REVIEW

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Regulatory T cells: magic bullets for immunotherapy?

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Abstract

In the past few years it has been become increasingly clear that T cells capable of actively suppressing immune responses are thought to be in part responsible for the maintenance of peripheral self tolerance. In healthy rodents and humans, $CD4^+$ T cells constitutively expressing the interleukin (IL)-2 receptor α-chain (CD25) are able to exert such suppressive function *in vitro* and *in vivo*. Despite great efforts in our understanding of the biology of such immunoregulatory T cells, there are still certain points incompletely understood. Although some authors suggest that immunoregulatory cytokines such as IL-10 or transforming growth factor-β are critical for the suppressive effect of these cells, this is controversial and the exact molecular nature and the targets of suppression are largely unknown. Thus far, until regulatory T cells can be used for diagnostic or therapeutic purposes many questions have to be answered. In this review we summarize the current knowledge on the function and properties of this T cell subset and discuss their potential role in human autoimmune or chronic inflammatory diseases.

Key words: T cell subset, regulatory T cell, autoimmune disease, inflammation, infection, immunoregulation

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INTRODUCTION

Maintaining tolerance to self antigens is a major function of the adaptive immune system. Avoidance of autoimmune reactivity is controlled at multiple stages. This is necessary because the antigen receptor of T and B lymphocytes is composed randomly out of different gene segments; this ensures on one hand a broad antigen specificity, but harbors, on the other hand, the risk of developing cells that recognize self antigens. Most of the autoreactive T cells are eliminated during thymic selection, but some escape this process and appear in the peripheral immune system, where their activation may induce autoimmune diseases. Among the several mechanisms that prevent the activation of these T cells (anergy, ignorance), the most important is now believed to be the action of regulatory T (T_{reg}) cells. The theory that immune reactions are actively controlled by specialized lymphocytes was developed in the early 1970s, when Gershon and Kondo [37] demonstrated that transfer of lymphocytes from animals tolerized against a certain antigen suppresses the humoral immune response against the same antigen in naïve recipients. Although elaborate interactions between suppressor cells, contrasuppressor cells, acceptor cells, and the action of T cell suppressor factor have been proposed, the exact phenotype and the mechanism of suppression have remained unknown for almost 20 years. The inability to characterize molecules or cells involved in suppressor activity eventually led to a decline of this concept, the word "suppression" vanishing from the immunologist's vocabulary.

WHAT IS A REGULATORY T CELL?

It was the work of Sakaguchi's group to reintroduce the concept of suppressor T cells (now renamed as regulatory T cell) by demonstrating that reconstitution of lymphopenic mice with splenocytes depleted of CD25- (interleukin (IL)-2 receptor α-chain) expressing cells from naïve donors results in a poly-autoimmune syndrome in the host, and that this can be prevented by transfer of purified $CD4^+CD25^+$ T_{reg} cells [82]. The autoimmune phenomena which occur after neonatal thymectomy are also the result of the absence of this $CD4^+CD25^+$ T_{reg} cell subset [7, 90]. Likewise, in a model of inflammatory bowel disease induced by adoptive transfer of $CD45RB^{high}$ $CD4^+$ cells into severe combined immunodeficiency (SCID) mice, the regula-

tory activity of CD45RB^{low} T cells is concentrated within the CD25$^+$CD45RB^{low} T_{reg} cell population [3, 8, 80]. Since then, numerous other studies have shown that CD4$^+$CD25$^+$ cells have immunoregulatory activity in mice (reviewed in [81]), rats, and humans (for instance references [49, 88]), indicating that the T_{reg} cell activity is an evolutionarily conserved mechanism for the maintenance of peripheral tolerance. These cells represent approximately 5–10% of the peripheral CD4$^+$ T cells and are considered to be a distinct, specialized T cell lineage. Since they exist in naïve, unmanipulated animals, they are now designated as naturally occurring T_{reg} cells [15]. In contrast to these, T cells with immunoregulatory or suppressive activity can also be induced by stimulation of naïve T cells with antigen *in vitro* or *in vivo* under particular, tolerogenic conditions (Fig. 1). Such conditions are antigen presentation by immature or semi-mature dendritic cells (DCs) (expressing low levels of co-stimulatory molecules) or stimulation of the T cells in the presence of immunosuppressive cytokines or drugs. Their effector mechanism seems to be the secretion of suppressive cytokines, such as IL-10 or transforming growth factor (TGF)-β (reviewed in [15]). Since these cells can be characterized by function rather than by expression of a specific surface marker, this review will focus on naturally occurring CD4$^+$CD25$^+$ T_{reg} cells.

