

Chapter 1

Natural and Induced T CD4+CD25+FOXP3+ Regulatory T Cells

Lucienne Chatenoud

Abstract

Evidence has been accumulated to show that the forkhead/winged-helix transcription factor Foxp3 is a good marker for specialized CD4⁺ T cells that regulate immune responses to self as well as to a variety of foreign antigens including infectious or tumor antigens, alloantigens, allergens, and commensal antigens. It is now well established that CD4⁺CD25⁺Foxp3⁺ regulatory T cells encompass two categories of lymphocytes that are distinct in their origin, antigen specificity, as well as the stimuli driving their differentiation and homeostasis. Natural CD4⁺CD25⁺Foxp3⁺ regulatory T cells are an independent lineage generated in the thymus through major histocompatibility class II molecules-dependent MHC class high avidity interactions with their T cell receptor. They are specific for self-antigens. Adaptive or induced CD4⁺CD25⁺Foxp3⁺ regulatory T cells stem from mature CD4⁺CD25⁻Foxp3⁻precursors at the periphery following adequate stimulation. They have been shown to develop *in vivo* following suboptimal antigen stimulation, in situations characterized by chronic inflammation (autoimmunity, allergy, immune responses to tumors and transplants) and also as physiological actors of the mucosal immune system. Although major progress has been accomplished over the last years in our understanding of the central role of CD4⁺CD25⁺Foxp3⁺ regulatory T cells in the control of immune responses, major issues are still elusive. In particular, there are still no reliable phenotypic or functional markers that make it possible to distinguish between natural and induced CD25⁺Foxp3⁺ regulatory T cells.

Key words: Natural regulatory T cells, Induced regulatory T cells, Foxp3, Immune regulation

1. Introduction

As all biological systems the immune system is redundant and finely tuned. Immune regulatory mechanisms make it possible to face extreme situations in which discrete signals must be amplified or conversely the intensity of some immune responses, which could be the source of “damage,” must be tempered. These immune regulatory mechanisms are complex; some are antigen-specific

and others not. Major players in mediating immune regulation are specialized subsets of T lymphocytes some of which exert helper functions, thus amplifying effector B and T cell responses, and others which operate as suppressors. The concept of a distinct subset of lymphocytes mediating suppressive or regulatory functions was initially proposed by Gershon and Kondo in the 1970s, and they coined the term “*infectious tolerance*” (1). For many years, the tools to approach suppressive or regulatory T cells remained more than elusive, which explains the difficult situation of the whole field for about 20 years until, in the 1990s, important technical steps were accomplished that drove the revival of the field of regulatory T cells based on robust molecular grounds.

It is now well established that depending on the experimental model or the clinical situation, distinct cell types, including subsets of CD4+ and CD8+ T cells and invariant NK T cells, which all may be presented in detail in the following chapters, may afford downregulation of immune responses. Our aim, here, is to focus on CD4+ Foxp3-expressing regulatory T cells, also termed Tregs, which are effective at containing immune responses to self and foreign antigens as well as to commensal microorganisms. According to the present state of the art, one may distinguish essentially two sets of CD4+Foxp3+ Tregs: “natural” CD4+ Foxp3+ Tregs (nTregs) emerging from the thymus as a distinct lineage (2–4) and “adaptive” or “induced” CD4+CD25+ regulatory T cells (iTregs) that develop outside the thymus, from mature CD4+CD25– T cell precursors, under particular conditions, i.e., antigenic stimulation and cytokine environment (5–9).

It would be unproductive and tedious to draw the long list of methodological details that allow purification and characterization of nTregs and iTregs in the many in vitro and in vivo studies, which have been reported over the last 15 years. In addition, it is fair to admit that we are still in the quest of reliable phenotypic and/or functional markers to distinguish nTregs from iTregs. Therefore, we shall address in parallel the salient steps that lead to the characterization of these two categories of Tregs, which in fact were closely related to the methodological advances gained through the multiplicity of experimental approaches used, and that involved the study of conventional and genetically modified murine models.

