Emerging Challenges in Regulatory T Cell Function and Biology

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Several cell-biological questions are of interest concerning these receptors: (i) Are they internalized, and where do they deliver bound antigens? (ii) Are some receptors better at programming cross-presentation? (iii) Can individual receptors target functionally distinct DC subpopulations? (iv) Does antibody binding to a given receptor trigger DC maturation, and if so, does receptor activation help to control the balance between immunity and tolerance? In general, we must ask what the relationship is between the mode of antigen endocytosis and the function of antigen presentation to T cells (Fig. 1).

In recent work published in *Science*, Dudziak *et al.* have shown that two lectin receptors on mouse DCs, DEC-205 and DCIR (33D1), are differentially expressed by two different populations of DCs in the spleen: $CD8\alpha^+$ and $CD8\alpha^-$ DCs, respectively (*22*). Targeting antigens via DEC-205 to the $CD8\alpha^+$ subset was found to selectively prime MHCI-restricted responses by cross-presentation, whereas MHCII-restricted responses were more efficiently triggered by DCIR targeting to the $CD8\alpha^-$ subset. This finding is consistent with the idea that cross-presentation is, in general, more the purview of $CD8\alpha^+$ DCs (*23*). Reversing cell-type restriction of receptor expression did not appear to change this conclusion substantially, suggesting that specializations associated with $CD8\alpha^+$ DCs may favor their ability for cross-presentation.

At the same time, in vitro work suggested that another lectin (the mannose receptor) was also quite effective at inducing the formation of peptide-MHCI complexes from exogenous antigen in bone marrow cultures and macrophages, where subpopulation identities are less clear (*24*). The mannose receptor appeared to deliver bound antibody to early endocytic compartments, suggesting that cross-presentation may occur from here as opposed to in late endosomes and lysosomes, which was the case for loading onto MHCII. Although these results suggest that the receptor used and route of antigen entry may also help determine the resulting form of antigen presentation, the data relied only on low-resolution qualitative immunofluorescence to define the intracellular localization of delivered antigens—criteria too limited to support a firm conclusion. Moreover, the data did not account for the fact that DEC-205, which also efficiently mediates MHCI-restricted cross-presentation, has been extensively characterized as delivering its bound antigens to late endosomes and lysosomes as opposed to early compartments (*25*). In any event, these findings highlight a whole new problem set involving the relative contributions of endocytosis and DC subpopulations in determining the nature of the immune response.

Looking Forward by Looking Backward

There are many other problems that would benefit immediately from a more effective and bidirectional relationship between immunologists and cell biologists. For example, the dynamics and function of the immunological synapse remain incompletely understood, in part because these critically important structures have yet to be subjected to the type of rigorous analysis applied to "simpler" problems of cell adhesion. The relationship between autophagy and antigen presentation in viral immunity is also emerging as critical (*26*, *27*). Signaling in immune cells will provide a rich area to mine, and some direct interchange over what lipid rafts can and cannot do would be of great value in itself. Finally, there is the issue that immunologists have always appreciated far better than most molecular cell biologists: the in vivo or systems-level context. Immunology exists to study the way the immune system works as a whole to confer protection against disease. Broad and conceptually profound, it is understandably difficult for the field to devote equivalent attention to the cellular mechanisms involved. However, further progress will require such effort, and the best path forward will be to take steps to make the language, concepts, and culture of immunology more accessible to colleagues in cell biology to attract them in and to outsource what may be too diversionary to learn. One area that is particularly ripe for spectacular advance is in the area of in vivo or "intravital" imaging. Although still in a largely descriptive phase of development, immunologists are nicely demonstrating to cell biologists the conceptual value of this platform. When this area is combined with emerging technologies to permit interventional experiments using actuation switches and quantitative molecular reporters, we will have entered a new age of "systems cell biology," combining the best of both worlds.

Like Elyot and Amanda, immunology and cell biology were once intimate partners; we find ourselves again in close proximity, but this time with the chance to rekindle a beautiful relationship.

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PERSPECTIVE

Emerging Challenges in Regulatory T Cell Function and Biology

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Much progress has been made in understanding how the immune system is regulated, with a great deal of recent interest in naturally occurring $CD4^+$ regulatory T cells that actively engage in the maintenance of immunological self-tolerance and immune homeostasis. The challenge ahead for immunologists is the further elucidation of the molecular and cellular processes that govern the development and function of these cells. From this, exciting possibilities are emerging for the manipulation of regulatory T cell pathways in treating immunological diseases and suppressing or augmenting physiological immune responses.

