Sliding set-points of immune responses for therapy of autoimmunity

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Although recent developments in the treatment of autoimmune disease have dramatically improved patient outcomes, these medications are not curative. Two studies in this issue demonstrate the feasibility of curing spontaneous autoimmunity in animal models via short-term enhancement of naturally arising regulatory T (T reg) cells, a subset of CD4$^+$ T cells needed for maintaining self-tolerance. Importantly, these therapies seemed to generate a new equilibrium, or "set-point," at which self-tissue damage no longer occurred long after the drug was eliminated from the body.

The development of novel biological therapies has fostered a renaissance in the treatment of autoimmune disease. For example, tumor necrosis factor–blockers, anti–B cell therapy, CTLA4-imunoglobulin (Ig), and interleukin 6 (IL-6) blockade, all introduced within the past decade, have made diseases such as rheumatoid arthritis much easier to control by limiting joint pain, swelling, and stiffness, as well as preventing joint damage and deformity (Smolen et al., 2010). However, because these treatments are not curative, years of therapy result in high costs for patients and the potential for serious infections, cancer, and other adverse outcomes. In this issue, studies by Grinberg-Bleyer et al. and Nishio et al. demonstrate (using animal models of type I diabetes) that long-lasting cure of autoimmune disease may be feasible through treatments that depend on IL-2 to expand or improve the function of CD4$^+$ T reg cells.

Antigen-specific versus nonspecific treatments for autoimmunity

As autoimmunity is often restricted to certain tissues, an appealing treatment would specifically target the pathogenic cells that cause autoimmunity without inducing global immunosuppression. Although this approach has been successful in certain animal models, the diversity of HLA proteins and possible autoantigens recognized by self-reactive T cells has made it difficult to generalize these approaches to treating individual human patients. More recently, the realization that T reg cells suppress effector T cell responses led to the suggestion that administration of self-antigen specific T reg cells may represent a "magic bullet" that shuts down autoimmune responses without globally affecting immunity to foreign pathogens (Roncarolo and Battaglia, 2007; Bluestone et al., 2010). This approach is particularly appealing, as T reg cells also appear to suppress the general local immune response, rather than just individual responses to the antigens recognized by the T reg cells. In this case, knowing the exact self-antigens being targeted by an individual patient's T cells may not be required. This approach was successfully tested in mice; T reg cells expressing a transgenic TCR specific for a single islet-specific antigen that were expanded in vitro protected against autoimmune diabetes in a disease model where it is clear that other self-antigens are also likely to be involved (Tang et al., 2004; Tarbell et al., 2004). This approach may soon be testable in humans (Putnam et al., 2009).

However, translating these murine studies into tailored therapies for individual human patients will require overcoming several technical hurdles. First, some suitable target antigens need to be identified. Although this process is advanced in certain diseases, such as type 1 diabetes (Roncarolo and Battaglia, 2007; Bluestone et al., 2010), these antigens are poorly characterized in other diseases, such as rheumatoid arthritis. Second, good manufacturing processes are required to ensure that in vitro culture results in a very pure population of antigen-specific T reg cells. Certainly, one adverse outcome to be avoided is the accidental introduction of effector cells that may exacerbate autoimmunity. However, this is complicated by the fact that Foxp3, the best available T reg cell–specific marker, is intracellular and cannot be directly used to purify human T reg cells. Finally, there is the possibility that this "individualized medicine" would be prohibitively expensive, precluding treatment of large numbers of patients. Thus, developing antigen-nonspecific approaches to manipulating the T reg cell population may be a more practical and useful treatment modality.

IL-2: from T cell growth factor to the "everything" factor for T reg cells

IL-2 was originally identified as crucial factor for T cell growth in vitro, as it facilitated the development of primary T cell clonal lines. It is also important for natural killer (NK) cell maturation and function. However, the observation that IL-2–deficient mice developed spontaneous autoimmunity rather than the predicted immunodeficiency

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suggested that the major role of this cytokine may be in immune tolerance (for review see Malek, 2008). Several studies demonstrated a role for IL-2 in virtually all aspects of T reg cell biology (Malek et al., 2002), including development (Burchill et al., 2008; Lio and Hsieh, 2008; Schallenberg et al., 2010), survival, expansion, and function (D'Cruz and Klein, 2005; Fontenot et al., 2005; Knoechel et al., 2005), as well as phenotypic stability (Duarte et al., 2009; unpublished data). Moreover, a deficiency in local IL-2 has been suggested to play a role in the progression of autoimmune diabetes in the murine NOD model (Tang et al., 2008).