2. General Considerations

There is no single method that affords characterizing in a reliable fashion nTregs and iTregs, neither in mice nor in humans. Therefore, the usage of a combination of tests is needed to assess in parallel the phenotypic pattern as well as the functional activity

of the selected T-cell subset and, importantly, to interpret the data based on the physiological or pathological situation under study.

The phenotypic characterization is possible using whole mononuclear cell or lymphocyte populations, recovered mostly from peripheral blood in humans and from various lymphoid organs in mice, as it is feasible by fluorescence-activated cell analysis using combinations of membrane markers to gate on individual T lymphocyte subsets (e.g., CD4+CD25+ T cells) looking for the expression of other key markers (Foxp3, CTLA-4, GITR within gated cells (10–13).

At variance with cell phenotyping, the analysis of the functional capacities of putative Tregs populations requires the purification of the given subset prior to testing by means of in vitro culture techniques or, in the case of experimental studies, in vivo models. Cell purification is performed by classical techniques using monoclonal antibodies to trace specific subsets; labeled cells are then separated from the unlabelled ones by magnetic bead cell sorting or fluorescence-activated cells sorting (FACS) (14–17). The latter method allows a much better purification of the selected subset especially when the marker used is expressed at variable density in the population of interest. This is, for instance, the case for CD4+CD25+ nTregs, which express a high density of CD25 (the α chain of the IL-2 receptor).

3. In Vitro Functional Analysis

Both in humans and in experimental models the method that has been and is widely used for the analysis of the in vitro functional capacity of Tregs to suppress is the so-called coculture assay. The principle is simple and consists in culturing purified “responder” T cells together with putative Tregs, also purified, to assess the capacity of the latter population to refrain the response of the former. The most commonly used marker of response is cell proliferation although the production of a given cytokine has also been adopted. For cell proliferation analysis, the two methods commonly used are conventional incorporation of tritiated thymidine. The pulse labeling of cells with radioactive thymidine provides a means to determine the distribution of times of entry into the first cell division. Alternatively, staining with 5-,6-carboxyfluorescein diacetate succinimidyl ester (CFSE) of the responder T cell subset is also used. CFSE is a vital dye whose intracellular concentration will decrease proportionally with cell division and serves visualizing (by FACS) and classifying proliferating cell populations into groups according to the number of divisions each cell has undergone.

In practice, as originally described the coculture test used as responder T cells purified polyclonal CD4⁺CD25⁻ T cells (or when needed purified CD8⁺ T cells) stimulated either with antigen and low numbers of antigen-presenting cells (APCs) or anti-T-cell receptor (mostly anti-CD3) antibody. When needed, in order to analyze what are the mechanisms underlying the effect observed, namely, cell–cell contact interactions and/or soluble mediators or both, transwell devices are used which physically separate the two cell subsets. This method was used in the case of both mouse and human cells.

Although it is obvious that in vitro systems will never fully recapitulate the in vivo situation, important properties of Tregs were approached with this method. It could thus be established that first Tregs suppress the proliferation and interleukin-2 (IL-2) production of responder cells in a dose-dependent fashion; second, both CD4⁺ and CD8⁺ cells could be suppressed; third, the suppressive activity of Tregs could be bypassed following addition of exogenous interleukin (IL)-2 or by triggering costimulatory pathways (e.g., CD28:B7); and fourth, depending on the situations, the suppressive ability of Tregs was or was not impaired through blockade of given immune regulatory cytokines such as TGF- β or IL-10. Although exceptions may be found in the literature, the consensus is that thymic-derived nTregs exert their in vitro function in a cell–cell contact-dependent but cytokine-independent way (18), while iTregs use both pathways.

