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Regulatory T Cells: Key Controllers of Immunologic Self-Tolerance

Minireview

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Our immune system protects us from a myriad of potentially pathogenic microorganisms while avoiding reacting with constituents of our body; i.e., we are tolerant of "self." Failure of immunologic self-tolerance often leads to the development of autoimmune disease, which is estimated to afflict up to 5% of the population. Although the etiology of autoimmune disease is at present largely unknown, it is well documented that T cells are the key mediators of many autoimmune diseases, such as insulin-dependent diabetes mellitus (IDDM), autoimmune thyroiditis, and autoimmune gastritis accompanying pernicious anemia. Furthermore, there is mounting evidence that normal healthy individuals harbor potentially pathogenic self-reactive T cells. For example, immunization of normal animals with a self-antigen in potent adjuvant can induce dormant self-reactive T cells to attack the antigen, eliciting inflammatory tissue damage. Self-reactive T cell clones can also easily be isolated from the peripheral blood of normal healthy individuals by repeated in vitro stimulation with self-molecules. Moreover, many tumor-associated antigens recognized by autologous T cells in cancer patients have now turned out to be normal self-constituents, not abnormal products of mutated genes, indicating that tumor immunity is in part an autoimmunity. One of the current key issues in immunology is therefore to elucidate how potentially hazardous (or sometimes beneficial) self-reactive T cells are generated in physiological or disease states, and how they are regulated to avoid autoimmune disease (or not to attack autologous tumor cells). A better understanding of the mechanisms underlying immunologic tolerance will lead to better treatments for autoimmune disease, cancer, and transplant rejection.

During T cell maturation in the thymus, immature T cells express an enormously diverse range of T cell antigen receptors (TCRs) formed by random rearrangements of TCR α and β chain gene segments, but only T cells expressing TCRs that recognize major histocompatibility complex (MHC) and associated self-peptides with moderate affinity can differentiate (positive selection). T cells whose TCRs fail to bind the MHC/self-peptide complex and T cells expressing TCRs that bind the complex too strongly are subjected to programmed cell death (death by neglect and negative selection, respectively). However, thymic negative selection does not seem to be sufficient to control self-reactive T cells and thereby prevent autoimmune disease. Self-reactive T cells that have somehow escaped thymic negative selection are further subjected to control in the periphery; T cells can be rendered anergic (i.e., functionally inactivated without death) or deleted upon encounter with self-antigens in the periphery. In addition to these "passive" mechanisms of controlling self-reactive T cells, there appears to be a "dominant" control mechanism—certain T cells actively downregulate the activation/proliferation of self-reactive T cells. The existence of such regulatory (or suppressor) T cells has been a great controversy among immunologists and has been given little credibility for many years, largely because of the paucity of reliable markers for defining the cell, the ambiguity in the molecular basis of suppressive phenomena, and even the elusive nature of some suppressive phenomena themselves. Nevertheless, if one asks which mechanism of self-tolerance should, when it goes awry, directly lead to autoimmune disease or which one, when strengthened, can prevent autoimmune disease, accumulating experimental evidence now suggests that removal or inactivation of a certain regulatory T cell population can break natural self-tolerance, leading to spontaneous development of various autoimmune diseases. Studies on transplantation tolerance have also documented that T cells actively inhibit effector T cells from rejecting grafts in a certain tolerant state. Furthermore, recent advances in cytokine research have revealed that T cells secreting immunosuppressive cytokines behave as regulatory T cells and indeed downregulate autoimmunity and responses to transplants. This review will discuss recent advances in our understanding of the key role of regulatory T cells in immunologic tolerance.