An explanation for the pro- and antiinflammatory effects of IL-2 may arise from the fact that most T reg cells express surface CD25, the IL-2 receptor α chain component of the high-affinity IL-2 receptor and the marker by which T reg cells were originally defined on their surface (Sakaguchi et al., 1995). This competitive advantage for capturing IL-2 in the local microenvironment has been suggested to be one mechanism by which T reg cells prevent spontaneous immune activation (Pandiyan et al., 2007). The constant "soaking-up" of IL-2 by T reg cells is supported by the observation that exogenous IL-2 can dramatically increase the size of the peripheral T reg cell population, which implies that in vivo levels of IL-2 are normally limited (Fontenot et al., 2005; Knoechel et al., 2005). Furthermore, naive T cell activation in peripheral lymph nodes results in IL-2 production, which induces a rapid activation of the resident T reg cells (O'Gorman et al., 2009); this observation suggests that IL-2 capture is not simply a cytokine clearance mechanism. Thus, it may be hypothesized that initial activation of naive T cells automatically triggers cell-extrinsic T reg cell–mediated negative feedback regulation (Fig. 1 A) in the absence of other factors that signal pathogenic infection (Pasare and Medzhitov, 2003). Recognition of self-antigens by these IL-2–stimulated T reg cells would further enhance suppression, thereby preventing inappropriate immune activation.

Grinberg-Bleyer et al. (2010) exploited the established T reg cell–boosting activities of IL-2 by using a short-term, low-dose IL-2 treatment strategy to preferentially target T reg cells rather than effector T or NK cells in NOD mice. This 5-d treatment was used at the onset of diabetes, analogous to when patients would clinically present with clinical symptoms. Remarkably, this resulted in long lasting normoglycemia in 30% of the mice 2.5 mo after treatment. A small cohort of mice followed for a long-term period was still diabetes-free for up to 8 mo. This treatment boosted T reg cell markers on T reg, but not effector, cells in the pancreatic islets, which is consistent with the differential sensitivity of these cell populations to IL-2. Interestingly, low-dose IL-2 appeared to primarily affect the activation status, rather than dramatically increase the number, of pancreatic T reg cells. Thus, these data suggest that short-term treatment of diabetic mice with low-dose IL-2 can result in a long-term cure in some cases.

The relatively low frequency of cure may be related to the efficiency with which low-dose IL-2 generates a