4. In Vivo Functional Analysis

These methods are, for obvious reasons, mostly restricted to mouse experimental models. Among in vivo manipulations that allow testing for the suppressive effect of Tregs one may cite: (1) in vivo depletion experiments whereby elimination of Tregs, following thymectomy on day 3 after birth or upon administration of monoclonal antibodies that deplete the target population (anti-CD25) (19), an exacerbation of given immune responses is observed and, (2) adoptive transfer experiments, mostly through i.v. injection, of purified subsets of putative Tregs to analyze their capacity to downregulate in vivo immune responses that develop in the recipients. Most commonly, immune-mediated inflammatory, autoimmune, allergic, or tumor responses were targeted. These adoptive transfers may be performed either in immune-competent or immune-deficient recipients (mice bearing the *scid*, *nu/nu*, or *Rag*^{-/-} mutations and therefore devoid of T cells).

4.1. Neonatal Thymectomy Experiments

The seminal experiment showing that cells expressing suppressive functions emerged from the thymus was that performed in the late 1960s by the group of Nishizuka and Sakakura (20).

These authors showed that neonatal thymectomy of female mice resulted in developmental arrest of the ovary leading to sterility. The ovaries of thymectomized mice were small, completely lacked follicles and corpora lutea, a histopathological pattern now recognized as autoimmune oophoritis (20). Disease was observed when mice were thymectomized at 3 days of age, but not at 7 days or later, and it was fully prevented by thymus grafting. As shown later, disease was also prevented by adoptive transfer of syngeneic T splenocytes from adult normal donors. In addition, more detailed analysis showed that the day 3 thymectomy induced in fact a more generalized autoimmune syndrome, also including a high proportion of mice showing gastritis and thyroiditis. Then started the long quest for cell markers which could afford characterizing among mature T lymphocytes the subset endowed with the regulatory properties; cells were negatively selected based on given surface molecules and then transferred to neonatally thymectomized mice to test their potential to prevent the autoimmune syndrome. CD5 was the first marker identified; removing CD5+ cells from donor splenocytes reduced the capacity of peripheral T cells to protect day 3 thymectomized mice from autoimmune oophoritis and gastritis. As a mirror image, CD5-depleted splenocytes adoptively transferred into immunodeficient/lymphopenic mice induced multiorgan autoimmunity (21).

A major finding in the mid-1990s was that CD25, the high-affinity subunit of the IL-2 receptor (IL2R α), was another important marker of the thymic-derived suppressive cells. Almost undetectable levels of peripheral CD4+CD25+ T cells were present a week after day 3 thymectomy, and tenfold less splenic CD4+CD25+ T cells were detected in thymectomized adult mice compared to controls (22).

Importantly, CD4+CD25+ regulatory T cells were capable of preventing autoimmunity not only in neonatally thymectomized mice but also in lymphopenic animals infused with pathogenic effector T lymphocytes (2, 23). This was demonstrated in models whereby transferring CD25+ T-cell-depleted splenocytes into lymphopenic hosts induced a multiorgan autoimmunity syndrome that resembled the one observed in neonatally thymectomized mice (2). Similarly, transfer of purified CD4+CD45RB^{high} cells into immunodeficient hosts induced severe inflammatory colitis leading to wasting syndrome (24, 25). The major breakthrough that gave molecular “credit” to the concept that thymic-derived suppressor T cells constituted a *bona fide* individual lineage of thymocytes was the description in 2003, by three independent laboratories (26–28), that the Foxp3 transcription factor was highly expressed constitutively by CD4+CD25+ regulatory T cells, while CD4+CD25– T cells did not upregulate Foxp3 when they acquired CD25 expression following TRC stimulation. Foxp3 is a forkhead/winged-helix transcription factor family member.

Mutations in the *Foxp3* encoding gene had been identified as responsible for the Immune dysregulation, Polyendocrinopathy, Enteropathy, X-linked (IPEX) syndrome (29), described in 1982 characterized by a cohort of autoimmune manifestations that develop during early infancy in affected males. A similar X-linked pathology was described back in 1959 in the scurfy mouse which was later on also attributed to a mutation in *Foxp3* gene. Using bone marrow chimeras and also using Foxp3 reporter mice (see below), it was ascertained that FoxP3 was mandatory for nTreg development in the thymus and constituted a valuable marker for this independent lineage of T cells. In fact, a significant proportion of developing T cells likely commit to the regulatory T-cell lineage within the thymus, as CD4+CD25+ with suppressive activity are detected in neonatal mice prior to peripheral generation of activated CD25+ cells in the spleen (3). In keeping with this conclusion were also the data showing that adoptive transfer of nTregs isolated from normal wild-type mice significantly prevented disease and related mortality in Foxp3 mutant mice (3).