ARE THERE SPECIFIC MARKERS FOR T_{REG} CELLS?

While CD25 remains the best available surface marker to characterize T_{reg} cells, CD4$^+$CD25$^+$ T_{reg} cells express a number of other surface molecules, such as glucocorticoid-induced tumor necrosis factor-receptor family-related protein (GITR) [67, 87], cytotoxic T lymphocyte-associated antigen (CTLA)-4 [80, 97], low levels of CD45RB [8, 57, 80], the integrin $\alpha_E\beta_7$ (CD103) [10, 45, 59], and other adhesion molecules (CD11a, CD44, CD54) [46, 67]. Thus, CD4$^+$CD25$^+$ T_{reg} cells are phenotypically different from conventional effector T cells. Strikingly, however, none of these markers is specific to T_{reg} cells, since conventional T cells up-regulate all of these marker upon activation. The difference lies in the fact that CD4$^+$CD25$^+$ T_{reg} cells display this activated phenotype even in the absence of any obvious antigenic stimulation.

In search of a more specific and unambiguous marker, several groups have found that the forkhead/winged helix transcription factor 3 (Foxp3) is predominantly expressed by CD4$^+$CD25$^+$ T_{reg} cells [33, 43, 53]. Foxp3 seems to be an essential master switch for the commitment to the T_{reg} cell lineage, since Foxp3-deficient mice lack CD4$^+$CD25$^+$ T_{reg} cells; furthermore, retroviral transfection of naïve T cells with Foxp3 converts them

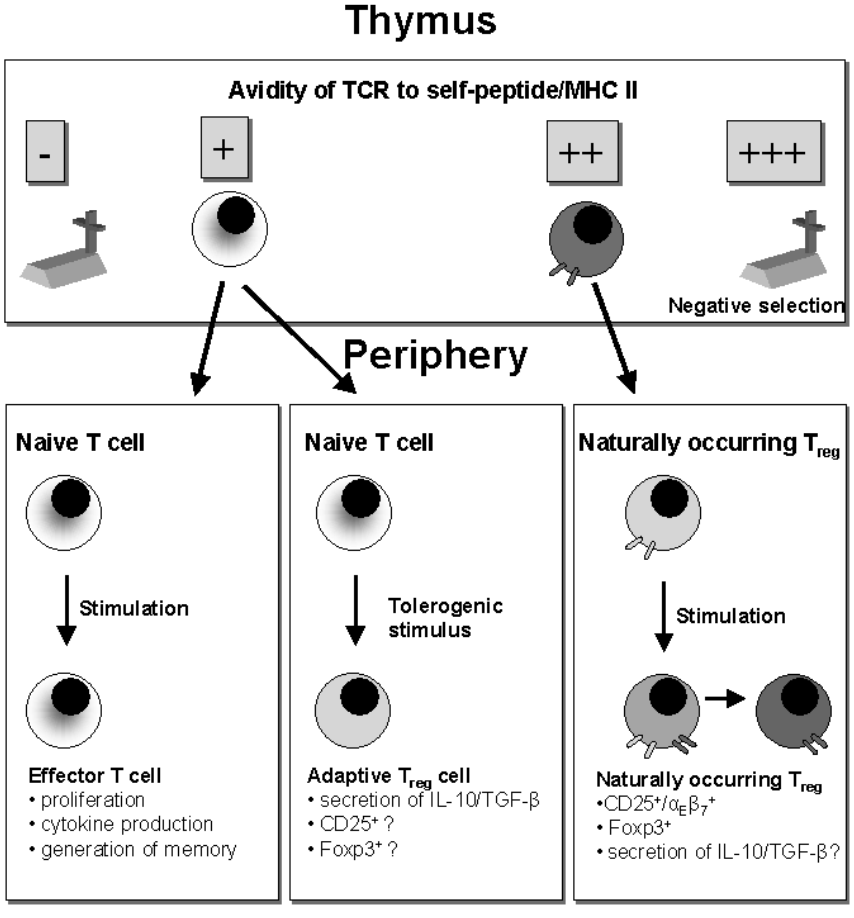

Fig. 1. Adaptive and naturally occurring regulatory T cells. T cell precursors with a higher avidity for self-peptide/MHC II complexes than conventional T cells (but not high enough to cause negative selection) are committed to the T_{reg} cell lineage in the thymus (upper panel). Since these cells leave the thymus as fully competent suppressor cells, they are designated as naturally occurring regulatory T cells (lower panel, right). Naïve T cells leave the thymus and enter the periphery. When stimulated with antigen in the presence of appropriate co-stimulation, naïve T cells proliferate and acquire effector functions (left). If antigen is administered in a "tolerogenic" fashion, i.e. via mucosal surfaces or with manipulated APCs, naïve T cells can be converted into so-called adaptive regulatory T cells (middle).

