, but showed an increased susceptibility to the induction of allergic and autoimmune diseases (33, 36, 37). Although the phenotype of transgenic mice differs in the various models described, these experiments demonstrate that TGF-β signaling in T cells is required for the maintenance of T-cell homeostasis.
This review will focus on the relation between TGF-β and the generation and function of Treg.

3.1. TGF-β is not required for thymic generation of $CD4^+CD25^+$ Treg

Neonatal thymectomy results in the development of mouse strain-dependent, tissue-specific autoimmunity (1, 9). $CD4^+CD25^+$ Treg were shown to appear in the periphery only after day three of life (22). Neonatal thymectomy seems to induce a transient elimination of Treg and the subsequent development of autoimmune disease; tolerance to self could be restored by the transfer of Treg into neonatally thymectomized mice (1, 9, 38). These data demonstrated that one key function of the thymus is the generation of $CD4^+CD8^-CD25^+$ Treg, called thymic or central Treg, which participate in the maintenance of peripheral tolerance (3, 39).

The role of TGF-β1 for the generation of thymic $CD4^+CD8^-CD25^+$ Treg was studied by several groups using TGF-$\beta1^{-\backslash-}$ mice. Up to an age of 2 weeks, thymic Treg developed normally in TGF-$\beta1^{-\backslash-}$ mice and displayed the characteristic expression of CTLA-4, GITR and Foxp3 (40, 41). However, because of the rapid onset of inflammation, which begins around day 10-12 in TGF-$\beta1^{-\backslash-}$ mice (30, 31), it is difficult to study the development of Treg in adult mice. Therefore, the role of endogenous TGF-β for the thymic development of $CD4^+CD8^-CD25^+$ Treg was analysed in transgenic mice with impaired TGF-β signaling specifically in T cells and thymocytes. No difference in the numbers of thymic $CD4^+CD8^-CD25^+$ Treg was observed between wild type and hCD2-dnTβRII transgenic mice at various ages tested (42-44). These results suggest, that thymic generation of murine $CD4^+CD8^-CD25^+$ Treg occurs independent from the production of TGF-β1 by Treg or other cells such as thymic stroma cells at a young age. Also, these results indicate that TGF-β signaling in Treg is not involved in their thymic generation (42-44).

Transgenic mice which overexpress active TGF-β1 specifically in T cells do not have altered numbers of thymic $CD4^+CD8^-CD25^+$ Treg; however, the numbers of $CD4^+CD25^-$ thymocytes were reduced. An almost complete reversal of this effect was seen in double transgenic mice overexpressing both, active TGF-β1 and a dnTβRII in T cells, suggesting direct autocrine effects of TGF-β1 on T cells (42). These results indicated a differential susceptibility of $CD25^-$ and $CD25^+$ thymocytes towards increased levels of active TGF-β1. Although there is no evidence for a role of TGF-β in thymic generation of Treg, these data demonstrate that an additional effect of TGF-β for the thymic generation of Treg cannot be completely ruled out.

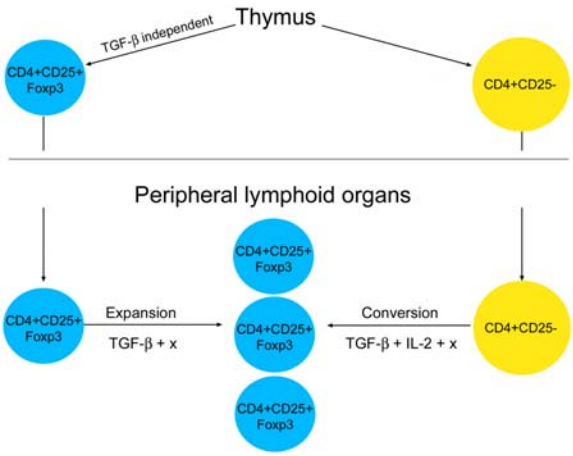

Figure 1. Regulation of Treg numbers *in vivo*. The thymus generates effector as well as regulatory T cells. The generation of thymic Treg seems to be independent from TGF-β. In contrast, the maintenance of the peripheral Treg pool is regulated by TGF-β: TGF-β signaling in Treg is required for their expansion in the setting of inflammation; in addition, TGF-β is able to convert naive $CD4^+CD25^-$ T cells into Treg and to induce the expression of Foxp3.