Walter B. Cannon, the originator of the concept of homeostasis, emphasized in his book *The Wisdom of the Body* that "when a factor is known which can shift a homeostatic state in one direction it is reasonable to look for a factor or factors having an opposing

effect." The immune system is not an exception to this. It harbors not only effector lymphocytes capable of attacking invading microbes but also an inhibitory population of T cells, called regulatory T (T_{reg}) cells. These lymphocytes are specialized in suppressing excessive or misguided immune responses that can be harmful to the host; for example, against normal self-constituents in autoimmune disease, innocuous environmental substances in allergy, or commensal microbes in certain inflammatory diseases (*1*, *2*). On the other hand, overzealous T_{reg} responses can impede host protective immunity in infectious disease and cancer. Recent advances in our understanding of the molecular mechanisms that control T_{reg} cell development have opened new avenues of investigation, but key questions concerning the antigen specificity of T_{reg} cells, their homeostasis, and mechanism of action remain. Here we discuss our current understanding of the biology and function of T_{reg} cells and how they might be clinically exploited to control physiological and pathological immune responses to self- and nonself-antigens.

Naturally occurring $CD4^+$ T_{reg} cells, which constitute approximately 10% of peripheral $CD4^+$ T cells in normal individuals, characteristically express CD25 [the interleukin-2 (IL-2) receptor α chain, which is a component of the high-affinity IL-2 receptor] (*1*, *2*). $CD4^+CD25^+$ T_{reg} cells play a nonredundant role in maintaining immunological self-tolerance and immune homeostasis. Their importance is made evident by the fact that the depletion of this population from normal rodents produces a variety of autoimmune inflammatory diseases, whereas reconstitution with $CD4^+$ $CD25^+$ T cells can inhibit disease development (*1*, *2*). They are produced by the normal thymus as a functionally distinct and mature population, although there is evidence that T cells with similar immune suppressive activity can be generated from naïve T cells in the periphery.

The identification of the transcription factor forkhead box p3 (Foxp3) as being specifically expressed by T_{reg} cells and crucial for their function has provided a molecular framework for dissecting T_{reg} function (*3*–*5*) (Fig. 1). Mutations in the gene encoding Foxp3 in humans and mice result in impaired development and function of $CD4^+CD25^+$ natural T_{reg} cells and lead to autoimmune inflammatory pathology. This is best exemplified by a human genetic disease called IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) syndrome, which is characterized by autoimmune disease (including type 1 diabetes and thyroiditis), allergy, and inflammatory bowel disease (IBD) (*6*). Further evidence for Foxp3 as a key controller of the development and suppressive function of natural T_{reg} cells comes from experiments in which transduction of the gene is sufficient to convert naïve T cells into T_{reg}-like cells (*3*–*5*). Notably, Foxp3 inhibits transcription of the gene encoding IL-2 and up-regulates the expression of CD25 and other T_{reg} cell–associated molecules (*3*, *4*). The resulting inability of $Foxp3^+$ T_{reg} cells to produce IL-2 appears to make them highly dependent on exogenous IL-2 for survival (*7*–*9*). Accordingly, mice genetically deficient in IL-2, CD25, or CD122 (the IL-2 receptor β chain) and humans with genetic deficiency of CD25 have both reduced numbers and impaired function of $Foxp3^+$ T_{reg} cells and succumb to severe autoimmune inflammatory disease (*8*, *10*).

A key question that has emerged from these findings is how Foxp3 orchestrates the cellular and molecular programs involved in T_{reg} function. Recent studies have shown that Foxp3 binds to other transcription factors such as NFAT (nuclear factor of activated T cells) and AML1 (acute leukemia-1)/Runx1 (runt-related transcription factor 1) and potentially interacts with activator protein 1 and nuclear factor κB (*11*–*13*). It is this Foxp3/NFAT/Runx1 complex, together with other coactivator or corepressor proteins, that is responsible for the observed repression of the IL-2 and other cytokine genes, as well as the activation of the genes for CD25, cytotoxic lymphocyte–associated antigen-4 (CTLA-4), and glucocorticoid-induced TNF receptor family–related protein (GITR) by binding to their respective promoters (*11*, *12*). MicroRNA genes also appear to be important in T_{reg} cell development; for example, T cell–specific depletion of Dicer, a ribonuclease enzyme required for processing double-stranded RNA, hampers thymic development of $Foxp3^+$ T cells and elicits IBD (*14*). In addition, it has been shown by genome-wide analysis combining chromatin immunoprecipitation with mouse genome tiling array profiling that Foxp3 directly or indirectly controls hundreds of genes, which include those that encode nuclear factors controlling gene expression and chromatin remodeling, membrane proteins, and signal transduction molecules (*15*, *16*). Assuming that the proteins encoded by Foxp3-controlled genes contribute to the suppressive activity of T_{reg} cells, it seems likely that further analysis of these pathways will provide insight into T_{reg} mechanisms of action.