towards a phenotype and function comparable to that of naturally occurring $CD4^+CD25^+$ T_{reg} cells [43]. Foxp3--deficient or -mutant mice succumb to an autoimmune syndrome due to absence of $CD4^+CD25^+$ T_{reg} cells and can be protected from disease by T_{reg} cell transfer from normal mice [33, 53]. Foxp3 acts as a reppressor of IL-2 transcription [85] and may be therefore responsible for the inability of T_{reg} cells to produce this cytokine [101]. In the murine system, this molecule is not induced in activated T cells and can be regarded therefore as a robust marker for T_{reg} cells. In the human system, in contrast, Foxp3 has been shown to be weakly expressed in effector T cells after activation [109]. Interestingly, the immune dysregulation, polyendocrinopathy, enteropathy, X-linked autoimmune syndrome is due to mutations in the *Foxp3* gene, clearly demonstrating the critical role of T_{reg} cells for the maintenance of immune homeostasis in humans [14].

ORIGIN AND ANTIGEN SPECIFICITY OF T_{REG} CELLS

Several lines of evidence suggest that $CD4^+CD25^+$ cells leave the thymus as committed mature suppressors. First, thymic $CD4^+CD25^+$ T_{reg} cells share the same phenotype and have similar function as their peripheral counterparts, including Foxp3, CTLA-4, and GITR expression [43, 87, 89]. Furthermore, CD25-depleted thymocytes can induce the same autoimmune syndrome as CD25-depleted splenocytes after adoptive transfer into lymphopenic hosts [43, 46].

It seems that recognition of self antigens in the thymus is required for the generation of T_{reg} cells: in double-transgenic mice bearing a single transgenic T cell receptor (TCR) and the transgenically expressed cognate antigen in the thymus, high-level antigen expression result in deletion of the TCR transgenic cells. Antigen expression at lower levels, however, results in a much lower degree of deletion, and up to 50% of the remaining cells are $CD25^+$. Using a TCR with the same specificity but lower affinity does not result in the selection of $CD25^+$ cells [50]. Similar findings in comparable transgenic animals [5, 52] favor a model in which T_{reg} cells are selected by interaction with self-peptide/MHC complexes within an affinity range higher than for conventional $CD4^+CD25^-$ T cells, but not high enough to cause negative selection (Fig. 1). However, peripheral generation of T_{reg} cells from naïve precursors is also possible. Intravenous injection of antigen, low-dose antigen infusion by osmotic pumps, or induction of oral tolerance have been shown to convert naïve T cells into $CD4^+CD25^+$ T_{reg} cells [6, 103]. Also, direct targeting of antigens to immature DCs can induce T_{reg} cells [64]. Furthermore, tolerance induction with anti-CD3 or anti-CD4 monoclonal antibodies involves $CD4^+CD25^+$ T_{reg} cells [12, 23, 51].

One critical factor in the conversion of peripheral T cells to T_{reg} cells seems to be TGF-β: transgenic tissue- or T cell-specific over-expression of TGF-β increases the number of $Foxp3^+$ T_{reg} cells, whereas these are reduced in mice with a T cell-specific defect in TGF-β signaling [76, 84]. Moreover, stimulation of naïve T cells *in vitro* in the presence of TGF-β induces a T_{reg}-like phenotype, including Foxp3 expression, in the murine as well as in the human system [21, 31, 114].

The above-described experiments in the TCR transgenic mice imply that T_{reg} cells may be autoreactive. Indeed, a recent paper demonstrates preferential recognition of self antigens by peripheral T_{reg} cells from non-transgenic mice using retroviral TCR gene transfer from T_{reg} cells to conventional T cells [44]. However, T_{reg} cells can regulate responses against allotransplants (reviewed in [110]) and exogenous antigens, i.e. of microbial origin (reviewed in [69]). This indicates that the TCR repertoire of T_{reg} cells is not strictly limited towards self antigen and also allows regulation of non-self responses.

