[Frontiers in Bioscience 14, 333-343, January 1, 2009]

Natural Tregs and autoimmunity

Antonio La Cava

Department of Medicine, University of California Los Angeles, 1000 Veteran Avenue 32-59, Los Angeles, California 90095-1670

TABLE OF CONTENTS

1. ABSTRACT

The subset of $CD4^+$ T lymphocytes that coexpress high levels of the interleukin (IL)-2 α receptor and the transcription factor Foxp3 ($CD4^+CD25^{high}Foxp3^+$ cells), commonly called regulatory T cells (Tregs), have a key role in the mechanisms of peripheral immune tolerance. Tregs modulate innate and adaptive immune responses *in vitro* and *in vivo* by suppressing the proliferation and cytokine production in different subsets of immune cells. Their key role in autoimmunity is suggested by the finding that reconstitution of normal numbers and/or function of Tregs in autoimmune animals associates with a delay of disease development and progression, whereas the elimination of Tregs can anticipate or precipitate disease. Since naturally occurring ("natural") Tregs represent only a small fraction of peripheral blood cells, the investigations for possible therapeutic use of Tregs in autoimmunity has largely focused on the use of "adaptive" Tregs, which can be induced through several different modalities. This review discusses the role of natural Tregs in the suppression of autoimmune responses and the relevance of these cells for possible therapeutic applications in autoimmunity.

2. INTRODUCTION

Two arms of the immune system act in concert to fight the challenges that come from the environment (i.e. pathogens) and/or from the host itself (i.e. transformed cells). One arm is the innate immunity - a first-line, primordial defense against bacterial or viral pathogens. The other arm is the adaptive immunity, which provides a somehow delayed, yet more efficient and specific response. While the innate immune system lacks a fine specificity in lieu of a broader recognition of common features shared by many pathogens, the adaptive immune system typically employs cells that have a defined antigenic specificity. Because of the vast number of epitopes that can be encountered – and their possible structural similarity with self antigens - the adaptive immune system carries an intrinsic risk for generating autoreactive cells potentially capable to trigger autoimmune responses (1-2). To limit this risk, one mechanism to keep self-reactive lymphocytes under control is immune tolerance, which helps to avert autoimmunity by preventing and/or shifting potentially deleterious autoreactive immune response towards non-injurious immune responses. Immune tolerance operates at a central level (i.e. thymus, bone marrow) and in the

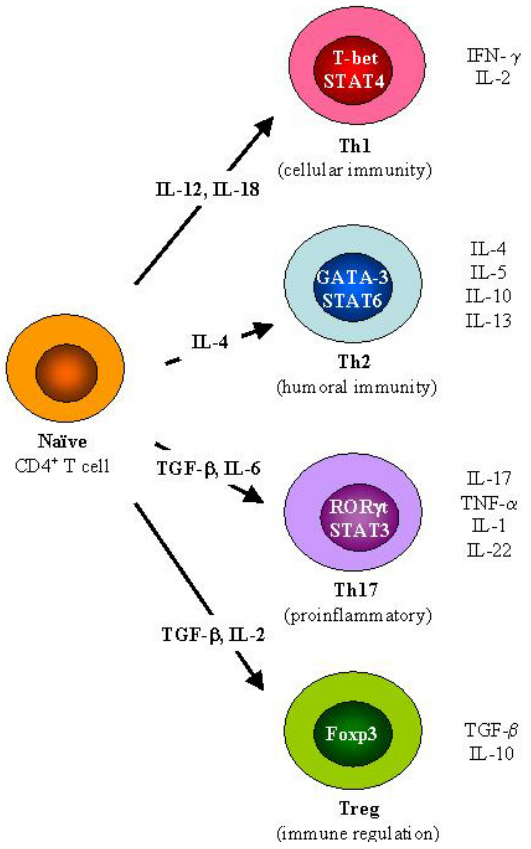

Figure 1. Schematic representation of the differentiation of naïve CD4 T cells towards the Th1, Th2, Th17 and Tregs phenotypes.

periphery, through mechanisms that include the deletion of potentially pathogenic clones, the hyporesponsiveness to antigenic stimulation (anergy), an ignorance for the antigen, and the suppression of immune responses by regulatory cells.

Regulatory/suppressor cells are phenotypically heterogeneous and include subsets of $CD4^+$ T cells (see below) but also natural killer (NK) and NK T (NKT) cells, $CD4^-CD8^-$ T cells, CD8 cells ($CD8^+CD28^-$, $CD8^+CD122^+$, $CD8^+CD25^+$ and Qa-1-restricted $CD8^+$ T cells), dendritic cells (DC), B cells, and γδ T cells (3-10). The mechanism of action of each of these immunoregulatory cell populations may be specific for a subset or common to other subsets, i.e. the secretion of transforming growth factor (TGF)-β and interleukin (IL)-10 can have a central role in the suppressive activity of Tr1 cells (see below) and NKT cells (5), while natural Tregs need a cell-to-cell contact to exert their suppression on target cells (see below). Aspects such as the interplay between Tregs and DC have been reviewed elsewhere (11).

In general, Tregs can be important in suppressing autoimmune reactivity but can be deleterious in tumor immune surveillance because of their capacity to suppress the activation, proliferation, and effector function of tumor-infiltrating T-cells (12-13). Analogous considerations on possible undesired effects of the Tregs can be made in regard to the capacity of these cells to suppress the effector function of immune cells reactive to pathogens in infection (as considered at the end of the chapter).

3. REGULATORY T CELLS (TREGS)

As schematically shown in Figure 1, naïve $CD4^+$ T cells can differentiate towards T helper (Th)1, Th2, Th17, and regulatory T cells (Tregs), both *in vitro* and *in vivo*, depending on the type of stimulation, antigen concentration, co-stimulation, and cytokine milieu where the immune response takes place (14-15). In general, the presence of interleukin (IL)-12 skews the $CD4^+$ T cells towards a Th1 phenotype, IL-4 towards Th2, TGF-β (+/- IL-2) towards Tregs, and IL-6 and TGF-β towards Th17 - with possible mutually exclusive skewing between Tregs and Th17 phenotypes (16). The committed cells typically express specific transcription factors: T-bet for Th1, GATA-3 for Th2, Foxp3 for Tregs, and RORγt for Th17 cells (17-22).

The best characterized subsets of $CD4^+$ T cells with immune suppressive capacity described so far are the T-regulatory 1 (Tr1) cells (23), the Th3 cells (24), and the $CD4^+CD25^+$ T cells (25-26).

Tr1 cells are IL-10 producing $CD4^+$ regulatory T cells induced from $CD4^+$ T cells by repetitive antigenic stimulation in the presence of IL-10 (23) or immature DC (27). Tr1 cells produce high levels of IL-10 and can be generated by chronic activation in the presence of IL-10, both in humans and in mice (23, 28).

Th3 regulatory T cells are TGF-β-producing $CD4^+$ T cells that suppress $CD4^+$ T cells in an antigen-nonspecific manner (24). These cells secrete TGF-β, IL-4 and IL-10 (29), and can be induced *in vitro* after exposure of $CD4^+CD25^-$ T cells to TGF-β or *in vivo* following oral or intravenous administration of antigen (30). The suppressive effect of *ex vivo*-induced Th3 cells appears mediated by suppressive cytokines and is thus, at least in part, cell-contact independent (30-31).

Finally, the most widely studied and best characterized regulatory $CD4^+$ T cells are the $CD4^+CD25^+$ T cells. In organ-specific autoimmunity, a deficiency of Tregs typically associates with development of organ damage (32-34), and a restoration of the number and/or function of Tregs confers protection from autoimmunity (33-37). In systemic autoimmunity, Treg-depleted animals typically develop multi-organ autoimmune disease (38), while supplementation of syngeneic Tregs or adoptive transfer of *in vitro* expanded Tregs abrogates the development of autoimmune disease manifestations (39).