In addition to the thymic production of natural $Foxp3^+$ T_{reg} cells, naïve T cells in the periphery acquire Foxp3 expression and T_{reg} function in several experimental settings, including in vitro antigenic stimulation in the presence of transforming growth factor–β (TGF-β) or after chronic antigen stimulation in vivo (*17*, *18*) (Fig. 1). Recent studies indicate that the intestine is a site of $Foxp3^+$ T_{reg} cell development and that specialized intestinal dendritic cells (DCs) promote Foxp3 expression via a mechanism that is dependent on local TGF-β and retinoic acid, a vitamin A metabolite (*19*–*21*). Peripheral development of $Foxp3^+$ T_{reg} cells may therefore represent a mechanism that helps broaden the T_{reg} repertoire in specialized anatomical sites. Recent studies have also revealed a reciprocal relationship between the development of $Foxp3^+$ T_{reg} and effector T cells, so that naïve $CD4^+$ T cells differentiate into $Foxp3^+$ T_{reg} cells in the presence of TGF-β or into T helper 17 (T_H17) cells (which secrete IL-17, a potent proinflammatory cytokine) in the presence of TGF-β and IL-6 (*22*, *23*). Therefore, TGF-β, which can be ubiquitously expressed in tissues, has the paradoxical effect of inducing distinct T cell subsets that appear to have opposing effects on immune responses. Moreover, IL-2 facilitates the differentiation of naïve $CD4^+$ T cells into T_{reg} cells but inhibits their differentiation into T_H17 cells, whereas IL-6 suppresses Foxp3 expression in T_{reg} cells in addition to enhancing T_H17 cell development (*23*, *24*). These results serve to illustrate the complexity of cytokine-mediated control of the differentiation of $Foxp3^+$ T_{reg} cells in the periphery, and further work is required to identify tissue-specific factors that influence the balance between T_{reg} and effector T cells in distinct tissue sites.

Although peripherally induced T_{reg} cells resemble thymically derived T_{reg} cells in phenotype and aspects of their function, future comparative studies of their functional and genetic stability, including the status of chromatin remodeling of the Foxp3 locus, need to be performed with the two populations (*25*). It should also be noted that, in contrast to mouse naïve T cells, in which it is difficult to induce Foxp3 by in vitro T cell receptor (TCR) stimulation, human naïve peripheral blood T cells readily express Foxp3 upon TCR stimulation although the expression level is generally much lower and more transient than in natural T_{reg} cells (*26*). Indeed, it is not yet established whether induced T_{reg} cells have identical functions to those of natural T_{reg} cells, to what extent they contribute to the pool of $Foxp3^+$ T_{reg} cells in the periphery, and whether this activation-induced Foxp3 expression in non-T_{reg} cells serves as a T cell–intrinsic brake on immune responses.

$Foxp3^+$ T_{reg} cells can both directly and indirectly suppress the activation and proliferation of many cell types, including T cells, B cells, DCs, natural killer (NK) cells, and NKT cells in vivo and/or in vitro (*27*, *28*). In vitro suppression of TCR-stimulated proliferation of other T cells is a commonly used assay for assessing T_{reg} cell suppressive activity; however, the mechanisms involved are incompletely understood. A number of different mechanisms have been linked to T_{reg} activity, including cell contact–dependent inhibition of the activation and proliferation of antigen-presenting cells (APCs) and T cells, the killing of either APCs or T cells or both, and suppression via cytokines such as IL-10 and TGF-β (*2*, *27*, *28*). These results suggest that $Foxp3^+$ T_{reg} cells do not suppress

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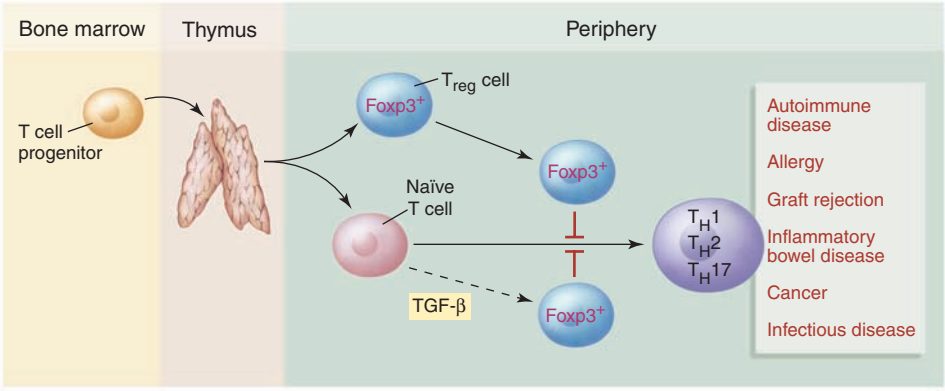