MECHANISMS OF SUPPRESSION

Cell contact-dependent versus cytokine-mediated suppression

The intriguing question of how T_{reg} cells suppress immune responses cannot be answered conclusively. When studied *in vitro,* $CD4^+CD25^+$ T_{reg} cells are unresponsive to TCR triggering in terms of proliferation and cytokine production. This state of anergy can be broken by addition of high amounts IL-2 or by increasing the level of co-stimulation in culture. When purified $CD4^+CD25^+$ T_{reg} cells are cultured together with responder $CD4^+CD25^-$ cells, the T_{reg} cells are able to suppress the proliferation of the responder T cells. This suppressive activity can be abrogated when both cell populations are physically separated by a Transwell® membrane insert [46, 57, 66, 96, 101, 102]. This phenomenon is usually interpreted as a cell contact-dependent suppressor mechanism; however, in such an experiment the distance between suppressor and responder T cells is in the range of millimeters. Thus it cannot be excluded that molecules acting in a paracrine fashion are the biological basis of suppression. Indeed, the absolute requirement of cell contact, affirmed by so many authors, has yet to be experimentally demonstrated. The suppressive activity in the T_{reg}/responder-T cell co-culture cannot be blocked by addition of blocking antibodies against the anti-inflammatory cytokine IL-10 [96, 101]. The role of TGF-β, another potent anti-inflammatory cytokine, is also still controversial: one group has demonstrated a surface-bound form of TGF-β in T_{reg} cells and shown that their activity *in vitro* can be blocked by addition of high-dose anti-TGF-β [72, 73], whereas other studies do not support these results [77, 96].

In contrast to these *in vitro* studies, there is evidence for a role of these cytokines in *in vivo* models. Inflammatory colitis, induced by transfer of naïve $CD45RB^{high}$ T cells into SCID mice, cannot be inhibited

by T_{reg} cells isolated from IL-10-deficient mice [91]. When isolated from wild-type mice, the inhibitory effect of T_{reg} cells on colitis can be abrogated by blocking antibodies against the IL-10 receptor or TGF-β [62, 65]. Furthermore, inhibition of diabetes by T_{reg} cells requires TGF-β responsiveness of the pathogenic T cells [40]. A requirement for IL-10 and/or TGF-β for T_{reg} cell activity has been described also in some other models [3, 13, 55].

Finally, it has also been demonstrated that human T_{reg} cells can convey IL-10/TGF-β-dependent suppressive function to naïve T cells, a phenomenon designated as infectious tolerance [27, 48].

Choosing the right model for the study of T_{reg} cell function

Most of the models in which T_{reg} cell function *in vivo* was assessed have utilized autoimmunity induced by complete lymphopenia (neonatal thymectomy, SCID colitis) or partial lymphopenia (non-obese diabetic mouse) [54]. Since lymphocytes exhibit an abnormal proliferation in the course of these conditions, and immune regulation by competition may play a major role in lymphopenic animals [11], the data may only poorly represent the situation in non-lymphopenic animals. However, depletion of $CD4^+CD25^+$ cells by treatment with monoclonal antibodies against CD25 in normal animals prior to or after immunization with autoantigens such as collagen type II or myelin basic protein results in much more severe collagen-induced arthritis or experimental allergic encephalomyelitis, respectively [70, 113]. These findings suggest an important suppressive role of T_{reg} cells also in non-lymphopenic models, as the treatment only leads to depletion of 5–10% of the CD4 compartment and therefore does not result in extreme lymphopenia. As mentioned above, T_{reg} cells do not only regulate immune responses specific to autoantigens, but also responses against exogenous antigens of, for instance, viral origin [93, 94]. Our own studies in the antigen-induced arthritis (AIA) model, which is induced by injection of xenogenic protein antigen into pre-immunized mice, corroborate these findings: depletion of T_{reg} cells in the interval between immunization and induction of arthritis exacerbate the arthritis severity, whereas adoptive transfer of purified $CD4^+CD25^+$ T_{reg} cells ameliorate disease. A detailed examination of the mechanisms involved in T_{reg} cell function in non-lymphopenic disease models is lacking [35].

A role for IL-2?