Self-Tolerance Is Maintained by CD4$^+$ Regulatory T Cells

The existence of regulatory T cells with autoimmune-inhibitory activity has been suggested in various animal models of autoimmune disease since the early 1980s (references cited in Itoh et al., 1999). For example, in NOD (non-obese diabetic) mice or BB (Bio-Breeding) rats, which spontaneously develop IDDM and autoimmune thyroiditis, inoculation with CD4$^+$ T cells from histocompatible normal animals effectively prevented IDDM. On the other hand, characterization of effector T cells mediating these organ-specific autoimmune diseases has firmly documented that CD4$^+$ helper T cells cause the damage by helping B cells to form autoantibodies and by inducing cell-mediated immune responses to self-antigens. Recent experiments with transgenic mice that harbor CD4$^+$ T cells expressing transgenic TCRs reacting with self-antigens, such as myelin basic proteins (one of the target self-antigens in multiple sclerosis in humans), have also shown that inoculation of normal CD4$^+$ T cells can prevent the autoimmunity (Olivares-Villagomez et al., 1998; Van de Keere and Tonegawa, 1998). These findings, when taken together, suggest that normal individuals may harbor two functionally distinct populations of CD4$^+$ T cells, one capable of mediating autoimmune disease and the other dominantly inhibiting it, and that the interactions between the two populations, the latter being dominant

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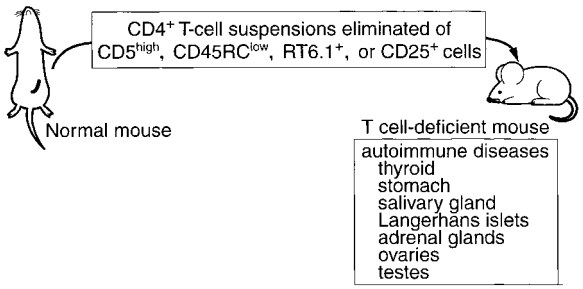

Figure 1. Induction of Autoimmune Diseases in T Cell–Deficient Mice (or Rats) by Transferring $CD4^+$ T Cell Suspensions Eliminated of a Particular Subpopulation Defined by the Expression Levels of Various Cell Surface Molecules

See text for details.

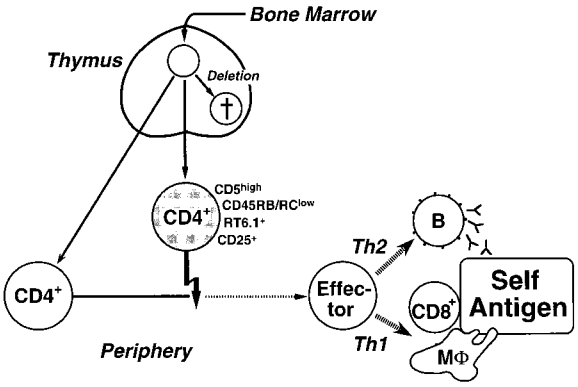

Figure 2. Immunologic Self-Tolerance Maintained by $CD4^+$ Regulatory T Cells

See text for details. Mϕ, macrophages.

in the physiological state, can be a key mechanism in maintaining self-tolerance. To test this hypothesis directly, attempts have been made from the mid 1980s to dissect the normal $CD4^+$ T cell population into smaller subpopulations by expression levels of a particular cell surface molecule and to examine the potential correlation of the separation with autoimmune induction or inhibition (Sakaguchi et al., 1985; Itoh et al., 1999; and references therein). The cell surface molecules that have been employed to date are CD25 (the interleukin [IL]-2 receptor α chain generally expressed on activated T cells), CD45RB/RC (a protein tyrosine phosphatase expressed in almost all hematopoetic cells), CD5 (expressed at high levels on mature T cells and a possible ligand for CD72), and RT6.1 (expressed on the majority of mature T cells in rats, and having ADP-ribosylation activity). As shown in Figure 1, when $CD4^+$ splenic T cell suspensions prepared from normal mice or rats were depleted of $CD25^+$, $RT6.1^+$, $CD5^{high}$, or $CD45RB/RC^{low}$ cells and the remaining $CD4^+$ T cells were transferred to syngeneic T cell-deficient or -depleted mice or rats, the recipients spontaneously developed various organ-specific autoimmune diseases (including IDDM, thyroiditis, and gastritis) and systemic wasting disease in a few months. Reconstitution of the eliminated population inhibited the autoimmune development. Inflammatory bowel disease (IBD) can also be induced in T and B cell–deficient SCID mice by transferring similarly treated T cell suspensions from normal histocompatible mice, although it remains to be determined whether this murine IBD is due to autoimmunity or heightened immune responses to commensal bacteria in the bowel (Groux and Powrie, 1999).