3.2. TGF-β maintains the peripheral $CD4^+CD25^+$ Treg pool

3.2.1. *In vitro* conversion of $CD4^+CD25^-$ T cells into $CD4^+CD25^+$ Treg through TGF-β1

Although thymic function seems to decline with advancing age, the numbers of peripheral Treg are maintained (45). Two different mechanisms might be involved in the regulation of the peripheral pool of Treg: expansion of thymic Treg and *de novo* generation of Treg in the periphery (Figure 1).

This question of *de novo* generation of Treg was approached by investigating the potential conversion of $CD4^+CD25^-$ T cells into Treg *in vitro*. Yamagiwa *et al.* demonstrated that the costimulatory effect of TGF-β1 on naïve human peripheral blood $CD4^+CD45RA^+$ T cells enhanced their expression of CD25 and CTLA-4 and accelerated their differentiation into the $CD45RA^-$ activated phenotype *in vitro*. The phenotype and *in vitro* suppressive capacity of these peripheral Treg turned out to be similar to thymic Treg (14).

Foxp3 is a transcription factor essential for the generation of $CD4^+CD25^+$ Treg cells. TGF-β1 treatment was shown to induce Foxp3 gene expression in $CD4^+CD25^-$ T cells in the presence of TCR stimulation and IL-2 *in vitro* and was able to convert $CD4^+CD25^-$ responder T cells to anergized $CD4^+CD25^+$ T cells (46-48). These newly generated T cells produced neither Th1 nor Th2 cytokines, but suppressed T-cell proliferation in a cell contact dependent manner (46). In addition, naïve $CD4^+$ T cells induced to become $CD25^+$ Treg by TGF-β *in vitro* were themselves capable of inducing the conversion of $CD4^+CD25^-$ T cells into Treg *in vitro*. The conversion required cell contact as well as the presence of TGF-β and IL-10, thereby providing the possibility of a positive feedback loop in order to rapidly augment $CD4^+CD25^+$ Treg numbers at sites needed (49). The induction of suppressive capacity in the $CD25^-$ T-cell fraction by human $CD4^+CD25^+$ Treg *in vitro* had previously been termed infectious tolerance (50).

However, to date it is unclear whether *in vitro* effects induced by nanomolar concentrations of TGF-β1 can be translated into the *in vivo* situation. In a study by Chen *et al*, $CD4^+CD25^+$ T cells generated in the presence of TGF-β1 inhibited the expansion of antigen-specific naïve CD4+ T cells *in vivo* and prevented house dust mite-induced allergic airway disease in a murine asthma model (46). These results indicate the potential usefulness of *in vitro* generated $CD4^+CD25^+$ Treg for the therapeutic manipulation of autoimmune and allergic diseases.

The capacity of TGF-β1 to induce the expression of Foxp3 in human and murine $CD4^+CD25^-$ T cells *in vitro* was confirmed by Fantini *et al.* (47). In these experiments, Foxp3 was shown to downregulate Smad7, an inhibitory Smad protein induced by TGF-β1. Thereby, in Treg the inhibition of TGF-β1 signaling could be antagonized and signaling via Smad3/4 enhanced in order to further stimulate Foxp3 expression (47). It should be mentioned that an additional effect of TGF-β1 on contaminating Treg can not be excluded, reaching a purity of the $CD25^-$ T cell fraction after negative selection of over 98%. In addition, the effects of $CD25^-$ Foxp3+ T cells are unclear. However, besides suggesting potentially therapeutic strategies, these results raise the question whether TGF-β1 regulates Foxp3 expression and peripheral Treg numbers also *in vivo*.

3.2.2. TGF-β promotes the expansion of peripheral $CD4^+CD25^+$ Treg *in vivo*

In vitro, Treg are anergic and unresponsive to proliferative effects of TGF-β1 (43, 46). *In vivo*, however, it is now accepted that antigen-triggered expansion of Treg occurs (51-57). As an example, Mottet *et al* demonstrated the proliferation of Treg in mesenteric lymph nodes and inflamed colon of mice in a transfer model of inflammatory bowel disease (51).