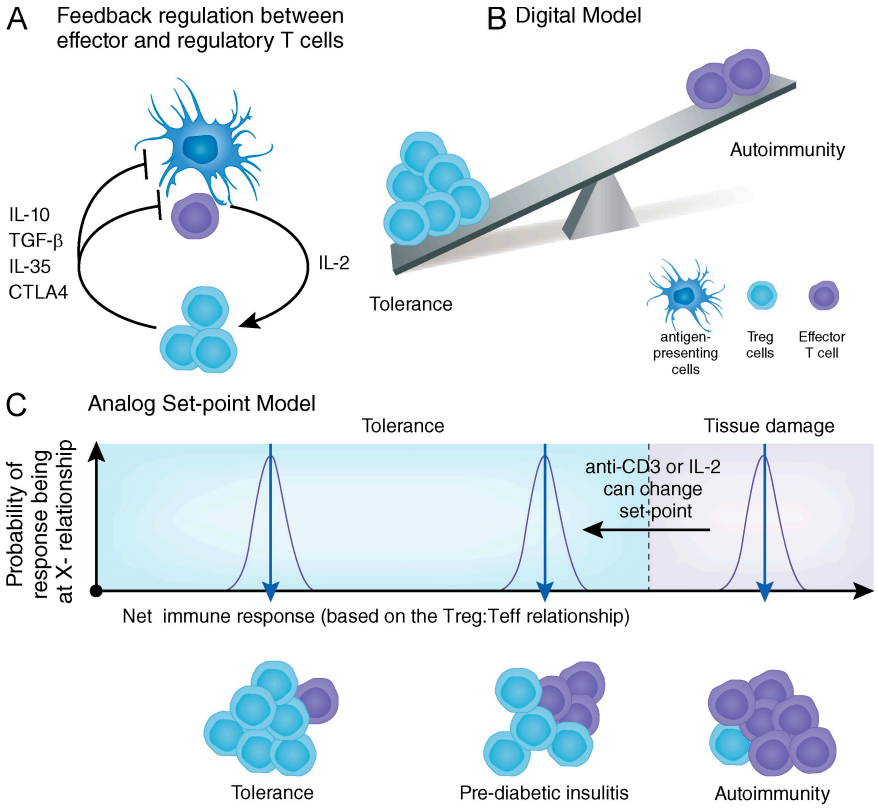

Figure 1. Simple models of T reg cell–dependent immune homeostasis. (A) Simplified diagram of feedback regulation illustrating effector T cell activation leading to IL-2 production, which can enhance T reg cell development, expansion, survival, stability, and function. T reg cells inhibit the effector response via myriad mechanisms on both T cells and APCs. In the absence of external forces, equilibrium may be established between effectors and regulators. (B) Digital model. Here, T reg and effector cells represent opposing forces that weigh against each other. In this model, the equilibrium point is quite small, resulting in essentially a binary response. (C) Analogue model with set-point autoregulation. Feedback regulation (A) would stabilize the net immune response around a "set-point" (blue arrows); set-points can move along a continuous scale (x axis). The net immune response reflects effector versus T reg cell numbers (depicted below x axis), as well as function (not depicted). In normal individuals, thymic production of self-reactive T reg cells would establish a tolerant set-point. However, genetics or environmental insults may change the set-point such that insulitis may occur, with or without tissue damage. Treatment with IL-2, anti-CD3, etc., can alter the set-point such that it falls within the tolerant range.

tolerogenic state. However, although higher levels of cytokines achieved using IL-2–anti–IL-2 monoclonal antibody complexes resulted in substantial increases of T reg cell frequency, it did not increase the cure rate beyond low-dose IL-2 (Grinberg-Bleyer et al., 2010). This may be caused by the concurrent activation of effector T or NK cells at higher levels of IL-2, resulting in no further shift toward immune tolerance. Pharmacologically improving the selectivity of IL-2R ligands for T reg versus effector or NK cells may overcome this limitation. Another possible reason for treatment failure is the high degree of tissue damage required to observe hyperglycemia; there may simply be too few islets left to save. Thus, identifying patients at prediabetic stages of inflammation will likely be important for increasing the efficacy of immunomodulatory therapies with long lasting benefits.

The T reg cell niche: cytokines and antigens

Nishio et al. (2010) used a different reagent, anti-CD3, which is currently being evaluated for the treatment of autoimmune disease. In the BDC2.5 TCR transgenic $Rag^{-/-}$ model of type I diabetes, they found that anti-CD3 treatment could reverse hyperglycemia in ∼50% of the mice for up to 80 d, the length of the observation period. This protection was dependent on the dramatic ∼30-fold increase in the percentage of T reg cells induced by anti-CD3 therapy, as T reg depletion resulted in relapse of diabetes within 5 d. Somewhat surprisingly, this T reg expansion was not dependent on transforming growth factor-β, which would have been expected based on previous studies (Li et al., 2006; Marie et al., 2006). T reg cell expansion was also dependent on IL-2 signaling. Although not proof of causality, these data also support the notion that IL-2 augmentation can result in enhanced T reg cell numbers and function for the treatment of autoimmunity.

An interesting observation was that the enhancement of T reg cells persisted long after the anti-CD3 treatment was stopped, with the percentage of T reg cells stabilizing ∼10-fold higher relative to pretreatment levels. Adoptive transfer studies suggested that the BDC2.5 TCR $Rag^{-/-}$ transgenic line normally contains few T reg cells because of intraclonal competition for a small antigen-dependent niche, akin to results observed for thymic T reg cell development (Bautista et al., 2009; Leung et al., 2009). Continued competitive pressure in the periphery for these antigenic niches likely explains why the thymic and peripheral T reg TCR repertoires, although largely overlapping, are not identical (Hsieh et al., 2006; Pacholczyk et al., 2006); and why T reg cells from different anatomical locations often use different TCR repertoires, presumably reflective of the nature of the local antigen pool (Lathrop et al., 2008). However, although an antigen-specific niche may be important for limiting the number of T reg cells in untreated mice, it is unclear why the niche size is stably increased after anti-CD3 treatment.