4.2. Generation of FoxP3 Reporter Mice

Once Foxp3 was found to represent the lineage-specific marker for Tregs, a major effort was devoted to create a model which could allow purification of T cells based on Foxp3 expression. In fact, Foxp3 being a transcription factor, staining of Foxp3+ cells in conventional mice necessitated cell fixation, which certainly allowed cell phenotyping but totally excluded in vitro or in vivo manipulations for which living cells were needed. This was the rationale for creating reporter Foxp3GFP mice in which the green fluorescent protein-coding sequence was inserted in-frame within exon2 of *Foxp3*. In these reporter mice, approximately 96% of Foxp3+ cells resided within the CD4+ T-cell compartment; 60–88% also expressing high levels of CD25, depending on anatomical location (3). Interestingly, the analysis of these mice directly validated the reports describing that Tregs were also included within the CD4+CD25– or CD4+CD25^{low} compartments. Comparison of the gene “signatures” of Foxp3+CD4+CD25^{high} and Foxp3+CD4+CD25^{low/-} T cells showed a greater similarity between these two subsets than between Foxp3+CD4+CD25^{high} and Foxp3–CD4+CD25– T cells. Additionally, low yet detectable numbers of Foxp3+ cells were detected among a small TCRαβ+ that stained CD8+.

Finally, it is important to mention that thymic differentiation of nTregs is CD28-dependent (30).

4.3. Regulatory T Cells Recognize Self-Determinants

Since the neonatal thymectomy-induced autoimmunity model was described, nTregs were hypothesized to recognize self-antigens. As mentioned above, transferring total splenocytes from normal syngeneic donors as a source of Tregs protected thymectomized neonates from the autoimmune syndrome. In contrast, adoptive transfer of splenocytes from donors in which a target tissue was

removed (in the case for instance of splenic preparations from athyroid donors) prevented all autoimmune manifestations but thyroiditis (31). The conclusion proposed was that the presence of the target antigens was mandatory to maintain nTregs.

Concerning their thymic development, compelling evidence has been accumulated to show that the TCR affinity threshold that induces nTregs was placed immediately after or during signal strengths that negatively select self-reactive thymocytes. Experimental models used mice that coexpress a transgenic TCR and its transgene encoded cognate ligand in the thymus (32). In these mice, cognate ligand-induced engagement of TCR on developing thymocytes resulted in significant thymic deletion, but remaining thymocytes contained significantly higher percentages of nTregs.

4.4. Extrathymic Regulatory T-Cell Differentiation

Studies using polyclonal populations (7) and suggesting that extrathymic generation of Tregs was possible were challenged by the description of Foxp3-expressing CD4+CD25⁻ cells (3) that could become Foxp3-expressing CD25⁺ cells.

The initial experimental evidence in support of the generation of regulatory T cells from peripheral CD4+CD25⁻Foxp3⁻ T cells came from studies of adoptive transfer of T cells from TCR transgenic recombinase (RAG)-deficient mice that are devoid of regulatory T cells, which are not generated in the absence of endogenous TCR gene rearrangements. Upon transfer of these TCR transgenic T cells into hosts harboring the cognate antigen, intense proliferation was observed followed by apoptosis/contraction of most dividing T cells; surviving cells expressed Foxp3 and suppressor functions.

The group of A. Abbas transferred naïve DO11 RAG^{-/-} T-cells (anti-ovalbumin (OVA)) into recipients in whom the cognate antigen is expressed ubiquitously as a secreted soluble protein (33). The transferred T cells expanded and differentiated into pathogenic effectors causing a transient graft-versus-host disease. In the surviving mice, once the syndrome resolved, OVA-specific CD25⁺Foxp3⁺ Tregs were detected (33).