To study the role of TGF-β for the expansion of $CD4^+CD25^+$ Treg *in vivo*, TGF-$\beta1^{-/-}$ mice were analysed. No changes in peripheral numbers of Treg were initially found in 5-7 day old mice (58), suggesting that TGF-β is not critical for the generation or expansion of Treg. Accordingly, 6-8 week old TGF-$\beta1^{-/-}$ TCR transgenic mice exhibited similar numbers of splenic Treg and Foxp3 mRNA levels as compared to TGF-$\beta1^{+/-}$ mice (44). The role of TGF-β in converting $CD4^+CD25^-$ T cells into Treg was investigated by the transfer of wild type $CD4^+CD25^-$ T cells into $Foxp3^{-/-}$ mice (20). No conversion into Treg was observed, although high levels of TGF-β1 were described in $Foxp3^{-/-}$ mice in another study (19). These results may serve as indirect evidence against an *in vivo* role for TGF-β1 in the maintenance of peripheral Treg numbers.

However, a significant reduction of Treg numbers including a reduction in Foxp3-expression was

recently observed in 8-10 day old TGF-$\beta1^{-/-}$ mice as compared to wild type mice (41). This discrepancy might be explained by the different time points as well as different genetic backgrounds analysed.

A role for TGF-β1 in the regulation of $CD4^+CD25^+$ Treg numbers *in vivo* was confirmed by elegant experiments using the mouse model of type I diabetes. Peng *et al* could show, that a transient pulse of TGF-β1 in the islets during the priming phase of diabetes was sufficient to inhibit disease onset by stimulating the expansion and Foxp3 expression of intraislet $CD4^+CD25^+$ Treg (59).

In order to analyse the direct effects of TGF-β on peripheral Treg, transgenic mice with impaired TGF-β signaling specifically in T cells were studied (42-44). Up to an age of 4 weeks, $CD4^+CD25^+$ Treg numbers seemed to be stable in these mice (43, 44). However, we found that the number of peripheral Treg decreased in mice with impaired TGF-β signaling between the age of 4-6 weeks, but remained stable thereafter. This reduction in peripheral Treg numbers was not due to a reduced generation of $CD4^+CD8^-CD25^+$ Treg in the thymus since transgenic mice with impaired TGF-β signaling in T cells did not show alterations in thymic $CD4^+CD8^-CD25^+$ Treg numbers over time (42, 43). These discrepant results might again relate to different promoters and genetic backgrounds analysed.

In order to address the role of TGF-β signaling in Treg expansion *in vivo*, we transferred Treg with impaired TGF-β signaling which had been labelled with CFSE into mice before the induction of colitis by dextrane sodium sulphate (DSS). Treg proliferation was significantly decreased in cells with impaired TGF-β signaling (43). Clearly, these data demonstrated that TGF-β signaling in $CD4^+CD25^+$ Treg themselves was required for their proper *in vivo* expansion. However, an additional effect of TGF-β on the *in vivo* conversion of $CD4^+CD25^-$ T cells into $CD4^+CD25^+$ Treg can not be ruled out to date.

The possible contribution of TGF-β to the regulation of Foxp3 expression *in vivo* was analysed in $CD4^+CD25^+$ T cells isolated from transgenic mice with impaired TGF-β signaling. In adult mice with impaired TGF-β signaling, $CD4^+CD25^+$ Treg had reduced Foxp3 mRNA expression levels (42). In addition, reduced expression of Foxp3 was recently found in peripheral Treg isolated from 8-10 day old TGF-$\beta1^{-/-}$ mice (41). In contrast, increased Foxp3 expression levels were reported in Treg isolated from transgenic mice overexpressing active TGF-β1 in T cells, supporting the notion that TGF-β significantly contributes to the regulation of Foxp3 *in vivo* (42).

In summary, these data show that TGF-β is an important regulator of the peripheral Treg pool, at least in mice, by promoting the expansion of precommitted Treg in the setting of inflammation. In addition, TGF-β1 seems to regulate the expression of Foxp3 *in vivo*. Further studies are needed on the potential conversion of $CD4^+CD25^-$ T cells into Treg and on the role of additional factors contributing to this conversion *in vivo*.

3.3. TGF-β and *in vitro* function of $CD4^+CD25^+$ Treg

$CD4^+CD25^+$ Treg are able to inhibit the proliferation of $CD4^+CD25^-$ T cells *in vitro* via the inhibition of IL-2 production (60-62). *In vitro*, the suppressive capacity of Treg was shown to be dependent on direct cell to cell contact, but not on the production of soluble cytokines (for review see (52)). In the following, we will focus on the role of TGF-β for the suppressive function of $CD4^+CD25^+$ Treg *in vitro*.