It is obviously not by chance that T_{reg} cells express CD25, the α-chain of the IL-2 receptor. Mice deficient in IL-2 or components of its receptor (i.e. CD25 or CD122) have strongly reduced T_{reg} cell numbers and develop a fatal lymphoproliferative disease [2, 36]. Since T_{reg} cells are not capable of producing IL-2 themselves, they are dependent on exogenous IL-2 (produced by conventional T cells) for survival and possibly, for proliferation. Two recent studies have demonstrated the absolute requirement of IL-2 for T_{reg} cell function *in vitro* [26, 100]. Blockade of IL-2 uptake in T_{reg} cells by selective inhibition of their IL-2 receptor completely abrogates their suppressor function *in vitro.* Naïve T cells co-cultured with T_{reg} cells do not up-regulate CD25 expression after activation, which is positively regulated by autocrine IL-2, indicating a deprivation of IL-2 in the co-culture. Furthermore, the effects of T_{reg} on T cells can all be mimicked by anti-IL-2 [26]. Also, after activation in the presence of IL-2, T_{reg} cells secrete IL-10 *in vitro* [26]. Thus, these data conclusively show that competition for IL-2 is an essential part of the regulatory activity of T_{reg} cells *in vitro* and that IL-2 itself triggers additional effector functions in T_{reg} cells after a period of time.

However, one has to mention that IL-2 receptor-deficient target T cells can be efficiently suppressed by T_{reg} cells *in vivo*, questioning whether suppression by T_{reg} cells is mediated solely by IL-2 consumption and indicating that the role of IL-2 *in vivo* might be predominantly the maintenance of peripheral T_{reg} cell number and, perhaps, triggering of other suppressive mechanisms.

Are T_{reg} cells really anergic?

Based on numerous *in vitro* studies, T_{reg} cells have been long considered to be anergic. More recent studies, however, demonstrate that this population has a high turnover *in vivo*. T_{reg} cells proliferate not only in lymphopenic conditions or after immunization with antigen or transgenically expressed antigens *in vivo* [3, 56, 71, 108, 111], but they also proliferate spontaneously in non-lymphopenic normal mice, i.e. without obvious contact with exogenous antigens [32]. Since at least a part of the T_{reg} cells is specific for autoantigens, it is tempting to speculate that this proliferation is driven by contact with autoantigens.

Similarly to *in vitro* conditions, T_{reg} cells do not produce IL-2 *in vivo*, even when proliferating; also, CD25 on the "suppressed" T cells is quickly down-regulated after immunization, probably reflecting IL-2 starvation [56, 108]. A proportion of T_{reg} cells secretes IL-10 when isolated after immunization [56] or from sites of inflammation [13, 41], which is compatibe with findings that IL-2 primes T_{reg} cells for IL-10 secretion *in vitro* [26].

Effects on target cells

T_{reg} cells can suppress not only $CD4^+$ T cells, but also $CD8^+$ T cells and B cells [17, 73, 78]. It is unknown whether these effects *in vivo* are mediated directly or via modulation of APC function by T_{reg} cells. It has been demonstrated that T_{reg} cells are found in clusters with $CD11c^+$ DCs and target T cells [71]. T_{reg} cells can modulate the expression of the co-stimulatory molecules B7.1 and B7.2 on APCs and suppress their function [19,

68]. CTLA-4, an inhibitory member of the CD28 superfamily, is over-expressed in T_{reg} cells and critical for their function *in vivo* [55, 62, 80], whereas its role *in vitro* remains controversial [4, 20, 29, 60, 62]. The high and constitutive expression of CTLA-4 on T_{reg} cells could enable them to out-compete normal T cells for co-stimulatory signals via CD28, since CTLA-4 has a much higher binding affinity to the CD28-ligands B7.1 or B7.2 [1]. Cross-linking of CTLA-4 can induce TGF-β production in T cells, suggesting a possible link between these molecules [22]. On the other hand, CTLA-4-mediated cross-linking of B7 molecules by T_{reg} cells can induce the immunosuppressive catabolism of tryptophan in DCs [30]. However, APCs are not absolutely required for the T_{reg} cell suppressor function at least *in vitro*, since this also works in APC-free systems [29, 78, 102].

Studies at a cellular level have shown that T_{reg} cells inhibit the differentiation of naïve T cells into effector T cells in primary responses [56, 58, 66, 83, 94] and interfere with the reactivation of memory T cells in secondary responses [35, 94]. This inhibition results in the lack of expression of homing molecules, and perhaps in a failure of these effector cells to migrate to target tissues [93]. T_{reg} cells do not reduce the number or modify the function of already primed T cells [35].