These attempts to search for cell surface markers specific for the regulatory $CD4^+$ T cells are still at an early stage. The profile of the $CD4^+$ regulatory T cells (such as being $CD5^{high}$, $CD45RB/RC^{low}$, $RT6.1^+$, or $CD25^+$) is not directly associated with the regulatory function itself, but may indicate that the cells are in an "activated," "primed," or "memory" state. Nevertheless, the results obtained to date show that a subpopulation of $CD4^+$ T cells suppresses the activation and expansion of potentially pathogenic self-reactive T cells in the normal immune system, thereby contributing to the maintenance of self-tolerance. Removal or reduction of such a regulatory $CD4^+$ T cell population, which may constitute at most 10% of mature $CD4^+$ T cells in the thymus and the periphery, may directly lead to spontaneous activation/expansion of self-reactive T cells and consequently to the development of various autoimmune diseases (Itoh et al., 1999) (Figure 2).

In organ transplantation, tolerance can often be adoptively transferred by lymphocytes from long-term survivors to naive recipients (Zhai and Kupiec-Weglinski, 1999; and references therein). For example, allograft tolerance can be established by a short course of administering cyclosporin A, a T cell–specific immunosuppressant, or monoclonal antibody blocking the CD4 molecule (or other molecules on the T cell surface) at the time of grafting; and this tolerance can be transferred to naive animals by $CD4^+$ T cells from tolerant donors (Hall et al., 1990; Qin et al., 1993). Interestingly, by the transfer of $CD4^+$ T cells from anti-CD4 antibody-treated tolerant mice, naive $CD4^+$ T cells in the recipients also gradually become tolerant and suppressive in the presence of the donor $CD4^+$ T cells and the allograft, potentiating the tolerant state as long as the graft is present (Qin et al., 1993). It remains to be determined whether the $CD4^+$ regulatory T cells involved in this "dominant" transplantation tolerance are similar in the phenotype, the function, and the origin to those involved with the natural self-tolerance.

Thymic Production of Regulatory T Cells: Another Key Function of the Thymus in Maintaining Self-Tolerance

Does the thymus produce regulatory T cells as a functionally mature population or do certain T cells acquire such a regulatory activity in the periphery? Recent studies have shown that the normal thymus contains mature $CD4^+$ $CD8^-$ thymocytes with autoimmune-preventive activity (Itoh et al., 1999; Seddon and Mason, 2000; and references therein). An attempt to delineate them by cell surface markers (as for peripheral T cells [Figure 1]) showed that transfer of $CD25^+$ cell-depleted mature $CD4^+$ $CD8^-$ thymocyte suspensions produced similar autoimmune diseases in syngeneic T cell–deficient mice, and reconstitution of the depleted population prevented the autoimmune development (Itoh et al., 1999). The normal thymus thus seems to be continuously producing not only pathogenic self-reactive T cells but also functionally mature regulatory $CD4^+$ T cells controlling them (Figure 2).

The thymus is also able to produce transplantation antigen-specific regulatory $CD4^+$ T cells when allogeneic thymic epithelial cells are transplanted to athymic nude mice; T cells that have developed through the selection process on the engrafted thymic epithelial cells can suppress naive T cells to respond to allogeneic skin grafts expressing the same MHC haplotype as the grafted thymic epithelial cells (Modigliani et al., 1995). The thymus also appears to be required for establishing transplantation tolerance by cyclosporin A or anti-CD4 antibody treatment described above, since removal of the thymus before treatment substantially diminishes the efficacy of inducing tolerance (Zhai and Kupiec-Weglinski, 1999; and references therein).

Taken together, these results indicate that the thymic selection mechanism may not only delete self-reactive T cells but also give rise to regulatory T cells, presumably specific for self-antigens in the case of natural self-tolerance or regulatory T cells specific for allo-antigens in the case of transplantation tolerance. Elucidation of this mechanism of thymic generation of regulatory T cells is an important and interesting area of future research.