Using nonlymphopenic recipients, the group of H. van Boehmer demonstrated that regulatory T-cell differentiation is favored when stimulating T cells under poorly immunogenic conditions. Thus, a subset of CD25⁺Foxp3⁺ Tregs could be established in TCR-hemagglutinin (HA) Tg RAG^{-/-} mice in which the cognate antigen was delivered by an osmotic pump (34). Similarly, hemagglutinin (HA)-specific CD25⁻ T cells transferred into nonlymphopenic hosts exposed to variable doses of antigen coupled to the dendritic cell-specific anti-DEC205 monoclonal antibody promoted the appearance of CD25⁺FoxP3⁺ Tregs, the highest proportion of Tregs being observed when using low doses of antigen (35). Moreover, the *in vivo* Foxp3 induction

is significantly impaired in T cells insensitive to TGF- β signaling (expressing a dominant-negative TGF- β receptor) (35).

Differentiation of iTregs has also been described in a variety of other situations including: induction of oral tolerance (36), autoimmunity (14, 37), allergic inflammation (38), tumor responses (39), and transplant responses (9). Moreover, they appear as an essential “physiological” component of the gut immune system generated by the response to microbiota and food antigens (40, 41).

At this point, it is important to mention that it was shown quite early that CD4+CD25+ Tregs, which subsequently were identified as Foxp3+, could be expanded in vitro from naïve conventional T cells following TCR-mediated stimulation in presence of TGF- β (5). These iTregs were shown to acquire functional suppressive capacities in vitro, in coculture, and also in vivo in an allergic asthma model. The mechanisms that mediate the capacity of TGF- β to induce Foxp3 have now been dissected at the molecular level and involve the transcription factors STAT 5 and NF-AT (42). At this point, it is interesting to mention the work of the group of H. Weiner showing that following distinct stimulations regulatory CD4+Foxp3+ cells expressing membrane TGF- β (LAP+ T cells) that are derived from peripheral precursors, especially from T cells in the gut, may be induced (43, 44). Another cytokine that is important for iTreg differentiation and/or survival is IL-2. IL-2 is essential for the in vitro generation of TGF- β -induced iTregs.

Aside from dedicated cytokines, additional microenvironmental factors have been reported as important to drive the differentiation of iTregs. This is the case for retinoic acid. Dendritic cells expressing the integrin CD103 present in the lamina propria and mesenteric lymph nodes that produce both TGF- β and retinoic acid are highly effective at mediating conversion of naïve T cells into Foxp3+ iTregs (40).

5. Conclusions

In spite of a long controversy, it is now well accepted that CD4+CD25+Foxp3+ play an essential role in immune regulation. It is also well established that these cells come in two flavors, thymically-derived nTregs and iTregs emerging from peripheral precursors.

Their precise respective roles in the physiological and pathological settings must still be fully elucidated and new tools must be generated to approach a more precise characterization of the two subsets. This will be important not only from the fundamental point of view but also in terms of clinical translation as great hope

is focused (as discussed in detail in other chapters of this volume) on the use of expanded Tregs as therapeutic tools in various pathological conditions (45).

It is important to add before closing that here we exclusively concentrated on Foxp3⁺iTregs. There is, however, in the literature compelling evidence to show the existence of subsets of CD4⁺ T cells mediating effective regulation that are induced at the periphery following adequate antigenic stimulation and lack Foxp3. These include, in particular, Tr1 cells (described in more detail elsewhere in this volume) that have been characterized by the group of M.G. Roncarolo and that play a major role in the regulation of transplantation responses and autoimmunity (46–48).