Several studies have investigated the *in vitro* function of Treg isolated from newborn TGF-$\beta1^{-/-}$ mice. The results obtained clearly demonstrated that the production of TGF-β1 is not required for the *in vitro* suppressive function of central or peripheral Treg (40, 41, 44, 58). However, these experiments did not exclude a role for TGF-β in the mechanism of suppression since TGF-β could be aquired by Treg from other sources within the *in vitro* suppressor assay. Conflicting data have emerged on the potential inhibition of Treg function by the addition of neutralizing anti-TGF-β1 antibody *in vitro*. Nakamura *et al* reported that *in vitro*, suppression induced by Treg could be abolished by the presence of high concentrations of anti-TGF-β1 antibody (50-100 μg/ml), presumably by blocking membrane bound TGF-β on the Treg cell surface (63). In contrast, it was reported that neutralisation of TGF-β1 with either monoclonal antibody (50 μg/ml) or soluble TGF-βRII-Fc was not able to reverse the *in vitro* suppression mediated by resting or activated murine Treg (58). The two most recent studies on the issue analysing Treg isolated from TGF-$\beta1^{-/-}$ mice confirmed, that the production of TGF-β1 is not required for maintaining the immunosuppressive function of Treg *in vitro*. However, the suppression observed could be blocked by the addition of anti-TGF-β1 antibody, strongly suggesting that TGF-β produced by non-Treg, presumably antigen presenting cells, is involved in suppression (41, 44).

In another approach investigating the role of TGF-β1 for the *in vitro* suppressive capacity of Treg, $CD4^+CD25^-$ responder T cells isolated from transgenic mice with impaired TGF-β signaling were analysed. *In vitro*, these responder cells were shown to be as susceptible to Treg mediated suppression as responder cells from wild type mice (42, 43, 58). Thus, several complementary pathways may guarantee the proper immunosuppressive function of Treg. This notion is supported by experiments showing that antibody to CTLA-4, but not to TGF-β, abrogated Treg suppression *in vitro*; however, CTLA-4-deficient Treg exhibited uncompromised suppression *in vitro* that could be inhibited by antibody to TGF-β1 (64).

Similar *in vitro* experiments were performed using human Treg. Jonuleit *et al.* reported that the presence of anti-TGF-β antibody could not reverse cell contact dependent suppression of $CD4^+$ T cells (50). However, others could completely block the suppressive effect of $CD4^+CD25^+$ human thymocytes by the combination of

antibody to CTLA-4 and to TGF-β, but not by either antibody alone (65).

Thus, the origin and the role of TGF-β in Treg suppression needs further clarification. Although widely used to assess the function of Treg, *in vitro* suppressor assays should be interpreted with caution, since Treg may function differently *in vitro* and *in vivo* (43, 44).

3.4. TGF-β and *in vivo* function of CD4+CD25+ Treg

TGF-β1 is essential for the maintenance of immune homeostasis in vivo, as demonstrated in TGF-$\beta1^{-/-}$ mice. These mice die within a few weeks of age due to a multifocal inflammatory syndrome (30, 31). In addition, the impairment of TGF-β signaling specifically in T cells has resulted in the loss of T-cell tolerance, either spontaneously (34, 35), or induced (33, 66). TGF-β1 has been previously shown to play a non redundant role in the control of various inflammatory disease models such as intestinal inflammation and type 1 diabetes (67, 68). A link between the loss of Treg function and TGF-β1 deficiency was suggested by the phenotype of Foxp3$^{-/-}$ mice which is similar to that of TGF-$\beta1^{-/-}$ mice (19, 21, 30, 31). Therefore, several groups have tried to define the importance of TGF-β1 for the in vivo function of $CD4^+CD25^+$ Treg.

Most studies using mouse models demonstrated that *in vivo*, the immunosuppressive capacity of Treg could be abrogated by injecting neutralizing anti-TGF-β1 antibody (69, 70). These results indicated that the presence of TGF-β1 might be required for the proper *in vivo* function of Treg. However, Treg mediated suppression of autoimmune gastritis could not be reversed by injecting neutralizing anti-TGF-β1 antibody, suggesting that the immunosuppressive mechanisms involved might vary depending on the nature of the immune response being suppressed (58).