3.1. Types of Tregs

$CD4^+$ regulatory T cells can be schematically divided into two groups: naturally occurring ("natural") Tregs, and induced ("adaptive") Tregs.

3.1.1. Natural Tregs

Natural Tregs arise from the thymus during ontogeny and, in the mouse, they seed the periphery around day 3 of neonatal life (40-41, 17). The number of natural Tregs circulating in the peripheral blood of adult mice remains virtually constant throughout life, comprising 5–10% of the $CD4^+$ cells. The fraction of Tregs is smaller in humans, where they represent the 1% to 2% of total $CD4^+$ T cells, e.g. the ones with highest CD25 expression ($CD25^{high}$) (42).

Natural Tregs constitutively express CD4, CD25 and Foxp3, and suppress the proliferation and cytokine production of target cells *in vitro* and *in vivo*. These cells contribute to avert autoimmune disease by suppressing activated immune cells through cell contact-dependent and cytokine-independent mechanisms (43-45). The cell-to-cell contact suppressive mechanisms used by Tregs include cytotoxic functions involving the synthesis of perforin, CD18, and granzyme A in a Fas-independent manner (46). The targets of the cytotoxic activity of the Tregs include $CD4^+CD25^-$ T cells, $CD8^+$ T cells, monocytes, antigen-presenting B cells, and DC (46-47, 25-26). A role for lymphocyte activation gene 3 (LAG-3) in the cell-to-cell mediated suppression of natural Tregs has been suggested by the blockade of the suppressive function of these cells using anti-LAG-3 antibody *in vitro* and *in vivo*, and by the finding that transduction of LAG-3 into $CD4^+CD25^-$ T cells conveys a suppressive function to these cells (48). Also, natural Tregs may require the ligation of B7 molecules on target T cells to mediate suppression of conventional T cells, since target cells deficient in B7 molecules (CD80 and, to a lesser degree, CD86) are resistant to Treg suppression (49).

Although anergic *in vitro*, natural Tregs can be expanded *ex vivo* and retain their functional suppressive activity when stimulated with anti-CD3 and IL-2 or when cultured with high ratios of antigen-loaded DC (50-51). Another mean to expand natural Tregs and increase their survival is through the combination of anti-CD3 and anti-CD28 antibodies (52).

Interestingly, $CD4^+CD25^+$ Tregs can promote infectious tolerance - a phenomenon by which they can induce conventional $CD4^+$ T cells to also become suppressor cells (53). The capacity of natural Tregs to amplify immune regulatory responses is shared with adaptive Tregs induced *ex vivo* using TGF-β and IL-2, as these cells can also "infectiously" tolerize $CD4^+CD25^-$ cells to become suppressor cells (54).

3.1.2. Adaptive Tregs

Adaptive Tregs can be generated in cultures or in the periphery. For example, $CD4^+CD25^+Foxp3^+$ Tregs can be induced *in vitro* from $CD4^+CD25^-$ T cells using TGF-β (54-55), and adoptively transferred polyclonal $CD4^+CD25^-Foxp3^-$ T cells can differentiate into $CD4^+CD25^+Foxp3^+$ T cells in recipient mice following homeostatic proliferation (56).

Additionally, $CD4^+$ cells can become IL-10-producing Tr1 cells when repeatedly stimulated with IL-10 or with immature DC (23), vitamin D3 and dexamethasone (57). Adaptive Tregs may have similar phenotype and function as natural Tregs since both types of lymphocytes can suppress immunological responses, yet they may differ in the mechanisms of action because natural Tregs can require direct cell–cell interaction for suppression (as shown in transwell experiments where supernatants from activated Tregs do not display suppressive properties) (49), whereas soluble factors (e.g. TGF-β) may be necessary for optimal action and maintenance of adaptive Tregs (54).

4. PHENOTYPE OF TREGS

Since CD25 – the α receptor for IL-2 - may not be unique to Tregs but is also expressed by conventional activated $CD4^+$ T cells, additional surface marker have been searched for reliable identification of suppressor Tregs. Recently, the α chain of the IL-7 receptor (CD127) has been proposed for a rapid phenotypic identification of the Tregs. This marker is present at low levels on the surface of Tregs ($CD127^{low}$) and at high levels on activated effector T cells ($CD127^{high}$) (58-59).

Other molecules that contribute to the phenotype of the $CD4^+CD25^+$ Tregs and that cannot yet be considered unique markers because of some overlap of expression with effector CD4 T cells include the glucocorticoid-induced tumor necrosis factor receptor (GITR), the cytotoxic T lymphocyte-associated antigen-4 (CTLA-4 or CD152 – high intracellularly, low on cell surface), $CD62L^{high}$, $CD45RB^{low}$, CD103 (integrin αEβ7), neuropilin-1, membrane-bound TGF-β (in form of latency-associated protein, LAP), CD5, CD27, CD38, CD39, CD69, CD73, CD122, OX-40 (CD134), CCR4, CCR7, CCR8, TNF-R2, LAG-3 and, last but not least, the intracellular forkhead/winged helix transcription factor Foxp3 (60-63), which is currently considered the most reliable marker to monitor functional Tregs (64).

The important role of Foxp3 in the activity of Tregs and in autoimmunity is best exemplified by the observation that genetic deficiency of $Foxp3^+$ cells in scurfy mice (17) or in humans affected by the IPEX syndrome (an X-linked syndrome characterized by immune dysregulation, polyendocrinopathy, enteropathy) (65) causes lymphoproliferative, rapidly lethal autoimmune disease.

5. MECHANISMS OF ACTION OF TREGS

Tregs may not require antigen specificity in their mechanisms of suppression, although the activation of these cells seems to occur preferentially via the engagement of the T-cell receptors (TCR) on these cells (69). Like conventional T cells, Tregs are selected with different affinity/avidity for the TCR and are enriched in self-reactive cells, as suggested by adoptive transfer studies (66-70). Overall, Tregs can be activated by self-antigens and non-self-antigens and may use or not antigen specificity for suppression *in vitro* and *in vivo* (71-75, 26).

Regarding IL-2, many studies have shown that this cytokine is important for the development, peripheral

survival, and suppressive function of Tregs through events that may not be linked to a modulated expression of Foxp3 (76). IL-2 produced at sites of inflammation could contribute to drive Treg suppression (77), and peripheral survival and expansion of Tregs could depend on the presence of IL-2 (78).

Notably, mice deficient in IL-2, IL-2R, or IL-2Rβ have reduced numbers of natural Tregs and die from a lymphoproliferative autoimmune syndrome (79) despite the fact that IL-$2^{-/-}$ and IL-$2R^{-/-}$ Tregs are functional and can suppress T-cell proliferation *in vitro* (80). These observations suggest that IL-2 can be required to sustain *in vivo* the homeostasis of natural Tregs (78) - possibly together with other signals for peripheral T cell survival including the encounter with MHC/self peptides (81), cytokines, and co-stimulatory molecules (82-84). Moreover, it has been shown that thymic generation of Tregs requires an intact IL-2/IL-2R pathway (85-86), yet the presence of a thymus is not an absolute requirement for the generation of Tregs and these cells can be generated in the periphery and/or from $CD4^+CD25^-$ T cells (87-88).

5.1. *In vitro* suppression

Activated Tregs suppress target cells in a cell-contact-dependent, cytokine-independent mechanism (89). CD80 and CD86 ligands on T cells might bind to CTLA-4 on the Tregs during suppression, and this might be a reason why cell contact is necessary. Other molecules influencing the susceptibility of target cells to suppression by Tregs include GITR and GITR-ligand (GITR-L) (90) and, possibly, cell surface-bound TGF-β (91).