References

1. Gershon, R.K., and K. Kondo. 1971. Infectious immunological tolerance. *Immunology* 21:903–914.
2. Sakaguchi, S., N. Sakaguchi, M. Asano, M. Itoh, and M. Toda. 1995. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J Immunol* 155:1151–1164.
3. Kim, J.M., and A. Rudensky. 2006. The role of the transcription factor Foxp3 in the development of regulatory T cells. *Immunol Rev* 212:86–98.
4. Fontenot, J.D., J.P. Rasmussen, L.M. Williams, J.L. Dooley, A.G. Farr, and A.Y. Rudensky. 2005. Regulatory T cell lineage specification by the forkhead transcription factor foxp3. *Immunity* 22:329–341.
5. Chen, W., W. Jin, N. Hardegen, K.J. Lei, L. Li, N. Marinos, G. McGrady, and S.M. Wahl. 2003. Conversion of peripheral CD4⁺CD25⁻ naive T cells to CD4⁺CD25⁺ regulatory T cells by TGF-beta induction of transcription factor Foxp3. *J Exp Med* 198:1875–1886.
6. Liang, S., P. Alard, Y. Zhao, S. Parnell, S.L. Clark, and M.M. Kosiewicz. 2005. Conversion of CD4⁺ CD25⁻ cells into CD4⁺ CD25⁺ regulatory T cells in vivo requires B7 costimulation, but not the thymus. *J Exp Med* 201:127–137.
7. Curotto de Lafaille, M.A., A.C. Lino, N. Kutchukhidze, and J.J. Lafaille. 2004. CD25⁻ T cells generate CD25⁺Foxp3⁺ regulatory T cells by peripheral expansion. *J Immunol* 173:7259–7268.
8. Karim, M., C.I. Kingsley, A.R. Bushell, B.S. Sawitzki, and K.J. Wood. 2004. 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.
9. Cobbold, S.P., R. Castejon, E. Adams, D. Zelenika, L. Graca, S. Humm, and H. Waldmann. 2004. Induction of foxP3⁺ regulatory T cells in the periphery of T cell receptor transgenic mice tolerized to transplants. *J Immunol* 172: 6003–6010.
10. Takahashi, T., T. Tagami, S. Yamazaki, T. Uede, J. Shimizu, N. Sakaguchi, T.W. Mak, and S. Sakaguchi. 2000. Immunologic self-tolerance maintained by CD25⁺CD4⁺ regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. *J Exp Med* 192:303–310.
11. Shimizu, J., S. Yamazaki, T. Takahashi, Y. Ishida, and S. Sakaguchi. 2002. Stimulation of CD25⁺CD4⁺ regulatory T cells through GITR breaks immunological self-tolerance. *Nat Immunol* 3:135–142.
12. Read, S., V. Malmstrom, and F. Powrie. 2000. 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.
13. Tang, Q., and J.A. Bluestone. 2006. Regulatory T-cell physiology and application to treat autoimmunity. *Immunol Rev* 212:217–237.
14. You, S., B. Leforban, C. Garcia, J.F. Bach, J.A. Bluestone, and L. Chatenoud. 2007. Adaptive TGF-β-dependent regulatory T cells control autoimmune diabetes and are a privileged target of anti-CD3 antibody treatment. *Proc Natl Acad Sci USA* 104:6335–6340.