Fig. 1. Foxp3$^+$ natural T_{reg} cells produced by the normal thymus suppress the activation and expansion of naïve T cells and their differentiation to effector T cells, including T_H1, T_H2, and T_H17 cells, which mediate a variety of pathological and physiological immune responses. Foxp3$^+$ T_{reg} cells can also be generated from naïve T cells in the periphery, although the physiological significance of this T_{reg}-generative pathway remains to be determined.

immune responses by a single mechanism but use a variety of pathways in a context-dependent manner; for example, depending on cytokine milieu, the activation status of APCs, and the strength of antigen stimulation. A key challenge therefore is to validate putative mechanisms of T_{reg} activity in vivo and define the circumstances in which these operate. An important factor may be the site of action of T_{reg} cells. Elegant studies by intra-vital imaging with two-photon microscopy to examine the in vivo behavior of activated T_{reg} cells in lymph nodes suggest that they may hamper the access of effector T cells to DCs (*29*, *30*). There is also evidence that T_{reg} cells act in tissues to control established inflammation and that T_{reg} cell production of IL-10 plays a functional role (*2*). IL-10–secreting Foxp3$^+$ T cells are rare in the spleen but abundant in the inflamed intestine and also become detectable at the site of inflammation in autoimmune disease or chronic infection (*31*). This indicates that there is compartmentalization of the T_{reg} response and that mechanisms of suppression may be influenced by the anatomical location and dictated by the nature of the inflammatory response being regulated. It is also imperative to the host that appropriate effector responses can be activated after infection with pathogens. The production of IL-6 by activated DCs has been shown to overcome T_{reg}-mediated suppression in vitro (*32*). However, further information on the cellular and molecular pathways that control the delicate balance between effector and regulatory T cells in vivo is required.

The specialized immunological properties of Foxp3$^+$CD4$^+$ T_{reg} cells suggest that they might be clinically exploited to control a variety of physiological and pathological immune responses (*2*, *10*). These cells can recognize a broad repertoire of self- and nonself-antigens including pathogens (*33*), although their total repertoire is apparently more skewed to recognizing self-antigens (*34*, *35*). Phenotypically they appear in an "antigen-activated" state in the thymus, as illustrated by their high expression levels of various accessory molecules, including adhesion molecules (*10*). Thus, they are poised to exert suppressive function whenever exposed to relevant antigens and thus are suited for controlling autoimmunity. In addition, in contrast to their in vitro hyporesponsiveness to TCR stimulation, many natural T_{reg} cells are in a proliferative state in vivo, presumably as a consequence of the recognition of self-antigens and possibly commensal microbes, and can be stimulated to proliferate by antigenic stimulation (*10*). They are also functionally stable, retaining their suppressive activity after clonal expansion (*10*). By exploiting this stable and robust suppressive activity as well as proliferative capacity, strategies that clonally expand antigen-specific natural T_{reg} cells while inhibiting the activation and expansion of effector T cells may be useful to strengthen or reestablish self-tolerance in autoimmune disease or induce tolerance to nonself-antigens in organ transplantation, allergy, and IBD, or augment feto-maternal tolerance in pregnancy (Fig. 1). As a reciprocal approach, selective reductions in the number or function of natural T_{reg} cells while retaining or enhancing effector T cells may be a strategy for provoking and augmenting tumor immunity in cancer patients or microbial immunity in chronic infection. Biologicals and small molecules with such differential effects on T_{reg} cells and effector T cells may represent a next generation of therapeutic reagents for suppressing or enhancing immune responses with a high level of selectivity (*36*).

Besides Foxp3$^+$ T_{reg} cells, there are a number of Foxp3-nonexpressing T cells with immune suppressive activity that are in the scope of clinical use. These include CD4$^+$ cells producing IL-10 or TGF-β as well as CD8$^+$ T_{reg} cells with different modes of suppression (*28*, *37*). Although the physiological role of these populations in immune homeostasis is not known, they do offer the advantage for clinical use that antigen-specific T_{reg} cells can be prepared relatively easily.

It is now firmly established that Foxp3$^+$ T_{reg} cells, naturally arising or induced, constitute an indispensable component of the immune system. Further elucidation of the molecular and cellular basis of their development and function will facilitate our understanding of immune tolerance and homeostasis and provide ways to better control immune responses for the benefit of the host.

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