Conflicting data have emerged regarding the *in vivo* function of Treg isolated from TGF-$\beta1^{-/-}$ mice. TGF-$\beta1^{-/-}$ Treg were able to suppress the development of colitis in T-cell transfer models, indicating that the synthesis of TGF-β1 by Treg themselves is not essential for their immunosuppressive capacity *in vivo* (40, 44). In contrast, Nakamura *et al* reported that TGF-$\beta1^{-/-}$ Treg failed to prevent colitis in the Scid mouse T-cell transfer model (71). In studies using the transfer of CD25-depleted TGF-$\beta1^{-/-}$ splenocytes into TGF-$\beta^{+/+}$ $Rag2^{-/-}$ mice, cotransfer of TGF-$\beta1^{-/-}$ Treg clearly attenuated autoimmune disease. However, suppression was less efficient as compared to the transfer of TGF-$\beta1^{+/+}$ Treg (40). Again, these experiments were performed on different genetic backgrounds, which might explain some of the contradictory results obtained.

Regarding TGF-$\beta1^{-/-}$ mice, it could be argued that endogenous Treg are not sufficiently functional to prevent onset and progression of inflammation. This implies that, even though the production of TGF-β1 by Treg might be dispensable, the presence of TGF-β1 within the system is not. Very recently, Fahlen *et al* demonstrated, that the repeated injection of large amounts of neutralizing anti-TGF-β1 antibody was able to abrogate the suppressive capacity of TGF-$\beta1^{-/-}$ Treg *in vivo*, indicating that TGF-β1 must be provided by a non Treg source, most probably antigen presenting cells (44).

The requirement for TGF-β signaling in Treg was analysed in transgenic mice with impaired TGF-β signaling in T cells. In contrast to wild-type Treg, peripheral Treg with impaired TGF-β signaling, when transferred, failed to protect recipients from colitis induced by DSS or transfer of $CD4^+CD45RB^{high}$ cells (43, 44). Interestingly, thymic $CD4^+CD8^-CD25^+$ Treg with impaired TGF-β signaling, but not peripheral Treg, were able to prevent colitis in the transfer model (44). In our own experiments, thymic Treg with impaired TGF-β signaling were not able to prevent colitis induced by DSS, suggesting that the requirement for TGF-β signaling within Treg might vary depending on the target cell population (own unpublished data). Until now, the functional differences between central and peripheral Treg, including possible interactions between the two populations, remain to be elucidated.

Membrane bound TGF-β plays a potential role for the immunosuppressive function of Treg in vitro. In vivo, the presence of latency associated peptide (LAP), which inactivates bound TGF-β dimer, was associated with the immunosuppressive function of $CD4^+$ T cells (72). It was recently reported that Treg isolated from 6 weeks old Ig-GAD donor mice, but not isolated from 8 or 26 weeks old donor mice, protected recipient mice against the development of type 1 diabetes. No difference in the expression of CTLA-4, CD62L, and Foxp3 could be detected between these protective or non-protective Treg. Interestingly, up to an age of 6 weeks, Treg expressed significant levels of active membrane bound TGF-β, which was followed by an abrupt decline in expression persisting up to an age of 26 weeks. Precoating functional 6 weeks old Treg with anti-TGF-β antibody resulted in a lack of protection against diabetes, supporting a role for membrane bound TGF-β in the suppression observed in vivo (73). To date, available data seems insufficient to support or refute the concept of membrane bound TGF-β for the in vivo function of Treg.

As mentioned above, TGF-$\beta1^{-/-}$ Treg require TGF-β for their proper *in vivo* function. However, it remains to be established, whether TGF-β induces TGF-β signaling in Treg or whether TGF-β attaches to the Treg surface to exert its function as an effector cytokine. If the latter is assumed, Treg overexpressing a dominant negative TGF-β type II receptor should demonstrate increased suppressive capacity due to their increased ability to bind TGF-β to their surface. However, these cells were found to be less functional than their wild-type counterparts (43, 44). In this respect it would be interesting to see whether Treg isolated from TGF-$\beta1^{-/-}$ mice with additional impairment in TGF-β signaling were functional *in vivo*.