15. Mchugh, R.S., M.J. Whitters, C.A. Piccirillo, D.A. Young, E.M. Shevach, M. Collins, and M.C. Byrne. 2002. CD4(+)CD25(+) immunoregulatory T cells: gene expression analysis reveals a functional role for the glucocorticoid-induced TNF receptor. *Immunity* 16:311–323.
16. Piccirillo, C.A., J.J. Letterio, A.M. Thornton, R.S. Mchugh, M. Mamura, H. Mizuhara, and E.M. Shevach. 2002. CD4(+)CD25(+) regulatory T cells can mediate suppressor function in the absence of transforming growth factor beta1 production and responsiveness. *J Exp Med* 196:237–246.
17. You, S., M. Belghith, P. Cobbold, M.A. Alyanakian, C. Gouarin, S. Barriot, C. Garcia, H. Waldmann, L. Chatenoud, and J.F. Bach. 2005. Autoimmune diabetes onset results from qualitative rather than quantitative age-dependent changes in pathogenic T cells. *Diabetes* 54(5):1415–1422.
18. Shevach, E.M., R.A. DiPaolo, J. Andersson, D.M. Zhao, G.L. Stephens, and A.M. Thornton. 2006. The lifestyle of naturally occurring CD4+ CD25+ Foxp3+ regulatory T cells. *Immunol Rev* 212:60–73.
19. Billiard, F., E. Litvinova, D. Saadoun, F. Djelti, D. Klatzmann, J.L. Cohen, G. Marodon, and B.L. Salomon. 2006. Regulatory and effector T cell activation levels are prime determinants of in vivo immune regulation. *J Immunol* 177:2167–2174.
20. Nishizuka, Y., and T. Sakakura. 1969. Thymus and reproduction: sex-linked dysgenesis of the gonad after neonatal thymectomy in mice. *Science* 166:753–755.
21. Sakaguchi, S., K. Fukuma, K. Kuribayashi, and T. Masuda. 1985. Organ-specific autoimmune diseases induced in mice by elimination of T cell subset. I. Evidence for the active participation of T cells in natural self-tolerance; deficit of a T cell subset as a possible cause of autoimmune disease. *J Exp Med* 161:72–87.
22. Asano, M., M. Toda, N. Sakaguchi, and S. Sakaguchi. 1996. Autoimmune disease as a consequence of developmental abnormality of a T cell subpopulation. *J Exp Med* 184:387–396.
23. Suri-payer, E., A.Z. Amar, A.M. Thornton, and E.M. Shevach. 1998. CD4+CD25+ T cells inhibit both the induction and effector function of autoreactive T cells and represent a unique lineage of immunoregulatory cells. *J Immunol* 160:1212–1218.
24. Powrie, F., R. Correa-oliveira, S. Mauze, and R.L. Coffman. 1994. Regulatory interactions between CD45R Bhigh and CD45 RBlow CD4+ T cells are important for the balance between protective and pathogenic cell-mediated immunity. *J Exp Med* 179: 589–600.
25. Stephens, L.A., C. Mottet, D. Mason, and F. Powrie. 2001. Human CD4(+)CD25(+) thymocytes and peripheral T cells have immune suppressive activity in vitro. *Eur J Immunol* 31:1247–1254.
26. Fontenot, J.D., M.A. Gavin, and A.Y. Rudensky. 2003. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat Immunol* 4:330–336.
27. Hori, S., T. Nomura, and S. Sakaguchi. 2003. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 299: 1057–1061.
28. Khattri, R., T. Cox, S.A. Yasayko, and F. Ramsdell. 2003. An essential role for Scurfin in CD4+CD25+ T regulatory cells. *Nat Immunol* 4:337–342.
29. Wildin, R.S., S. Smyk-Pearson, and A.H. Filipovich. 2002. Clinical and molecular features of the immunodysregulation, polyendocrinopathy, enteropathy, X linked (IPEX) syndrome. *J Med Genet* 39:537–545.
30. Salomon, B., D.J. Lenschow, L. Rhee, N. Ashourian, B. Singh, A. Sharpe, and J.A. Bluestone. 2000. B7/CD28 Costimulation is essential for the homeostasis of the CD4+CD25+ immunoregulatory T cells that control autoimmune diabetes. *Immunity* 12:431–440.
31. Seddon, B., and D. Mason. 1999. Peripheral autoantigen induces regulatory T cells that prevent autoimmunity. *J Exp Med* 189: 877–882.
32. Jordan, M.S., A. Boesteanu, A.J. Reed, A.L. Petrone, A.E. Hohenbeck, M.A. Lerman, A. Naji, and A.J. Caton. 2001. Thymic selection of CD4(+)CD25(+) regulatory T cells induced by an agonist self-peptide. *Nat Immunol* 2:301–306.
33. Knoechel, B., J. Lohr, E. Kahn, J.A. Bluestone, and A.K. Abbas. 2005. Sequential development of interleukin 2-dependent effector and regulatory T cells in response to endogenous systemic antigen. *J Exp Med* 202: 1375–1386.
34. Apostolou, I., and H. von Boehmer. 2004. In vivo instruction of suppressor commitment in naive T cells. *J Exp Med* 199:1401–1408.
35. Kretschmer, K., I. Apostolou, D. Hawiger, K. Khazaie, M.C. Nussenzweig, and H. von Boehmer. 2005. Inducing and expanding regulatory T cell populations by foreign antigen. *Nat Immunol* 6:1219–1227.
36. Mucida, D., N. Kutchukhidze, A. Erazo, M. Russo, J.J. Lafaille, and M.A. Curotto de Lafaille. 2005. Oral tolerance in the absence of naturally occurring Tregs. *J Clin Invest* 115:1923–1933.

