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Regulatory T Cells and Inflammation: Better Late Than Never

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In this issue of *Immunity*, Curotto de Lafaille et al. (2008) show that adaptive T regulatory cells control airway inflammation and that it is when they are generated that determines whether they function during acute or chronic inflammation.

The airways are constantly assaulted by aeroantigens, yet the vast majority of individuals are tolerant and mount no immune response. However, atopic individuals respond inappropriately to these innocuous aeroantigens and develop airway inflammatory disease (asthma) characterized by remodeling and hyperresponsiveness of the airways. That asthma is a disease of immune dysregulation is exemplified by the fact that affected individuals have an elevated number of T helper 2 (Th2) cells and Th2 cell-type cytokines in the lung as well as elevated amounts of IgE and lung eosinophilia (Wills-Karp, 1999). The ability of the majority of people to remain unresponsive to aeroantigens has been ascribed to the ability of regulatory T (Treg) cells and immunomodulatory cytokines (e.g., IL-10) to control responses (Akdis, 2006; Umetsu and Dekruyff, 2006). Although the role of Treg cells to control airway inflammation has been shown in transfer models, what role they play as disease develops remains to be determined. In addition, how aeroantigen-specific Treg cells, if they exist, develop and function is an area of intense investigation and discussion. The data in Curotto de Lafaille et al. (2008) begin to develop a framework for uncovering the role of Treg cells in both acute and chronic airway inflammation in the lung, and, by extension, other mucosal surfaces.

In naive mice, the outcome of an encounter with an inhaled antigen, in the absence of an inflammatory stimulus, is tolerance, not activation. However, this tolerance is not the result of a lack of a response to the antigen, as shown by the fact that robust $CD4^+$ T cell proliferation is seen in draining lymph nodes after treatment (Hammad and Lambrecht, 2008). Rather, it may be due to incomplete dendritic cell activation, resulting in either aborted T cell activation and subsequent deletion or the generation of T cells with regulatory activity (Hammad and Lambrecht, 2008). These tolerized mice are then resistant to subsequent challenge with the same antigen in the presence of an inflammatory stimulus. In addition, adoptive transfer of antigen-specific Treg cells can inhibit disease development, and depletion of $CD4^+CD25^+$ cells increases disease parameters in challenged mice (Lewkowitch et al., 2005), further supporting an important role for Treg cells in controlling inflammation in airways.

The mechanism by which these Treg cells control airway inflammation is not at all clear. Several reports have suggested that Treg cells block inflammation in an IL-10-dependent manner. For example, either transfer of Treg cells from IL-10-deficient mice or IL-10 blockade after transfer of Treg cells abolished inhibition of airway inflammation (Kearley et al., 2005). In contrast, Treg cells induced by helminth infection are capable of completely inhibiting allergic airway inflammation in an IL-10-independent manner (Wilson et al., 2005).

Several important issues remain to be resolved concerning the role of Treg cells in controlling airway inflammation. These include whether the Treg cells seen in these models are thymically derived, what the role of Foxp3 is in their generation and function, and what role, if any, inflammation plays in controlling Treg cell generation and function. By using a very elegant and simple system, Curotto de Lafaille et al. (2008) provide insight into these issues. They take advantage of mice whose T and B cell repertoires are non-self reactive and monoclonal through expression of a single T cell receptor (TCR) and BCR in a Rag-deficient background (referred to as T-Bmc mice). Similar to other TCR transgenic mice in a Rag-deficient host, these mice lack thymically derived, "natural," Treg cells, and thus can be used to determine the role of adaptive Treg cells in tolerance to aeroantigens. The other advantage of these mice is that they can be rendered Foxp3 deficient without causing the