Localization of T_{reg} cells

The question of where T_{reg} cells act is at least as controversial as many other issues discussed in this review. In some models, such as diabetes or graft-versus-host disease, lymph node homing T_{reg} cells (high expression of CD62L and CCR7) have been reported to be better regulators than their $CD62L^{low}$ counterparts [95, 99]. Using the integrin $\alpha_E\beta_7$, Huehn and colleagues subdivided the $CD4^+CD25^+$ T_{reg} cells into $CD4^+CD25^+\alpha_E\beta_7^-$ or $CD4^+CD25^+\alpha_E\beta_7^+$ T_{reg} cells [45, 59]. Detailed molecular and functional analysis have provided evidence that the $\alpha_E\beta_7$-expressing subset represents antigen-experienced effector/memory-like T_{reg} cells, whereas $\alpha_E\beta_7^-$ cells represent naïve-like T_{reg} cells. *In vivo*, there are striking differences in the chemokine responsiveness and in the migration behavior between these subsets: $\alpha_E\beta_7$-expressing T_{reg} cells over-express receptors for inflammatory chemokines and several adhesion molecules critically involved in entry into inflamed peripheral tissues [45]. When tested for their suppressive capacity, $\alpha_E\beta_7^+$ T_{reg} cells are more effective suppressors in the SCID colitis model and in murine AIA [45, 59]. Homing analyses have shown that $\alpha_E\beta_7^+$ T_{reg} cells are able to enter the synovial membrane of arthritic mice, in contrast to $CD4^+CD25^+$ $\alpha_E\beta_7^-$ T_{reg} cells. This indicates that the suppression at the site of inflammation may be more effective, at least in this experimental setting [45]. Functional T_{reg} cells have also been found in inflamed tissues in infection, transplant, and colitis models, supporting the view that suppression at the site of inflammation is an important part of T_{reg} cell activity [13, 39, 71].

In conclusion, there may exist a division of labor between naïve- and effector/memory-like T_{reg} cells: naïve-like T_{reg} cells inhibit the activation of effector T cells in lymphoid environments, whereas effector/memory-like T_{reg} cells suppress effector T cell responses directly in peripheral tissues.

Regulation of T_{reg} cell activity

Another important question is how T_{reg} cell activity is regulated to allow efficient adaptive immune responses. Since T_{reg} cells are attracted by activated APCs [17], it could be expected that they quickly suppress every ongoing immune reaction. Clearly, however, there must be means whereby conventional T cells escape suppression by T_{reg} cells. High local levels of IL-2 can overcome suppression by T_{reg} cells [26]. Stimulation of Toll-like receptor (TLR) pathways in APCs by bacterial products induces the production of IL-6 (and perhaps other factors), which renders conventional T cells unresponsive to suppression by T_{reg} cells *in vitro* [75]. Also, *in vivo* data suggest that continuously triggered TLR pathways are required for by-passing T_{reg} cell-mediated tolerance [112].

Early studies have demonstrated that stimulation of GITR (which is over-expressed by T_{reg} cells) abrogates their suppressive effect [67, 87]; however, more recent work demonstrates that GITR/GITR-ligand interactions are potent co-stimulatory signals for both conventional and regulatory T cells [104], and that GITR/GITR-ligand co-stimulation renders conventional T cells resistant to suppression by T_{reg} cells.

A hypothetical model of T_{reg} cell action

We propose that T_{reg} cells utilize different mechanisms to achieve suppression, depending on the strength and stage of the immune reaction to be regulated, the localization, and the developmental stage of the T_{reg} cells. Conceivably, suppression mechanisms consist of a dynamic interplay between regulatory and conventional T cells rather than a simple on/off suppressor function. Figure 2 depicts a hypothetical model of how T_{reg} cells act, based on the data discussed above.

T_{REG} CELLS IN HUMAN DISEASES

Although an important role of $CD4^+CD25^+$ T_{reg} cells has been demonstrated in various animal models, little is known about their function in human autoimmune diseases. It is an attractive hypothesis that T_{reg} cell function is altered in autoimmunity. However, animal models are associated with some important caveats: **A**) First, experimental animals are kept in clean animal facilities and used at young ages for analysis, resulting in $CD25^+$ compartments almost exclusively composed of T_{reg} cells. In humans, however, the $CD25^+$ compartment is always a mixture of T_{reg} and activated effector T cells, in that only cells with high expression of CD25 possess regulatory activity; this is in contrast to cells with intermediate CD25