Regarding the target cell, after the encounter with the Tregs the effector T cells can display an arrest in cell cycle progression caused by uncoupled IL-2 signaling (92). Moreover, the activation of Tregs by anti-CD3 and anti-CD46 results in the expression of granzyme A, which facilitates the killing of the targets by a perforin-dependent Fas-Fas ligand-independent mechanism (46, 43). A granzyme B-dependent perforin-independent mechanism has also been identified for the suppressive activity of the Tregs (93).

The specific mechanisms of action of Foxp3, GITR, and CTLA-4 in Treg suppression are currently under intense investigation. It is clear that abrogation of the activity of any of these molecules reduces significantly the suppressive capacity of the Tregs. For example, antibody-mediated inhibition of CTLA-4 can abrogate the protective effects of Tregs in murine inflammatory bowel disease (94), possibly because CTLA-4 on Tregs could transduce a costimulatory activating signal along with TCR signaling (95).

It has also to be considered that there are molecules that can counteract the suppressive activity of the Tregs. For example, ligation of Toll-like receptor-2, -4, -8 and -9 results in the abrogation of Treg-mediated suppression (see below). Toll-like receptors (TLR) are molecules that recognize pathogen-associated molecular patterns (PAMP) common to different pathogens. Upon activation, TLR can stimulate adaptive immune responses through the activation of DC and the upregulation of costimulatory molecules and inflammatory cytokines including IL-6, TNF-α and IL-12 (96). The engagement of TLR-4 or TLR-9 on murine splenic DC can abrogate the suppressive activity of Tregs *in vitro* - possibly via the induction of a resistance of target cells to Treg-mediated suppression (a phenomenon that is dependent in part on DC-derived IL-6) (97). Also, TLR-9 deficiency in MRL mice leads to exacerbated lupus apparently as a result of increased T-cell activation associated with impaired Treg suppressive capacity *in vitro* (98-99).

5.2. *In vivo* suppression

The *in vitro* hyporesponsiveness to antigenic stimulation of the Tregs can be overcome by stimulation with anti-CD3 antibody and IL-2. Although Tregs are anergic *in vitro*, they do proliferate *in vivo* in response to antigen (100-101, 68). Available data suggest that the suppression *in vitro* of the Tregs may not reflect their *in vivo* activity, and additional factors such as Tregs migration and homing might influence significantly the activity of these cells, particularly at sites of inflammation (75). In particular, the differential expression of CD62L and CD27 on human $CD4^+CD25^{high}$ Tregs could distinguish Treg subsets with migratory properties, i.e. $CD27^-CD62L^-$ Tregs could be destined to periphery, $CD62L^+CD27^+$ Tregs would home to lymph nodes (102-106), and Tregs expressing CCR6 and CD45RO would accumulate in tissue (105). Ultimately, the accumulation of Tregs in tissue could limit the activity of effector target cells and subsequent inflammation at sites of organ damage. These considerations could contribute to explain why soluble factors may not be involved in the *in vitro* suppression mediated by the Tregs whereas *in vivo* the Tregs may need IL-10 and TGF-β to suppress target cells (106-107), and/or why Tregs can inhibit target cell transcription of IL-2 *in vitro* (108) while *in vivo* they may suppress independently on IL-2 involvement (80).

6. NATURAL TREGS AND AUTOIMMUNITY

The dysregulation of immune tolerance has a central role in the development and progression of pathogenic autoimmunity, and mice with an autoimmune background typically have reduced numbers of $CD4^+CD25^+$ T cells when compared to non-autoimmune mice. We summarize below the role of Tregs in the pathogenesis of several organ specific and systemic autoimmune diseases.

6.1. Natural Tregs and organ-specific autoimmunity

6.1.1. Multiple sclerosis

Experimental autoimmune encephalomyelitis (EAE) is a disease model for human multiple sclerosis (MS), and is mediated by encephalitogenic $CD4^+$ T cells. Mice that harbor myelin basic protein (MBP)-specific $CD4^+$ T cells have high incidence of disease due to the failure of Tregs to control pathogenic T cells (109). Tregs can inhibit *in vitro* the proliferation and cytokine production by myelin oligodendrocyte glycoprotein

(MOG)-specific Th1 cells and can confer *in vivo* protection against EAE (33).

In humans, a functional dysfunction and a reduced number of Tregs has been described in patients with MS (110-111).

6.1.2. Type-1 diabetes

The progression of benign to aggressive insulitis in type-1 diabetes-prone female nonobese diabetic (NOD) mice is associated with a peripheral deficit of Tregs in pancreatic lymph nodes and pancreatic islets (112-115). In NOD mice, the functional potency and intra-pancreatic proliferative potential of natural Tregs declines with age, augmenting in turn the diabetogenic responses and the development of the disease (116). Notably, the adoptive transfer of Treg-depleted splenocytes in rodents exacerbates disease (113), whereas the transfer of either Tregs from diabetes-resistant animals (117) or Tregs expanded *in vitro* suppresses the disease (118). Since Tregs isolated from pancreatic lymph nodes - but not from peripheral lymph nodes - delay the progression of diabetes (119), it has been proposed to use antigen-specific Tregs for the prevention and/or reversal of experimental diabetes, with encouraging preliminary results (120).

In humans, the role of Tregs in type-1 diabetes is suggested by the finding of a functional deficit and impaired suppressive capacity of these cells (121). Importantly, rapamycin can allow the growth of functional Tregs while contributing to the depletion of effector T cells, envisioning a rapamycin-based, Treg-targeted immunotherapy in the disease (122).

6.1.3. Inflammatory bowel disease

The development of experimental colitis induced by the transfer of naïve $CD4^+CD45RB^{high}$ T cells into syngeneic immuno-deficient mice (123) is prevented by co-transfer of regulatory $CD4^+CD45RB^{low}$ T-cells (124). In a murine model for human inflammatory bowel disease (IBD), $CD4^+CD25^+Foxp3^+$ Tregs suppress effector T cell responses through mechanisms of cytokine deprivation-induced apoptosis (125), and their suppressive activity can be shown at the site of organ damage - the intestinal lamina propria (126).

In humans, the number of peripheral Tregs in IBD patients correlates with changes in disease activity, as it increases during remission and decreases with active disease (127).

6.1.4. Experimental autoimmune thyroiditis

Several studies suggest that Tregs might effectively modulate experimental autoimmune thyroiditis, a mouse model for Hashimoto's thyroiditis (128-130). This is relevant because patients with autoimmune thyroiditis may suffer from a reduced suppressive capacity of the Tregs.

6.2. Natural Tregs and systemic autoimmunity

6.2.1. Systemic lupus erythematosus

Studies in murine models of SLE have suggested that a deficit of Tregs can contribute to loss of self-tolerance and subsequent development of clinical manifestations of the disease (131). Additionally, adoptive transfer of *ex vivo* expanded Tregs can slow the progression of renal disease and decrease the mortality in transferred mice (39).

In view of the finding that some lupus-prone mice also have a reduced sensitivity of $CD4^+CD25^-$ effector T cells to the suppression by Tregs (132), it can be hypothesized that the dysfunction of immune homeostasis in murine lupus might be partly associated with an abnormal number/function of Tregs and/or a reduced resistance of the target cells to Treg suppression, depending on the animal model considered.

In human SLE, the number of natural Tregs is decreased during active disease flares (133-134) and in active SLE pediatric patients (135). Interestingly, those defects can be reversed *in vitro* by Treg activation - a process which renders the defective SLE Tregs functional suppressor cells (136).

6.2.2. Autoimmune rheumatic diseases

Depletion of Tregs accelerates collagen-induced arthritis in mice (137), whereas adoptive transfer of Tregs protects mice from the systemic, chronic joint inflammation (138).