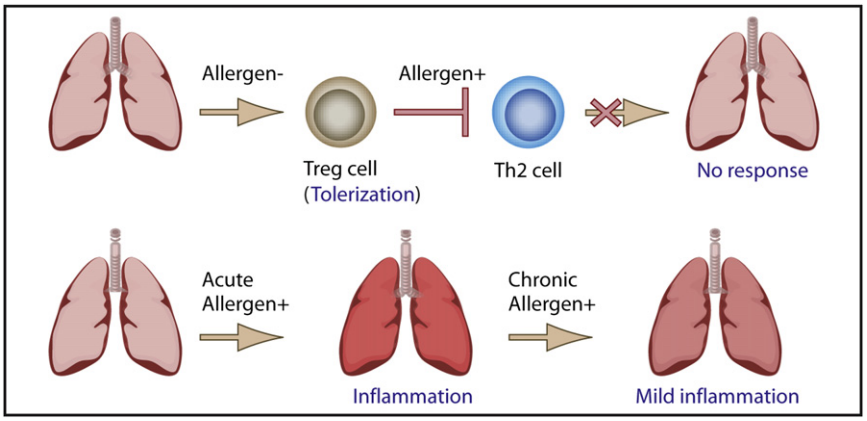

Figure 1. Timing of Adaptive Treg Cell Generation Determines Outcome
Top: OVA-specific adaptive Treg cells, generated via oral administration of antigen prior to sensitization and challenge (allergen −), can successfully inhibit inflammatory responses in the lung.
Bottom: In the absence of pre-existing allergen-specific Treg cells, sensitization and challenge (allergen +) results in the onset of acute inflammation, as well as the generation of allergen-specific Treg cells. Although these Treg cells are incapable of inhibiting acute inflammation, they can ameliorate inflammation if antigen is given chronically.

development of fatal autoimmunity, allowing a determination of Foxp3's role in this process. Although not a perfect system (e.g., the role of $CD8^+$ T cells cannot be addressed), it is ideal for a specific examination of the role of Foxp3 in the generation and function of adaptive Treg cells.

This group had previously shown that oral administration of ovalbumin (OVA) drove the generation of $Foxp3^+$ Treg cells in the T-Bmc mice (Mucida et al., 2005). In the current study they show that, not surprisingly, generation of these Treg cells requires Foxp3. An interesting feature of these experiments was the finding that in the T-Bmc mice lacking Foxp3, oral administration of OVA did not lead to the generation of effector T cells. These data are somewhat surprising given the recent report of a reciprocal relationship between adaptive Treg and Th17 cells (Zhou et al., 2008), and support instead the view that distinct microenvironments in the gut are responsible for Treg and effector T (Teff) cell differentiation. However, another possible explanation is that in the absence of adjuvant, DCs are not sufficiently mature to drive effector differentiation.

An important aspect of this work is a better understanding of not how, but when, Treg cells exert their influence over inflammatory responses (Figure 1). Not surprisingly, in mice given oral OVA prior to immunization and challenge, no appreciable airway inflammation was found. In these mice, the presence of antigen-specific Treg cells inhibits at the priming stage, and thus no response can develop. However, in naive animals (lacking both thymic and adaptive Treg cells), Teff and Treg cells develop side by side after sensitization. These Treg cells are ineffective at suppressing inflammation, as shown by the fact that the level of inflammation is the same in the presence and absence of Foxp3 in the mice. One reason for this difference may be timing—Teff, but not Treg, cells are capable of crossing the epithelial border and enter the airways. Treg cells enter the airways only after an antigen challenge, giving the effectors a "head start" on developing an inflammatory response. Where these Treg cells are effective is in suppressing responses after chronic exposure to antigen. T-Bmc mice lacking Foxp3 develop more severe inflammation that includes disseminated IL-4 production, the development of organized lymphoid follicles (BALT) in the lung, and smooth muscle hypertrophy. Interestingly, and in contrast to other systems (Kearley et al., 2005), IL-10 amounts decrease, along with cytokines, in the $Foxp3^+$ T-Bmc mice, and remain at high amounts in the T-Bmc mice lacking Foxp3, suggesting that IL-10 plays little or no role in resolving inflammation in this model. It should be noted that the $Foxp3^+$ Treg cells in these mice do not suppress all aspects of inflammation—IL-5 production and the resulting lung eosinophilia appear to be inhibited by a Foxp3-independent mechanism involving IFN-γ.

Taken as a whole, the data presented propose an important role for adaptive Treg cells in controlling inflammation and suggest that the timing of Treg cell generation ultimately determines the outcome of an encounter with antigen. Passive antigen encounter, for example through the airways, creates a tolerizing environment through the generation of antigen-specific Treg cells. These Treg cells can then suppress responses if a more active encounter with the same antigen occurs. If the first encounter with an antigen is active (via infection, for example), then acute inflammation can occur, but Treg cells generated during the initial response will inhibit subsequent chronic inflammation. In the airways, this could be from an early respiratory viral infection.