One possibility is that the anti-CD3 treatment enlarged the antigen-specific niche for BDC2.5 T reg cells via TCR activation. However, the niche size would be predicted to shrink once anti-CD3 was eliminated from the body. Alternatively, anti-CD3 could expand the antigen-specific niche by increasing the presentation of BDC2.5 antigens, perhaps via concurrent activation of effector cells. Sustaining this antigenic boost would presumably require continued islet destruction, which seems unlikely as the mice remain normoglycemic. The burst of IL-2 presumably responsible for anti-CD3–driven enhancement of T reg cell numbers is also likely to return to baseline after the drug has been cleared.

Another possibility is that anti-CD3 induces the generation of effector cells that produce greater levels of IL-2 and less effector cytokines. In addition, it may be possible that anti-CD3–expanded T reg cells are less dependent on IL-2 or TCR engagement for their survival and maintenance. Could this anti-CD3–mediated expansion and only partial contraction of T reg cell populations represent the generation of memory T reg cells? Gene expression profiling did not reveal gross differences between anti-CD3–expanded and naturally arising T reg cells, although there were a few. Nevertheless, both of these possibilities could be consistent with the observed lower levels of interferon-γ and higher levels of T reg cell CD25 expression after low-dose IL-2 therapy (Grinberg-Bleyer et al., 2010). The specific mechanisms by which anti-CD3 exerts long-lasting perturbation of the T reg cell niche remain to be determined.

The homeostasis between effector and regulatory T cells

In both of these studies, tolerance lasted well beyond the time frame in which the drug was active. This suggests that the balance between effectors and regulators can be altered such that a new set-point can be reached. One simplistic model is that effector and T reg cells represent opposing forces (Fig. 1 B), such as two weights on opposite ends of a teeter-totter (Bluestone et al., 2010). However, this model would predict a binary response; e.g., once the desired effector/regulator balance point is reached, the teeter-totter would flip from autoimmunity to tolerance. However, this simplistic "digital" model is inconsistent with the complex outcome that treatment with low-dose IL-2 did not reverse insulitis, a prediabetic inflammatory state in the pancreas.

We therefore favor a model in which effector and T reg cells are always in homeostasis because of the negative feedback loop mediated in part by effector cell–derived IL-2 (Fig. 1 a). This ensures that in the absence of external perturbation, the interaction between these two cell types gravitates toward a certain set-point, analogous to the body maintaining a fixed level of thyroid hormone. This set point, however, can exist in an "analogue" continuum of effector-to-T reg cell ratios and functional efficacies, ranging from complete suppression of immune activation to frank autoimmunity (Fig. 1 C), and can be influenced by environmental stimuli or genetics. At a certain set-point, tissue damage will occur, and over time, lead to autoimmune manifestations such

as diabetes. Perturbation of this set-point by anti-CD3 or IL-2 could therefore have long lasting and stable effects. A probabilistic element to the establishment of this set-point may explain why some, but not all NOD mice get diabetes.

Although future experiments will be required to assess the utility of these models, there is a direct analogy to observations in cancer immunobiology. It has been observed that the immune system is capable of rejecting some tumors, whereas other tumors grow and progress. These outcomes would correlate with efficient effector responses and immune tolerance to tumors, respectively, which is consistent with the observation that increased T reg cell frequency in a tumor portends a worse outcome (Curiel et al., 2004). Interestingly, equilibrium between the tumor and immune system can arise, resulting in a situation in which the tumor is neither expanding nor being rejected (Koebel et al., 2007). This equilibrium point would not be considered a true tolerant state, as some effector responses are likely required to eliminate some tumor cells to prevent tumor growth. However, this set-point of T reg and effector T cell function is maintained via autoregulatory mechanisms such that equilibrium is attained and overall tumor volume remains stable. Thus, these observations in tumor immunobiology also support the notion of variable set-points in effector/T reg cell homeostasis.

Concluding remarks

These and other studies suggest that T reg cell numbers and function are controlled by both cytokine- and antigen-dependent niches. Unraveling the mechanisms that control the intricate relationship between effector T cells and T reg cells will be important for understanding how T reg cells are involved in immune tolerance. Nonetheless, it is already clear that therapeutic manipulation of these T reg cell niches can stably change the balance between effector and regulatory T cells, thereby holding the potential to provide a cure for autoimmune disease.

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