In humans, early rheumatoid arthritis (RA) associates with a reduced number of peripheral Tregs (139), although functional Tregs can be found enriched in the synovial fluid of patients with RA (140). It is possible that these cells may not be fully functional also because they have a decreased *in vitro* capacity to suppress the production of IFN-γ and TNF-α in target $CD4^+CD25^-$ T cells (141).

Of note, patients with relatively benign oligoarticular juvenile idiopathic arthritis have higher frequency of Tregs than patients with oligoarticular juvenile arthritis, a disease with a less favorable prognosis (142).

7. CONCLUSION

Although Tregs have the potential to reestablish immune tolerance to self-antigens both in new-onset and established disease in several experimental settings, they carry at the meantime the potential for detrimental effects due to the suppression of effector immune responses to tumors and microorganisms. Therapeutic considerations for targeting Tregs in autoimmunity remain nonetheless an appealing possibility, particularly because of the encouraging data obtained in animal models. Future studies of Treg-based immunotherapy will need to focus on how to circumvent the current obstacles, i.e. to define more specific markers for these cells, to keep in mind the differences between human and rodent Tregs, and to consider their site of action and the interactions with other immune cells and cytokine milieu where they operate.

8. ACKNOWLEDGMENTS

A.L.C. is supported by the National Institutes of Health grants AR53239 and AI63515.

9. REFERENCES

1. Nossal, G. J. Negative selection of lymphocytes. *Cell* 76, 229–239 (1994)

2. von Boehmer, H. Positive selection of lymphocytes. *Cell* 76, 219–228 (1994)

3. La Cava, A., F.-D. Shi. Natural and adaptive immune cell-based therapies in autoimmunity. *Curr Med Chem* 13, 1557-1566 (2006)

4. Ghiringhelli, F., C. Menard, M. Terme, C. Flament, J. Taieb, N. Chaput, P. E. Puig, S. Novault, B. Escudier, E. Vivier, A. Lecesne, C. Robert, J. Y. Blay, J. Bernard, S. Caillat-Zucman, A. Freitas, T. Tursz, O. Wagner-Ballon, C. Capron, W. Vainchencker, F. Martin, L. Zitvogel. $CD4^+CD25^+$ regulatory T cells inhibit natural killer cell functions in a transforming growth factor-β-dependent manner. *J Exp Med* 202, 1075–1085 (2005)

5. Filaci, G., N. Suciu-Foca. $CD8^+$ T suppressors are back in the game: are they players in autoimmunity? *Autoimmun Rev* 1, 279-283 (2002)

6. Lu, L., M. B. Werneck, H. Cantor. The immunoregulatory effects of Qa-1. *Immunol Rev* 212, 51–59 (2006)

7. Rifa'I, M., Y. Kawamoto, I. Nakashima, H. Suzuki. Essential roles of $CD8^+CD122^+$ regulatory T cells in the maintenance of T cell homeostasis. *J Exp Med* 200, 1123–1134 (2004)

8. Hanninen, A., L. C. Harrison. γδ T cells as mediators of mucosal tolerance: the autoimmune diabetes model. *Immunol Rev* 173, 109–119 (2000)

9. Bacchetta, R., S. Gregori, M. G. Roncarolo. $CD4^+$ regulatory T cells: mechanisms of induction and effector function. *Autoimmun Rev* 4, 491-496 (2005)

10. Jiang, S., R. I. Lechler, X. S. He, J. F. Huang. Regulatory T cells and transplantation tolerance. *Hum Immunol* 67, 765-776 (2006)

11. Tang, Q., J. A. Bluestone. Regulatory T-cell physiology and application to treat autoimmunity. *Immunol Rev* 212, 217-237 (2006)

12. Itoh, M., T. Takahashi, N. Sakaguchi, Y. Kuniyasu, J. Shimizu, F. Otsuka, S. Sakaguchi. Thymus and autoimmunity: production of $CD25^+CD4^+$ naturally anergic and suppressive T cells as a function of the thymus in maintaining immunologic self-tolerance. *J Immunol* 162, 5317-5326 (1999)

13. Bluestone, J. A., A. K. Abbas. Natural versus adaptive regulatory T cells. *Nature* 3, 253-257 (2003)

14. Afzali, B., G. Lombardi, R. I. Lechler, G. M. Lord. The role of T helper 17 (Th17) and regulatory T cells (Treg) in human organ transplantation and autoimmune disease. *Clin Exp Immunol* 148, 32-46 (2007)

15. Constant, S. L., K. Bottomly. Induction of Th1 and Th2 $CD4^+$ T cell responses: the alternative approaches. *Annu Rev Immunol* 15, 297–322 (1997)

16. Bettelli, E., Y. Carrier, W. Gao, T. Korn, T. B. Strom, M. Oukka, H. L. Weiner, V. K. Kuchroo. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* 441, 235-238 (2006)

17. Fontenot, J. D., M. A. Gavin, A. Y. Rudensky. Foxp3 programs the development and function of $CD4^+CD25^+$ regulatory T cells. *Nat Immunol* 4, 330-336 (2003)

18. Gately, M. K., L. M. Renzetti, J. Magram, A. S. Stern, L. Adorini, U. Gubler, D. H. Presky. The interleukin-12/interleukin-12-receptor system: role in normal and pathologic immune responses. Annu Rev Immunol 16, 495–521 (1998)

19. Nelms, K., A. D. Keegan, J. Zamorano, J. J. Ryan, W. E. Paul. The IL-4 receptor: signaling mechanisms and biologic functions. Annu Rev Immunol 17, 701–738 (1999)

20. Szabo, S. J., S. T. Kim, G. L. Costa, X. Zhang, C. G. Fathman, L. H. Glimcher. A novel transcription factor, T-bet, directs Th1 lineage commitment. Cell 100, 655–669 (2000)

21. Zheng, W., R. A. Flavell. The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. Cell 89, 587–596 (1997)

22. Ivanov, I. I., B. S. McKenzie, L. Zhou, C. E. Tadokoro, A. Lepelley, J. J. Lafaille, D. J. Cua, D. R. Littman. The orphan nuclear receptor ROR□t directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell 126, 1121–1133 (2006)

23. Groux, H., A. O'Garra, M. Bigler, M. Rouleau, S. Antonenko, J. E. de Vries, M. G. Roncarolo. A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. Nature 389, 737-742 (1997)

24. Weiner, H. L. Induction and mechanism of action of transforming growth factor-□-secreting Th3 regulatory cells. Immunol Rev 182, 207-214 (2001)

25. Sakaguchi, S. Naturally arising CD4+ regulatory T cells for immunologic self-tolerance and negative control of immune responses. Annu Rev Immunol 22, 531-562 (2004)

26. Shevach, E. M. CD4+CD25+ suppressor T cells: more questions than answers. Nat Rev Immunol 2, 389-400 (2002)

27. Levings, M. K., M. G. Roncarolo. T-regulatory 1 cells: a novel subset of CD4 T cells with immunoregulatory properties. *J Allergy Clin Immunol* 106, S109-112 (2000)

28. Dieckmann, D., C. H. Bruett, H. Ploettner, M. B. Lutz, G. Schuler. Human $CD4^+CD25^+$ regulatory, contact-dependent T-cells induce interleukin-10-producing, contact-independent type 1-like regulatory T-cells. *J Exp Med* 196, 247–253 (2002)

29. Chen, Y., J. Inobe, V. K. Kuchroo, J. L. Baron, C. A. Janeway Jr., H. L. Weiner. Oral tolerance in myelin basic protein T-cell receptor transgenic mice: suppression of autoimmune encephalomyelitis and dose-dependent induction of regulatory cells. *Proc Natl Acad Sci USA* 93, 388-391 (1996)