Cytokines and Regulatory T Cells

Characterization of cytokine-secreting patterns of $CD4^+$ T cells has revealed in the past decade another aspect of T cell–mediated immunoregulation: the presence of $CD4^+$ subpopulations secreting distinct patterns of cytokines (Mosmann and Coffman, 1989; O'Garra et al., 1997). $CD4^+$ T cells can be subdivided into Th1 cells, which produce IL-2, interferon (IFN)-γ and lymphotoxin, and Th2 cells, which produce IL-4, IL-5, IL-6, and IL-13. This segregation well correlates with the effector functions of $CD4^+$ T cells; i.e., Th1 cells conduct cell-mediated immunity by activating macrophages or $CD8^+$ cytotoxic lymphocytes, while Th2 cells help B cells in antibody production (Figure 2). Furthermore, these specific cytokines produced by Th1 and Th2 cells (especially IFN-γ and IL-4, respectively) are also potent cross-inhibitors of the two cell types. Cytokine-secreting pattern and cross-inhibition are of great interest from the standpoint of self-tolerance and autoimmunity. For example, effector $CD4^+$ T cells mediating autoimmune disease tend to be deviated to Th1 or Th2 type depending on the type of autoimmune disease—Th1 cells are the main mediators of organ-specific autoimmune diseases (such as IDDM, thyroiditis, and gastritis), while Th2 cells mainly mediate systemic autoimmune diseases (such as lupus). Furthermore, control of such cytokine-secreting patterns of self-reactive T cells may be able to inhibit the development of autoimmune disease or downregulate the ongoing autoimmune responses. Indeed, in organ-specific autoimmune diseases, IDDM in particular, efforts have been made to divert effector T cells from pathogenic Th1 type to protective Th2 type by administering IL-4 and/or anti-IFN-γ antibody (Liblau et al., 1995) or a target self-peptide in adjuvant (Tian et al., 1996).

In addition to Th1 and Th2 cells, $CD4^+$ T cells predominantly producing transforming growth factor (TGF)-β or IL-10 (designated Th3 or Tr1 type, respectively) can also be propagated in vitro; and their inoculation was shown to be effective in treating autoimmune or T cell–mediated inflammatory disease in animal models (Chen et al., 1994; Groux et al., 1997). Furthermore, TGF-β-deficient mice or TGF-receptor-inactivated mice developed various autoimmunities (Gorelik and Flavell, 2000; and references therein). These findings suggest the possibility that regulatory $CD4^+$ T cells in the T cell–mediated natural self-tolerance or the transferable transplantation tolerance may mediate the suppression by secreting IL-4, IL-10, or TGF-β. Indeed, administration of neutralizing antibodies to IL-4 or TGF-β abrogated the in vivo autoimmune-preventive or tolerance-inducing activity of $CD4^+$ T cells in some models (Seddon and Mason, 1999; Zhai and Kupiec-Weglinski, 1999). Recent studies have also shown that these regulatory $CD4^+$ T cells, unlike other T cells, constitutively express CTLA-4, a costimulatory molecule in T cell activation, and that T cells stimulated via CTLA-4 predominantly secrete TGF-β (Chen et al., 1998; Solomon et al., 2000). These findings suggest that regulatory $CD4^+$ T cells activated through CTLA-4 might suppress other T cells by secreting TGF-β. On the other hand, there is substantial data that regulatory $CD4^+$ T cells control other T cells by a cognate cellular interaction on APCs (Itoh et al., 1999; and references therein). To further elucidate the role of cytokines in T cell–mediated immunoregulation, it must be determined whether cytokines mediate the suppressive activity itself of regulatory T cells or whether they influence the developmental milieu of regulatory T cells, since IL-4, IL-10, and TGF-β can induce the differentiation of T cells that produce these same cytokines (Groux et al., 1997; O'Garra et al., 1997).

Future Perspective

The phenomena of T cell–mediated suppression in immunologic tolerance have been controversial and remain an exciting area of active research. An approach from the analysis of autoimmune-preventive activity of normal T cells is now revealing a unique regulatory T cell population dominantly engaged in the maintenance of immunologic self-tolerance. Production of such regulatory T cells can be another key function of the thymus in mediating self-tolerance. Recent research has also revealed that a part of the suppressive phenomena can be attributed to immunosuppressive cytokines secreted by particular types of effector T cells. Therefore, more than one population of regulatory T cells seem to be engaged in the maintenance of self-tolerance and these populations function in different ways—some are locally induced as a result of immune responses, while others are naturally produced. Further characterization of the function and development of these regulatory T cells will contribute to our understanding of immunologic self-tolerance as an acquired process and of the cause and mechanism of autoimmune disease. Manipulation of regulatory T cells will lead to new strategies for the treatment or prevention of autoimmune disease, transplant rejection, and cancer.

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