37. You, S., N. Thieblemont, M.A. Alyanakian, J.F. Bach, and L. Chatenoud. 2006. Transforming growth factor-beta and T-cell-mediated immunoregulation in the control of autoimmune diabetes. *Immunol Rev* 212:185–202.
38. Curotto de Lafaille, M.A., N. Kutchukhidze, S. Shen, Y. Ding, H. Yee, and J.J. Lafaille. 2008. Adaptive Foxp3+ regulatory T cell-dependent and -independent control of allergic inflammation. *Immunity* 29:114–126.
39. Liu, V.C., L.Y. Wong, T. Jang, A.H. Shah, I. Park, X. Yang, Q. Zhang, S. Lonning, B.A. Teicher, and C. Lee. 2007. Tumor evasion of the immune system by converting CD4+CD25- T cells into CD4+CD25+ T regulatory cells: role of tumor-derived TGF-beta. *J Immunol* 178:2883–2892.
40. Sun, C.M., J.A. Hall, R.B. Blank, N. Bouladoux, M. Oukka, J.R. Mora, and Y. Belkaid. 2007. Small intestine lamina propria dendritic cells promote de novo generation of Foxp3 T reg cells via retinoic acid. *J Exp Med* 204:1775–1785.
41. Barnes, M.J., and F. Powrie. 2009. Regulatory T cells reinforce intestinal homeostasis. *Immunity* 31:401–411.
42. Josefowicz, S.Z., and A. Rudensky. 2009. Control of regulatory T cell lineage commitment and maintenance. *Immunity* 30:616–625.
43. Ochi, H., M. Abraham, H. Ishikawa, D. Frenkel, K. Yang, A.S. Basso, H. Wu, M.L. Chen, R. Gandhi, A. Miller, R. Maron, and H.L. Weiner. 2006. Oral CD3-specific antibody suppresses autoimmune encephalomyelitis by inducing CD4(+)CD25(-)LAP(+) T cells. *Nat Med* 12:627–635.
44. Chen, M.L., B.S. Yan, Y. Bando, V.K. Kuchroo, and H.L. Weiner. 2008. Latency-associated peptide identifies a novel CD4+CD25+ regulatory T cell subset with TGFbeta-mediated function and enhanced suppression of experimental autoimmune encephalomyelitis. *J Immunol* 180:7327–7337.
45. Tang, Q., K.J. Henriksen, M. Bi, E.B. Finger, G. Szot, J. Ye, E.L. Masteller, H. McDevitt, M. Bonyhadi, and J.A. Bluestone. 2004. In vitro-expanded antigen-specific regulatory T cells suppress autoimmune diabetes. *J Exp Med* 199:1455–1465.
46. Battaglia, M., A. Stabilini, E. Draghici, B. Migliavacca, S. Gregori, E. Bonifacio, and M.G. Roncarolo. 2006. Induction of tolerance in type 1 diabetes via both CD4+CD25+ T regulatory cells and T regulatory type 1 cells. *Diabetes* 55:1571–1580.
47. Groux, H., A. O'garra, M. Bigler, M. Rouleau, S. Antonenko, J.E. De Vries, and M.G. Roncarolo. 1997. A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* 389:737–742.
48. Battaglia, M., S. Gregori, R. Bacchetta, and M.G. Roncarolo. 2006. Tr1 cells: from discovery to their clinical application. *Semin Immunol* 18:120–127.