30. Thorstenson, K. M., A. Khoruts. Generation of anergic and potentially immunoregulatory $CD25^+CD4^+$ T-cells *in vivo* after induction of peripheral tolerance with intravenous or oral antigen. *J Immunol* 167, 188–195 (2001)

31. Zheng, S. G., J. D. Gray, K. Ohtsuka, S. Yamagiwa, D. A. Horwitz. Generation *ex vivo* of TGF-β-producing regulatory T cells from $CD4^+CD25^-$ precursors. *J Immunol* 169, 4183-4189 (2002)

32. Lepault, F., M. C. Gagnerault. Characterization of peripheral regulatory $CD4^+$ T cells that prevent diabetes onset in nonobese diabetic mice. *J Immunol* 164, 240-247 (2000)

33. Kohm, A. P., P. A. Carpentier, H. A. Anger, S. D. Miller. $CD4^+CD25^+$ regulatory T cells suppress antigen-specific autoreactive immune responses and central nervous system inflammation during active experimental autoimmune encephalomyelitis. *J Immunol* 169, 4712-4716 (2002)

34. Bach, J. F., L. Chatenoud. Tolerance to islet autoantigens and type 1 diabetes. *Annu Rev Immunol* 19, 131-161 (2001)

35. Wu, A., S. Hua, S. Munson, H. McDevitt. Tumor necrosis factor-α regulation of $CD4^+CD25^+$ T cell levels in NOD mice. *Proc Natl Acad Sci USA* 99, 12287-12292 (2002)

36. Szanya, V., J. Ermann, C. Taylor, C. Holness, C. G. Fathman. The subpopulation of $CD4^+CD25^+$ splenocytes that delays adoptive transfer of diabetes expresses L-selectin and high levels of CCR7. *J Immunol* 169, 2461-2465 (2002)

37. Mekala, D. J., T. L. Geiger. Immunotherapy of autoimmune encephalomyelitis with redirected $CD4^+CD25^+$ T lymphocytes. *Blood* 105, 2090-2092 (2005)

38. Sakaguchi, S., T. Takahashi, Y. Nishizuka. Study on cellular events in post-thymectomy autoimmune oophoritis in mice: II. Requirement of Lyt-1 cells in normal female mice for the prevention of oophoritis. *J Exp Med* 156, 1577-1586 (1982)

39. Scalapino, K. J., Q. Tang, J. A. Bluestone, M. L. Bonyhadi, D. I. Daikh. Suppression of disease in New Zealand Black/New Zealand White lupus-prone mice by adoptive transfer of *ex vivo* expanded regulatory T cells. *J Immunol* 177, 1451-1459 (2006)

40. Hori, S., T. Nomura, S. Sakaguchi. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 299, 1057-1061 (2003)

41. Khattri, R., T. Cox, S. A. Yasayko, F. Ramsdell. An essential role for scurfin in $CD4^+CD25^+$ T regulatory cells. *Nat Immunol* 4, 337-342 (2003)

42. Baecher-Allan, C., J. A. Brown, G. J. Freeman, D. A. Hafler. $CD4^+CD25^{high}$ regulatory cells in human peripheral blood. *J Immunol* 167, 1245–1253 (2001)

43. von Boehmer, H. Mechanisms of suppression by suppressor T cells. *Nat Immunol* 6, 338-344 (2005)

44. Thornton, A. M., E. M. Shevach. Suppressor effector function of $CD4^+CD25^+$ immunoregulatory T cells is antigen nonspecific. *J Immunol* 164, 183-190 (2000)

45. Thornton, A. M., E. M. Shevach. $CD4^+CD25^+$ immunoregulatory T cells suppress polyclonal T cell activation *in vitro* by inhibiting interleukin 2 production. *J Exp Med* 188, 287-296 (1998)

46. Grossman, W. J., J. W. Verbsky, W. Barchet, M. Colonna, J. P. Atkinson, T. J. Ley. Human T regulatory cells can use the perforin pathway to cause autologous target cell death. *Immunity* 21, 589–601 (2004)

47. Lim, H. W., P. Hillsamer, A. H. Banham, C. H. Kim. Direct suppression of B cells by $CD4^+CD25^+$ regulatory T cells. *J Immunol* 175, 4180-4183 (2005)

48. Huang, C. T., C. J. Workman, D. Flies, X. Pan, A. L. Marson, G. Zhou, E. L. Hipkiss, S. Ravi, J. Kowalski, H. I. Levitsky, J. D. Powell, D. M. Pardoll, C. G. Drake, D. A. Vignali. Role of LAG-3 in regulatory T cells. *Immunity* 21, 503–513 (2004)

49. Fehervari, Z., S. Sakaguchi. $CD4^+$ Tregs and immune control. *J Clin Invest* 114, 1209–1217 (2004)

50. Taylor, P. A., C. J. Lees, B. R. Blazar. The infusion of *ex vivo* activated and expanded $CD4^+CD25^+$ immune regulatory cells inhibits graft-versus-host disease lethality. *Blood* 99, 3493–3499 (2002)

51. Yamazaki, S., T. Iyoda, K. Tarbell, K. Olson, K. Velinzon, K. Inaba, R. M. Steinman. Direct expansion of functional $CD25^+CD4^+$ regulatory T cells by antigen-processing dendritic cells. *J Exp Med* 198, 235–247 (2003)

52. Tang, Q., K. J. Henriksen, E. K. Boden, A. J. Tooley, J. Ye, S. K. Subudhi, X. X. Zheng, T. B. Strom, J. A. Bluestone. Cutting edge: CD28 controls peripheral homeostasis of $CD4^+CD25^+$ regulatory T cells. *J Immunol* 171, 3348–3352 (2003)

53. Cobbold, S. P., L. Graca, C. Y. Lin, E. Adams, H. Waldmann. Regulatory T cells in the induction and maintenance of peripheral transplantation tolerance. *Transpl Int* 16, 66–75 (2003)

54. Zheng, S. G., J. H. Wang, J. G. Gray, H. Soucier, D. A. Horwitz. Natural and induced $CD4^+CD25^+$ cells educate $CD4^+CD25^-$ cells to develop suppressive activity: role of IL-2, TGF-β and IL-10. *J Immunol* 172, 5213-5221 (2004)

55. Chen, W., W. Jin, N. Hardegen, K. J. Lei, L. Li, N. Marinos, G. McGrady, S. M. Wahl. Conversion of peripheral $CD4^+CD25^-$ naïve T cells to $CD4^+CD25^+$ regulatory T cells by TGF-β induction of transcription factor Foxp3. *J Exp Med* 198, 1875-1886 (2003)

56. Curotto de Lafaille, M. A., A. C. Lino, N. Kutchukhidze, J. J. Lafaille. $CD25^-$ T cells generate $CD25^+Foxp3^+$ regulatory T cells by peripheral expansion. *J Immunol* 173, 7259-7268 (2004)

57. Barrat, F. J., D. J. Cua, A. Boonstra, D. F. Richards, C. Crain, H. F. Savelkoul, R. de Waal-Malefyt, R. L. Coffman, C. M. Hawrylowicz, A. O'Garra. *In vitro* generation of interleukin 10-producing regulatory $CD4^+$ T cells is induced by immunosuppressive drugs and inhibited by T helper type 1 (Th1)- and Th2-inducing cytokines. *J Exp Med* 195, 603–616 (2002)

58. Banham, A. H. Cell-surface IL-7 receptor expression facilitates the purification of $FOXP3^+$ regulatory T cells. *Trends Immunol* 27, 541-544 (2006)

59. Seddiki, N., B. Santner-Nanan, J. Martinson, J. Zaunders, S. Sasson, A. Landay, M. Solomon, W. Selby, S. I. Alexander, R. Nanan, A. Kelleher, B. Fazekas de St Groth. Expression of interleukin (IL)-2 and IL-7 receptors discriminates between human regulatory and activated T cells. *J Exp Med* 203, 1693-1700 (2006)