3.5. TGF-β as an effector cytokine of $CD4^+CD25^+$ Treg function *in vivo*

There is an ongoing controversy regarding the contribution of different cytokines such as TGF-β and IL-

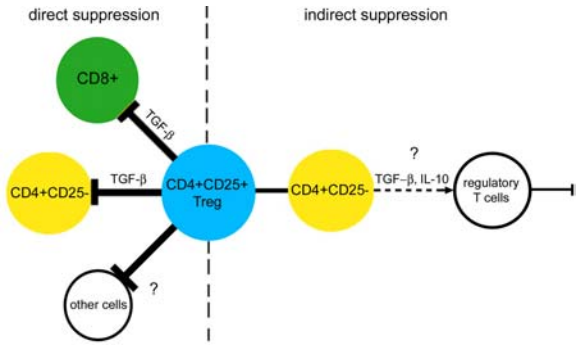

Figure 2. *In vivo* suppression induced by Treg. Treg are able to directly suppress $CD4^+$ as well as $CD8^+$ effector T cells. TGF-β seems to be involved in the suppression but the exact mechanism and origin of TGF-β needs to be clarified. More data are needed on the potential *in vivo* induction of T cells with a regulatory phenotype by Treg. Indirectly, Treg may be able to confer suppressor activity to conventional $CD4^+$ T cells by the induction of a Tr1-like, Th3-like or Treg-like phenotype.

10 to the function of the various populations of regulatory T cells. The role of TGF-β as an effector cytokine of *in vivo* immunosuppressive function can best be studied in models using responder cells with an impaired TGF-β signaling pathway. Green *et al* could first demonstrate that $CD8^+$ effector T cells require TGF-β signaling for being sensitive to Treg suppression. In these experiments, type 1 diabetes was induced by transfer of autoreactive islet-specific $CD8^+$ T lymphocytes. Cotransfer of Treg suppressed disease caused by the transfer of wild type effector T cells, but failed to suppress disease caused by the transfer of transgenic effector cells overexpressing a dnTβRII (74). However, from these experiments it remained unclear, whether effector mechanisms such as proliferation, cytokine release or cytolytic activity were affected by Treg-mediated TGF-β signaling. In another recent study, Treg abrogated $CD8^+$ T cell-mediated tumor rejection by specifically suppressing the cytotoxity of expanded $CD8^+$ T cells; production of INF-γ and expansion of tumor specific $CD8^+$ T cells was unaffected. This specific effect was induced by the direct activity of TGF-β on $CD8^+$ T cells, since the cytotoxicity of effector cells overexpressing a dnTβRII was not impaired in the presence of Treg (75). Similar results were recently obtained investigating $CD4^+$ T cells as targets of Treg mediated suppression. Colitis was induced by the transfer of $CD4^+CD45RB^{high}$ T cells into Scid mice. Cotransfer of Treg inhibited the accumulation of wild type $CD4^+CD45RB^{high}$ cells in the colon and also reduced the frequency of cells capable of secreting IFN-γ. In contrast, Treg were unable to significantly change the course of colitis induced by the transfer of $CD4^+CD45RB^{high}$ T cells overexpressing a dnTβRII receptor (44). In our studies, Treg were able to inhibit colitis induced by the ingestion of DSS in transgenic mice with impaired TGF-β signaling in T cells (43). However, it should be kept in mind that T cells probably do not constitute the main effector cell population in DSS-colitis (76).

Together, these data demonstrate that $CD4^+CD25^+$ Treg are able to suppress specific effector mechanisms of $CD8^+$ as well as $CD4^+$ T cells by the induction of TGF-β signaling. The origin of TGF-β and its mode of presentation remain to be clarified.

4. PERSPECTIVE

There is increasing evidence that there are two distinct populations of $CD4^+CD25^+$ Treg. Generation and function of central $CD4^+CD8^-CD25^+$ Treg which arise in the thymus seem to be independent from TGF-β. However, expansion and immunosuppressive function of peripheral Treg seems to depend on TGF-β both as an effector cytokine and as an inducer of Treg (Figure 2). The exact mechanism involved in the *in vivo* and *in vitro* function of Treg including the potential cellular source of TGF-β requires further study. Subpopulations and homing properties of peripheral Treg, as was suggested by the differential expression of integrins (77), are an important area of research. In addition, intracellular signaling pathways induced by TGF-β within Treg need to be analysed, since these may allow to manipulate Treg function for antigen specific immune modulation. Additional mediators of Treg function, generation and expansion of peripheral Treg should be investigated *in vitro* and *in vivo*. The prospect of antigen specific expansion of Treg for the therapeutic manipulation of immune mediated disease is intriguing and should stimulate further work in the field.

5. ACKNOWLEDGMENTS

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