What these data do not explain, and what remains the major question in this field, is what happens in individuals who develop chronic airway diseases like asthma? The model described above would posit that most individuals should be tolerized against aeroantigens through passive interactions and should also be protected from other antigenic challenges through generation of adaptive immunity. Because asthmatics develop chronic inflammatory disease as a response to innocuous aeroantigen, it would appear that neither of these pathways is operative. This could be due to a defect in the ability to generate adaptive Treg cells or (as is now becoming apparent in some autoimmune settings) due to the generation of effector T cells that are resistant to suppression. Other possibilities include defects in other cell populations in the lung, such as the airway epithelium, resulting in inappropriate production of cytokines (such as IL-25 or TSLP) that may drive Th2 cell-type differentiation at the expense of Treg cells. Obviously more work is needed to better understand these problems, and the T-Bmc model is a good place to start.

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With a Little Help from Their Friends: Interleukin-21, T Cells, and B Cells

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T follicular cells help B cells generate high-affinity antibodies. Two papers in this issue of *Immunity* (Nurieva et al., 2008, and Vogelzang et al., 2008) have identified a role for interleukin-21 in the development of these specialized cells and highlight questions about how this dedicated population is generated.

Since the initial description of the T helper 1 (Th1) and Th2 subsets, the study of T helper cells has been dominated by questions about the events that lead to Th cell differentiation and the role of cytokines and transcription factors that prompt individual T cells to adopt different fates. More recently, with the characterization of regulatory T cells (Treg) and Th17 cells, many of these same issues have been revisited in slightly different contexts. Moreover, with the advent of improved ways to assay polyfunctionality in T cells, it is becoming apparent that defining a T cell lineage based on the production of a single cytokine or expression of a particular transcription factor has its limitations. This in turn has led to debate about whether the paradigm of T cell subsets is still useful and how to characterize phenotypic plasticity within T cell populations. Neglected by many in the midst of these deliberations is the original reason that $CD4^+$ T cells were ascribed a helper function: their ability to provide cognate help to B cells required for the generation of high-affinity antibodies and memory. These interactions between T and B cells provide an important checkpoint for the development of protective antibody responses and are also critical for the maintenance of peripheral B cell tolerance. However, given the distinct compartmentalization of T and B cells within secondary lymphoid tissues, there needs to be a process that brings relevant lymphocytes together. Thus, antigen-stimulated B cells accumulate at the margins of the B cell areas where they elicit T cell help. This carefully choreographed event is dependent on the ability of T cells to provide costimulation to the B cells that leads to the seeding of follicles with B and T cells and lymphocyte proliferation accompanied by differentiation and somatic hypermutation to give rise to plasma cells secreting high-affinity antibodies. The migration of Th cells to the edge of the B cell zones and the follicular regions and germinal centers rich in the chemokine CXCL13 is linked to the expression of the chemokine receptor CXCR5 (Ansel et al., 2000).

Until relatively recently, there has been a poor understanding of the Th cells that are involved in this process, and one model was that Th1 or Th2 cells provided help to B cells through shared mechanisms that include costimulatory interactions through CD40-CD40L or ICOS-ICOSL or their ability to produce specific B cell growth factors. Consistent with this notion was the association of their signature cytokines IFN-γ and IL-4 with class switching and particular IgG isotypes. In contrast, the first transcriptional profiling of $CXCR5^+$ $CD4^+$ T cells was more in line with the idea that T follicular helper (Tfh) cells were a distinct subset (Chtanova et al., 2004). Indeed, from these studies came the ideas that Tfh cells make IL-10 and IL-21, which are not typically associated with Th1 or Th2 cells, but which provide proliferative signals to B cells.

In the last month, three manuscripts have addressed the relationship of Tfh cells with other T cell subsets and identify IL-21 as a key cytokine that promotes the development of these specialized effector cells (Nurieva et al., 2008; Suto et al., 2008; Vogelzang et al., 2008). IL-21 is closely related to other potent T cell growth factors, such as IL-2 and IL-15, and signals through the shared common γ chain. Previous studies had shown that B cells express the IL-21R, that IL-21 promotes growth of mature B cells, and that *Il21r*$^{-/-}$ mice had reduced B cell responses (Spolski and Leonard, 2008). Together, these studies were congruent with the idea that Tfh cells are a source of IL-21 that drives B cell expansion within germinal centers. However, these more recent publications have