60. Piccirillo, C. A., E. M. Shevach. Naturally-occurring $CD4^+CD25^+$ immunoregulatory T cells: central players in the arena of peripheral tolerance. *Semin Immunol* 16, 81-88 (2004)

61. Yi, H., Y. Zhen, L. Jiang, J. Zheng, Y. Zhao. The phenotypic characterization of naturally occurring regulatory $CD4^+CD25^+$ T cells. *Cell Mol Immunol* 3, 189-195 (2006)

62. Wing, K., E. Suri-Payer, A. Rudin. $CD4^+CD25^+$ regulatory T cells from mouse to man. Scand *J Immunol* 62, 1-15 (2005)

63. Miyara, M., S. Sakaguchi. Natural regulatory T cells: mechanisms of suppression. *Trends Mol Med* 13, 108-116 (2007)

64. Yagi, H., T. Nomura, K. Nakamura, S. Yamazaki, T. Kitawaki, S. Hori, M. Maeda, M. Onodera, T. Uchiyama, S. Fujii, S. Sakaguchi. Crucial role of FOXP3 in the development and function of human $CD25^+CD4^+$ regulatory T cells. *Int Immunol* 16, 1643-1656 (2004)

65. Bennett, C. L., J. Christie, F. Ramsdell, M. E. Brunkow, P. J. Ferguson, L. Whitesell, T. E. Kelly, F. T. Saulsbury, P. F. Chance, H. D. Ochs. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FoxP3. *Nat Genet* 27, 20-21 (2001)

66. Bensinger, S. J., A. Bandeira, M. S. Jordan, A. J. Caton, T. M. Laufer. Major histocompatibility complex class II-positive cortical epithelium mediates the selection of $CD4^+25^+$ immunoregulatory T cells. *J Exp Med* 194, 427–438 (2001)

67. Jordan, M. S., A. Boesteanu, A. J. Reed, A. L. Petrone, A. E. Holenbeck, M. A. Lerman, A. Naji, A. J. Caton. Thymic selection of $CD4^+CD25^+$ regulatory T cells induced by an agonist self-peptide. *Nat Immunol* 2, 301–306 (2001)

68. Fisson, S., G. Darrasse-Jeze, E. Litvinova, F. Septier, D. Klatzmann, R. Liblau, B. L. Salomon. Continuous activation of autoreactive $CD4^+CD25^+$ regulatory T cells in the steady state. *J Exp Med* 198, 737–746 (2003)

69. Kumar, V. Homeostatic control of immunity by TCR-specific Tregs. *J. Clin. Invest.* 114, 1222-1226 (2004)

70. Picca, C. C., A. J. Caton. The role of self-peptides in the development of $CD4^+CD25^+$ regulatory T cells. *Curr Opin Immunol* 17, 131-136 (2005)

71. Samy, E. T., L. A. Parker, C. P. Sharp, K. S. Tung. Continuous control of autoimmune disease by antigen-dependent polyclonal $CD4^+CD25^+$ regulatory T cells in the regional lymph node. *J Exp Med* 202, 771-781 (2005)

72. Seddon, B., D. Mason. Peripheral autoantigen induces regulatory T cells that prevent autoimmunity. *J Exp Med* 189, 877-882 (1999)

73. Tanchot, C., F. Vasseur, C. Pontoux, C. Garcia, A. Sarukhan. Immune regulation by self-reactive T cells is antigen specific. *J Immunol* 172, 4285-4291 (2004)

74. Shevach, E. M., R. A. DiPaolo, J. Andersson, D. M. Zhao, G. L. Stephens, A. M. Thornton. The lifestyle of naturally occurring $CD4^+CD25^+Foxp3^+$ regulatory T cells. *Immunol Rev* 212, 60-73 (2006)

75. Matarese, G., V. De Rosa, A. La Cava. Regulatory CD4 T cells: sensing the environment. *Trends Immunol* 29, 12-17 (2008)

76. D'Cruz, L. M., L. Klein. Development and function of agonist-induced $CD25^+Foxp3^+$ regulatory T cells in the absence of interleukin 2 signaling. *Nat Immunol* 6, 1152-1159 (2005)

77. Malek, T. R. The main function of IL-2 is to promote the development of T regulatory cells. *J Leukoc Biol* 74, 961-965 (2003)

78. Fontenot, J. D., J. P. Rasmussen, M. A. Gavin, A. Y. Rudensky. A function for interleukin 2 in Foxp3-expressing regulatory T cells. *Nat Immunol* 6, 1142-1151 (2005)

79. Nelson, B. H. IL-2, regulatory T cells, and tolerance. *J Immunol* 172, 3983-3988 (2004)

80. Fontenot, J. D., A. Y. Rudensky. A well adapted regulatory contrivance: regulatory T cell development and the forkhead family transcription factor Foxp3. *Nat Immunol* 6, 331-337 (2005)

81. Bensinger, S. J., A. Bandeira, M. S. Jordan, A. J. Caton, T. M. Laufer. Major histocompatibility complex class II-positive cortical epithelium mediates the selection of $CD4^+25^+$ immunoregulatory T cells. *J Exp Med* 194, 427-448 (2001)

82. Papiernik, M., M. L. de Moraes, C. Pontoux, F. Vasseur, C. Penit. Regulatory CD4 T cells: expression of IL-2Rα chain, resistance to clonal deletion and IL-2 dependency. Int *Immunol* 10, 371-378 (1998)

83. Takahashi, T., T. Tagami, S.Yamazaki, T. Uede, J. Shimizu, N. Sakaguchi, T. W. Mak, S. Sakaguchi. Immunologic self-tolerance maintained by $CD25^+CD4^+$ regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. *J Exp Med* 192, 303–310 (2000)

84. Salomon, B., D. J. Lenschow, L. Rhee, N. Ashourian, B. Singh, A. Sharpe, J. A. Bluestone. B7/CD28 costimulation is essential for the homeostasis of the $CD4^+CD25^+$ immunoregulatory T cells that control autoimmune diabetes. *Immunity* 12, 431–440 (2000)

85. Scheffold, A., J. Huhn, T. Hofer. Regulation of $CD4^+CD25^+$ regulatory T cell activity: it takes (IL-)2 to tango. *Eur J Immunol* 35, 1336-1341 (2005)

86. Malek, T. R., A. Yu, V. Vincek, P. Scibelli, L. Kong. CD4 regulatory T cells prevent lethal autoimmunity in IL-2Rβ-deficient mice. Implications for the non-redundant function of IL-2. *Immunity* 17, 167-178 (2002)

87. Apostolou, I., A. Sarukhan, L. Klein, H. von Boehmer. Origin of regulatory T cells with known specificity for antigen. *Nat Immunol* 3, 756–763 (2002)

88. Karim, M., C. I. Kingsley, A. R. Bushell, B. S. Sawitzki, K. J. Wood. Alloantigen-induced $CD25^+CD4^+$ regulatory T cells can develop *in vivo* from $CD25^-CD4^+$ precursors in a thymus-independent process. *J Immunol* 172, 923-928 (2004)

89. Shevach, E. M. Regulatory T cells in autoimmunity. *Annu Rev Immunol* 18, 423-449 (2000)

90. Stephens, G. L., R. S. McHugh, M. J. Whitters, D. A. Young, D. Luxenberg, B. M. Carreno, M. Collins, E. M. Shevach. Engagement of glucocorticoid-induced TNFR family-related receptor on effector T cells by its ligand mediates resistance to suppression by $CD4^+CD25^+$ T cells. *J Immunol* 173, 5008–5020 (2004)

91. Chen, W., S. M. Wahl. TGF-β: the missing link in $CD4^+CD25^+$ regulatory T cell-mediated immunosuppression. *Cytokine Growth Factor Rev* 14, 85-89 (2003)