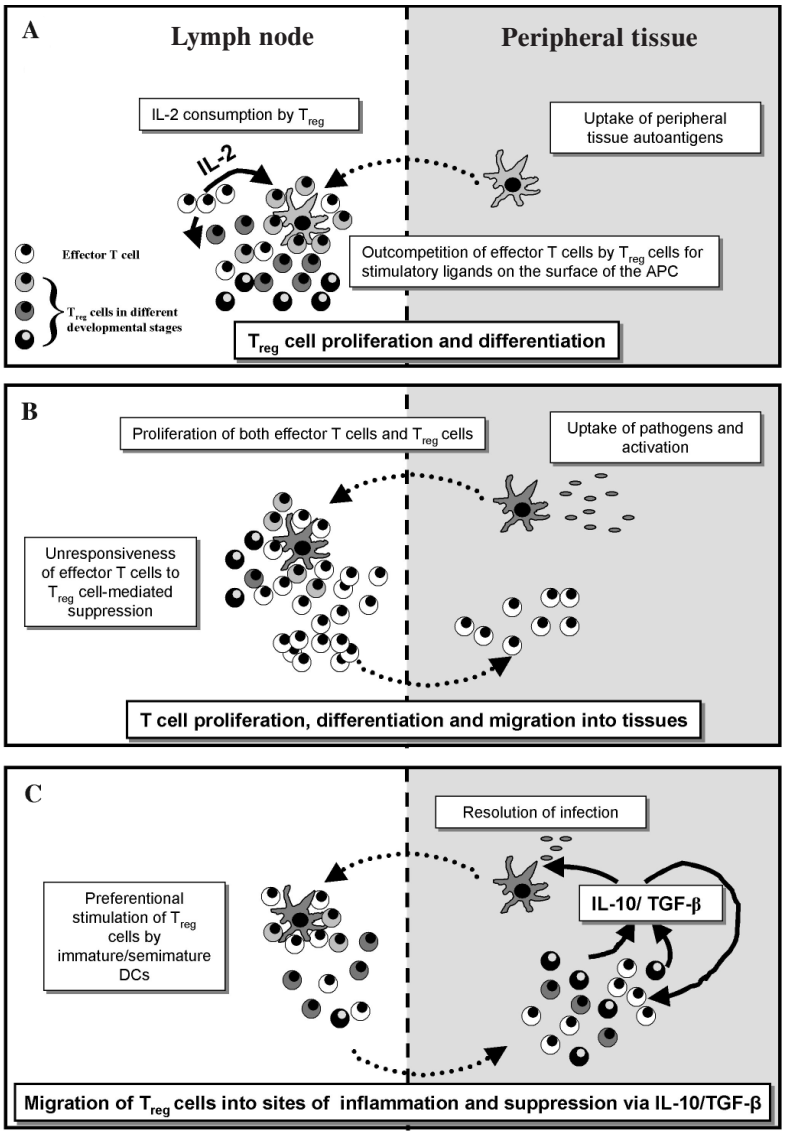

Fig. 2. Model of dynamics of conventional T cells and T_{reg} cells. **A** – in the steady-state, DCs take up antigens in peripheral tissues and transport them to lymph nodes. APCs recruit T_{reg} and conventional T cells; however, T_{reg} cells may be more efficiently recruited due to their expression of chemokine receptors, thus limiting the availability of co-stimulatory molecules to conventional T cells. Also, they may consume IL-2 produced by conventional T cells, thus inhibiting their proliferation by competition mechanisms. **B** – upon infection, DCs are stimulated via their TLRs, thus acquiring a highly activated phenotype. Under these circumstances, conventional T cells and T_{reg} cells are both stimulated; a sufficient immune response can be elicited because the suppression by T_{reg} cells can be overcome by high levels of co-stimulatory molecules (i.e. CD80/86 or GITR-L) on APCs or the production of cytokines such as IL-2 or IL-6. **C** – after resolution of infection, T_{reg} cells migrate to inflammatory sites and suppress inflammation. This suppression may be cytokine-mediated and less dependent on a blocked proliferation of conventional effector T cells. Previous priming of T_{reg} cells in the presence of high levels of IL-2 may enable their production of IL-10.

expression, which represent activated effector T cells [9]. Thus, even if FACS-sorted $CD25^{high}$ cells are used, it cannot be excluded that they are contaminated to some degree with activated effector T cells. **B)** Perhaps more importantly, T_{reg} cell activity is commonly measured by their ability to suppress the proliferation of responder T cells (usually conventional T cells) in *in vitro* co-cultures. As extensively discussed above, it is unclear how the *in vitro* suppressor activity relates to the *in vivo* activity. However, one can assume at least a certain degree of similarity in spite of the obvious differences in behavior (anergy versus proliferation, cell contact versus cytokines).