92. Duthoit, C. T., D. J. Mekala, R. S. Alli, T. L. Geiger. Uncoupling of IL-2 signaling from cell cycle progression in naïve $CD4^+$ T cells by regulatory $CD4^+CD25^+$ T lymphocytes. *J Immunol* 174, 155–163 (2005)

93. Gondek, D. C., L. F. Lu, S. A. Quezada, S. Sakaguchi, R. J. Noelle. Contact-mediated suppression by $CD4^+CD25^+$ regulatory cells involves a granzyme B-dependent, perforin-independent mechanism. *J Immunol* 174, 1783–1786 (2005)

94. Read, S., V. Malmstrom, F. Powrie. Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of $CD25^+CD4^+$ regulatory cells that control intestinal inflammation. *J Exp Med* 192, 295–302 (2000)

95. Paust, S., L. Lu, N. McCarty, H. Cantor. Engagement of B7 on effector T cells by regulatory T cells prevents autoimmune disease. *Proc Natl Acad Sci USA* 101, 10398–10403 (2004)

96. Medzhitov, R., P. Preston-Hurlburt, C. A. Janeway Jr. A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. *Nature* 388, 394-397 (1997)

97. Pasare, C., R. Medzhitov. Toll pathway-dependent blockade of $CD4^+CD25^+$ T cell-mediated suppression by dendritic cells. *Science* 299, 1033-1036 (2003)

98. Wu, X., S. L. Peng. Toll-like receptor 9 signaling protects against murine lupus. *Arthritis Rheum* 54, 336-342 (2006)

99. Christensen, S. R., M. Kashgarian, L. Alexopoulou, R. A. Flavell, S. Akira, M. J. Shlomchik. Toll-like receptor 9 controls anti-DNA autoantibody production in murine lupus. *J Exp Med* 202, 321-331 (2005)

100. Gavin, M. A., S. R. Clarke, E. Negrou, A. Gallegos, A. Rudensky. Homeostasis and anergy of $CD4^+CD25^+$ suppressor T cells *in vivo*. *Nat Immunol* 3, 33–41 (2002)

101. Walker, L. S., A. Chodos, M. Eggena, H. Dooms, A. K. Abbas. Antigen-dependent proliferation of $CD4^+CD25^+$ regulatory T cells *in vivo*. *J Exp Med* 198, 249–258 (2003)

102. Ermann, J., P. Hoffmann, M. Edinger, S. Dutt, F. G. Blankenberg, J. P. Higgins, R. S. Negrin, C. G. Fathman, S. Strober. Only the $CD62L^+$ subpopulation of $CD4^+CD25^+$ regulatory T cells protects from lethal acute GVHD. *Blood* 105, 2220-2226 (2005)

103. Ruprecht, C. R., M. Gattorno, F. Ferlito, A. Gregorio, A. Martini, A. Lanzavecchia, F. Sallusto. Coexpression of CD25 and CD27 identifies $FoxP3^+$ regulatory T cells in inflamed synovia. *J Exp Med* 201, 1793-1803 (2005)

104. Koenen, H. J., E. Fasse, I. Joosten. CD27/CFSE-based *ex vivo* selection of highly suppressive alloantigen-specific human regulatory T cells. *J Immunol* 174, 7573-7583 (2005)

105. Kleinewietfeld, M., F. Puentes, G. Borsellino, L. Battistini, O. Rotzschke, K. Falk. CCR6 expression defines regulatory effector/memory-like cells within the $CD25^+CD4^+$ T-cell subset. *Blood* 105, 2877-2886 (2005)

106. Dieckmann, D., C. H. Bruett, H. Ploettner, M. B. Lutz, G. Schuler. Human $CD4^+CD25^+$ regulatory, contact-dependent T cells induce interleukin 10-producing, contact-independent type 1-like regulatory T cells. *J Exp Med* 196, 247–253 (2002)

107. Longhi, M. S., M. J. Hussain, R. R. Mitry, S. K. Arora, G. Mieli-Vergani, D. Vergani, Y. Ma. Functional study of $CD4^+CD25^+$ regulatory T cells in health and autoimmune hepatitis. *J Immunol* 176, 4484–4491 (2006)

108. Belkaid, Y., C. A. Piccirillo, S. Mendez, E. M. Shevach, D. L. Sacks. $CD4^+CD25^+$ regulatory T cells control Leishmania major persistence and immunity. *Nature* 420, 502–507 (2002)

109. Paust, S., H. Cantor. Regulatory T cells and autoimmune disease. *Immunol Rev* 204, 195-207 (2005)

110. Viglietta, V., C. Baecher-Allan, H. L. Weiner, D. A. Hafler. Loss of functional suppression by $CD4^+CD25^+$ regulatory T cells in patients with multiple sclerosis. *J Exp Med* 199, 971–979 (2004)

111. Matarese, G., P. B. Carrieri, A. La Cava, F. Perna, V. Sanna, V. De Rosa, D. Aufiero, S. Fontana, S. Zappacosta. Leptin increase in multiple sclerosis associates with reduced number of $CD4^+CD25^+$ regulatory T cells. *Proc Natl Acad Sci USA* 102, 5150-5155 (2005)

112. Pop, S. M., C. P. Wong, D. A. Culton, S. H. Clarke, R. Tisch. Single cell analysis shows decreasing FoxP3 and TGF-β1 coexpressing $CD4^+CD25^+$ regulatory T cells during autoimmune diabetes. *J Exp Med* 201, 1333-1346 (2005)

113. Gregori, S., N. Giarratana, S. Smiroldo, L. Adorini. Dynamics of pathogenic and suppressor T cells in autoimmune diabetes development. *J Immunol* 171, 4040–4047 (2003)

114. You, S., N. Thieblemont, M. A. Alyanakian, J. F. Bach, L. Chatenoud. Transforming growth factor-β and T-cell-mediated immunoregulation in the control of autoimmune diabetes. *Immunol Rev* 212, 185-202 (2006)

115. Pop, S. M., C. P. Wong, Q. He, Y. Wang, M. A. Wallet, K. S. Goudy, R. Tisch. The type and frequency of immunoregulatory $CD4^+$ T-cells govern the efficacy of antigen-specific immunotherapy in nonobese diabetic mice. *Diabetes* 56, 1395-1402 (2007)

116. Tritt, M., E. Sgouroudis, E. d'Hennezel, A. Albanese, C. A. Piccirillo. Functional waning of naturally occurring $CD4^+$ regulatory T-cells contributes to the onset of autoimmune diabetes. *Diabetes* 57, 113-123 (2008)

117. Poussier, P., T. Ning, T. Murphy, D. Dabrowski, S. Ramanathan. Impaired post-thymic development of regulatory $CD4^+25^+$ T cells contributes to diabetes pathogenesis in BB rats. *J Immunol* 174, 4081-4089 (2005)

118. Tarbell, K. V., S. Yamazaki, K. Olson, P. Toy, R. M. Steinman. $CD25^+CD4^+$ T cells, expanded with dendritic cells presenting a single autoantigenic peptide, suppress autoimmune diabetes. *J Exp Med* 199, 1467–1477 (2004)

119. Green, E. A., Y. Choi, R. A. Flavell. Pancreatic lymph node-derived $CD4^+CD25^+$ Treg cells: highly potent regulators of diabetes that require TRANCE-RANK signals. *Immunity* 16, 183-191 (2002)

120. Bluestone, J. A., Q. Tang. Therapeutic vaccination using $CD4^+CD25^+$ antigen-specific regulatory T cells. *Proc Natl Acad Sci USA* 101, 14622-14626 (2004)

121. Lindley, S., C. M. Dayan, A. Bishop, B. O. Roep, M. Peakman, T. I. Tree. Defective suppressor function in $CD4^+CD25^+$ T-cells from patients with type 1 diabetes. *Diabetes* 54, 92-99 (2005)

122. Battaglia, M., A. Stabilini, B. Migliavacca, J. Horejs-Hoeck, T. Kaupper, M. G. Roncarolo. Rapamycin promotes expansion of functional $CD4^+CD25^+FOXP3^+$ regulatory T cells of both healthy subjects and type 1 diabetic patients. *J Immunol* 177, 8338-8347 (2006)

123. Powrie, F., D. Mason. $OX\text{-}22^{high}$ $CD4^+$ T cells induce wasting disease with multiple organ pathology: prevention by the $OX\text{-}22^{low}$ subset. *J Exp Med* 172, 1701-1708 (1990)

124. Powrie, F., J. Carlino, M. W. Leach, S. Mauze, R. L. Coffman. A critical role for transforming growth factor-β but not interleukin 4 in the suppression of T helper type 1-mediated colitis by $CD45RB^{low}$ $CD4^+$ T cells. *J Exp Med* 183, 2669-2674 (1996)

125. Pandiyan, P., L. Zheng, S. Ishihara, J. Reed, M. J. Lenardo. $CD4^+CD25^+Foxp3^+$ regulatory T cells induce cytokine deprivation-mediated apoptosis of effector $CD4^+$ T cells. *Nat Immunol* 8, 1353-1362 (2007)

126. Makita, S., T. Kanai, Y. Nemoto, T. Totsuka, R. Okamoto, K. Tsuchiya, M. Yamamoto, H. Kiyono, M. Watanabe. Intestinal lamina propria retaining $CD4^+CD25^+$ regulatory T cells is a suppressive site of intestinal inflammation. *J Immunol* 178, 4937-4946 (2007)

127. Maul, J., C. Loddenkemper, P. Mundt, E. Berg, T. Giese, A. Stallmach, M. Zeitz, R. Duchmann. Peripheral and intestinal regulatory $CD4^+CD25^{high}$ T cells in inflammatory bowel disease. *Gastroenterology* 128, 1868-1878 (2005)

128. Vasu, C., R. N. Dogan, M. J. Holterman, B. S. Prabhakar. Selective induction of dendritic cells using granulocyte macrophage-colony stimulating factor, but not fms-like tyrosine kinase receptor 3-ligand, activates thyroglobulin-specific $CD4^+CD25^+$ T cells and suppresses experimental autoimmune thyroiditis. *J Immunol* 170, 5511-5522 (2003)

129. Gangi, E., C. Vasu, D. Cheatem, B. S. Prabhakar. IL-10-producing $CD4^+CD25^+$ regulatory T cells play a critical role in granulocyte-macrophage colony-stimulating factor-induced suppression of experimental autoimmune thyroiditis. *J Immunol* 174, 7006-7013 (2005)

130. Verginis, P., H. S. Li, G. Carayanniotis. Tolerogenic semimature dendritic cells suppress experimental autoimmune thyroiditis by activation of thyroglobulin-specific $CD4^+CD25^+$ T cells. *J Immunol* 174, 7433-7439 (2005)

131. Wolf, D., K. Hochegger, A. M. Wolf, H. F. Rumpold, G. Gastl, H. Tilg, G. Mayer, E. Gunsilius, A. R. Rosenkranz. $CD4^+CD25^+$ regulatory T cells inhibit experimental anti-glomerular basement membrane glomerulonephritis in mice. *J Am Soc Nephrol* 16, 1360-1370 (2005)

132. Monk, C. R., M. Spachidou, F. Rovis, E. Leung, M. Botto, R. I. Lechler, O. A. Garden. MRL/Mp $CD4^+$, $CD25^-$ T cells show reduced sensitivity to suppression by $CD4^+$, $CD25^+$ regulatory T cells *in vitro*: a novel defect of T cell regulation in systemic lupus erythematosus. *Arthritis Rheum* 52, 1180-1184 (2005)

133. Miyara, M., Z. Amoura, C. Parizot, C. Badoual, K. Dorgham, S. Trad, D. Nochy, P. Debré, J. C. Piette, G. Gorochov. Global natural regulatory T cell depletion in active systemic lupus erythematosus. *J Immunol* 175, 8392-8400 (2005)

134. Mellor-Pita, S., M. J. Citores, R. Castejon, P. Tutor-Ureta, M. Yebra-Bango, J. L. Andreu, J. A. Vargas. Decrease of regulatory T cells in patients with systemic lupus erythematosus. *Ann Rheum Dis* 65, 553-554 (2006)

135. Lee, J. H., L. C. Wang, Y. T. Lin, Y. H. Yang, D. T. Lin, B. L. Chiang. Inverse correlation between CD4 regulatory T-cell population and autoantibody levels in pediatric patients with systemic lupus erythematosus. *Immunology* 117, 280-286 (2006)

136. Valencia, X., C. Yarboro, G. Illei, P. Lipsky. Deficient $CD4^+CD25^{high}$ T regulatory cell function in patients with active systemic lupus erythematosus. *J Immunol* 178, 2579-2588 (2007)

137. Morgan, M. E., R. P. Sutmuller, H. J. Witteveen, L. M. van Duivenvoorde, E. Zanelli, C. J. Melief, A. Snijders, R. Offringa, R. R. de Vries, R. E. Toes.. $CD25^+$ cell depletion hastens the onset of severe disease in collagen-induced arthritis. *Arthritis Rheum* 48, 1452-1460 (2003)

138. Morgan, M. E., R. Flierman, L. M. van Duivenvoorde, H. J. Witteveen, W. van Ewijk, J. M. van Laar, R. R. de Vries, R. E. Toes. Effective treatment of collagen-induced arthritis by adoptive transfer of $CD25^+$ regulatory T cells. *Arthritis Rheum* 52, 2212-2221 (2005)

139. Lawson, C. A., A. K. Brown, V. Bejarano, S. H. Douglas, C. H. Burgoyne, A. S. Greenstein, A. W. Boylston, P. Emery, F. Ponchel, J. D. Isaacs. Early rheumatoid arthritis is associated with a deficit in the $CD4^+CD25^{high}$ regulatory T cell population in peripheral blood. *Rheumatology (Oxford)* 45, 1210-1217 (2006)

140. Cao, D., V. Malmstrom, C. Baecher-Allan, D. Hafler, L. Klareskog, C. Trollmo. Isolation and functional characterization of regulatory $CD25^{bright}CD4^+$ T cells from the target organ of patients with rheumatoid arthritis. *Eur J Immunol* 33, 215-223 (2003)

141. Ehrenstein, M. R., J. G. Evans, A. Singh, S. Moore, G. Warnes, D. A. Isenberg, C. Mauri. Compromised function of regulatory T-cells in rheumatoid arthritis and reversal by anti-TNF-α therapy. *J Exp Med* 200, 277–285 (2004)

142. de Kleer, I. M., L. R. Wedderburn, L. S. Taams, A. Patel, H. Varsani, M. Klein, W. de Jager, G. Pugayung, F. Giannoni, G. Rijkers, S. Albani, W. Kuis, B. Prakken. $CD4^+CD25^{bright}$ regulatory T cells actively regulate inflammation in the joints of patients with the remitting form of juvenile idiopathic arthritis. *J Immunol* 172, 6435-6443 (2004)

Key Words : Regulatory T cells (Tregs), Immune tolerance, Autoimmunity, Review

Send correspondence to: Antonio La Cava, Department of Medicine at the University of California Los Angeles, 1000 Veteran Avenue 32-59, Los Angeles, California 90095-1670. Tel: 310-267-4975, Fax: 310-206-8606, E-mail: alacava@mednet.ucla.edu

http://www.bioscience.org/current/vol14.htm