Given these limitations, two studies have shown that in multiple sclerosis the number of T_{reg} cells is similar in patients and healthy controls. Analyses of the suppressor activity *in vitro* indicate a loss of suppressor function under limiting stimulation conditions [79, 106]. In rheumatoid arthritis (RA) and other chronic joint inflammations, an enrichment of $CD4^+CD25^+$ T_{reg} cells in inflamed joints has been described; also, these cells are functionally active when tested *in vitro* [18, 25, 105]. The reason why chronic inflammation persists in the presence of high numbers of T_{reg} cells is unknown, but it may be speculated that such inflammatory conditions abolish the suppressor function. Analyses of T_{reg} cells in peripheral blood of RA patients have revealed that they can suppress proliferation of effector T cells, but not the production of pro-inflammatory cytokines. Interestingly, anti-TNF therapy can increase the number of T_{reg} cells and restore their impaired function [28]. However, it cannot be excluded that T_{reg} cells activate synovial fibroblasts, perhaps via TGF-β secretion, and that in RA these cells have a disease-promoting role rather than a protective one.

T_{REG} CELLS AS A TARGET FOR THERAPEUTIC INTERVENTIONS

The use of T_{reg} cells for the re-establishment of peripheral tolerance is an attractive approach for the treat-

ment of autoimmune diseases. Therapeutic vaccination with *in vitro* expanded T_{reg} cells [42] may be one way to achieve this goal. However, T_{reg} cells are difficult to expand *in vitro* and evidence from animal models that polyclonal T_{reg} cells can cure autoimmune diseases is sparse and controversial. Adoptive transfer of $CD4^+CD25^+$ T_{reg} cells has been shown to cure murine colitis after disease onset in SCID mice [62, 71]. Apparently, however, this therapeutic efficacy can be achieved only in a limited period of time [34]; in addition, this model deals with extremely lymphopenic conditions. The transfer of polyclonal T_{reg} cells has failed in experimental diabetes, although transfer of antigen-specific T_{reg} cells isolated from TCR transgenic mice could reverse even established disease [16, 98]. In our own experiments in the AIA model we were unable to demonstrate a curative effect of T_{reg} cell transfer on established arthritis [35].

An alternative approach could be the *in vivo* expansion or *de novo* induction of T_{reg} cells by treatment with superagonistic anti-CD28 or anti-CD3 antibodies [12, 61] or low-dose antigen administration [6]. However, it is undoubtedly too early to speculate whether and how cellular therapy with T_{reg} cells or modulation of their function *in vivo* bears therapeutic potential in autoimmune diseases until we have reliable knowledge about the function of these cells in disease. At the same time, the augmentation of anti-tumor responses by depletion of T_{reg} cells seems quite promising. Several groups have reported that T_{reg} cell depletion allows rejection of otherwise non-rejected tumors in murine models [38, 47, 74, 86, 92]. In addition, patients with malignant diseases display elevated numbers of $CD25^+$ T_{reg} cells not only in the peripheral blood and within the tumor tissue, but also in the draining lymph nodes or malignant ascites [24, 63, 107]. The tumor-infiltrating cells express Foxp3 and are actively recruited by CCL22, a chemokine produced by tumor cells [24]. These tumor-derived T_{reg} cells inhibit tumor-specific immunity *in vitro* and *in vivo,* and there is a strong correlation between high T_{reg} cell numbers and poor survival in these patients [24]. Thus, the tumor data convincingly demonstrate that in humans (similarly to mice) T_{reg} cells actively inhibit tumor-specific immune responses; they also suggest that elimination of T_{reg} cells may be a powerful way to enhance anti-tumor responses and, perhaps, the effectiveness of tumor vaccinations.

CONCLUDING REMARKS

Even though the knowledge on regulatory T cells has substantially increased in the past few years, we are still beginning to understand the biological role of these cells. Strong efforts are needed to identify the molecular nature of suppression. Once identified, this knowledge may enable us to assess T_{reg} cell function more directly in patients with autoimmune diseases and to obtain reliable information on whether augmentation of T_{reg} cell function or numbers is therapeutically relevant. Indeed, the prominent role of T_{reg} cells in experimental models strongly suggests that they will be become an important target of diagnostic, prognostic, and therapeutic purposes in clinical research.

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