Foxp3$^+$ Regulatory T Cells: Selfishness under Scrutiny

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DOI 10.1016/j.immuni.2007.08.008

The Foxp3$^+$ T cell lineage is thought to arise from self-specific precursors during development. By using a unique mouse model, Pacholczyk et al. (2007) present compelling evidence that self-specific cells are exceedingly rare among Foxp3$^-$ and, surprisingly, Foxp3$^+$ subsets.

The critical roles of Foxp3$^+$ regulatory T (Treg) cells in dampening the potential catastrophic effects of unchecked immune responses have been demonstrated in numerous studies. Despite intensive investigation, the mechanism of Foxp3$^+$ Treg cell suppression still remains largely unknown. A critical obstacle to uncovering the mechanism of suppression has been a lack of knowledge of the target antigens recognized by the Foxp3$^+$ lineage. Early studies performed in models of autoimmunity suggested that Treg cells are activated by antigens derived from host tissues, and the view that Foxp3$^+$ Treg cells are specific for self-antigens became a well-entrenched dogma. One implication of such a model is that Treg cells originated from precursor thymocytes that received a unique instructive signal determined by the strength of T cell receptor (TCR) engagement during development. Signals stronger than those required for positive selection of conventional T (Tconv) cells, yet below the threshold resulting in deletion, have been hypothesized to lead to the generation of Foxp3$^+$ Treg cells.

This model of thymic selection of Treg cells was bolstered by studies of double-transgenic mice that expressed a neoself-antigen along with the corresponding high-affinity TCR specific for antigen in the thymus (Jordan et al., 2001). The proportion of suppressive CD4$^+$CD25$^+$ T cells dramatically increased relative to the conventional CD4$^+$CD25$^-$ subset. Importantly, an increase was not seen in mice expressing a transgenic TCR with a lower affinity for the same antigen. Later studies utilizing a neoself-antigen as a transgene under the control of a tetracycline-regulated promoter (van Santen et al., 2004) demonstrated that the amount of the agonist could be correlated with the development of antigen-specific CD4$^+$CD25$^+$ T cells. Increasing the expression of the agonist ligand led to a corresponding increase in the in the proportion of antigen-specific CD4$^+$CD25$^+$ T cells. However, this increase was not accompanied by an increase in their absolute number, but was secondary to a decrease in the numbers of antigen-specific CD4$^+$CD25$^-$ T cells selected. These results suggested that the Treg cell lineage was potentially more resistant to the effects of negative selection than were their Tconv counterparts. As a consequence, antigen-specific Treg cells become overrepresented when high amounts of the agonist peptide were present because of the preferential loss of the antigen-specific Tconv cells.

Although these studies provided invaluable insight into possible mechanisms of Treg cell development, the degree to which they truly represented the natural process leading to the selection of polyclonal Treg cells was unclear. Attempting to measure the diversity and specificity of polyclonal T cells poses an extraordinary challenge because of the enormity of the repertoire. Thus, comparisons of TCR diversity at this level must employ techniques that limit the repertoire to a more manageable size while simultaneously preserving a certain degree of diversity. One study took this general approach by analyzing TCR diversity and the self-reactivity of the Treg and Tconv cell subsets in mice expressing a variable Vα chain and a fixed transgenic TCR Vβ-chain (Hsieh et al., 2004). Direct sequencing of the TCRs cloned from Treg and Tconv cells from these mice demonstrated that each possessed a comparable degree of diversity. Although similarly diverse, a comparison of the TCR sequences present in each population suggested that they expressed largely distinct repertoires of receptors because only 20% of TCRs were shared between them. Pacholczyk et al. (2006) also concluded that the overlap between the Treg and Tconv was modest, with only 15%–20% of receptors shared, but observed a greater degree of repertoire diversity within the Treg cell subset than was reported by Hsieh et al. (2004). These studies were consistent with a model in which TCR specificity determines the lineage fate of the T cell.

The anergic phenotype of Treg cell has been an important obstacle to making determinations concerning their specificity. To overcome this difficulty, Hsieh et al. (2006) cloned ten TCRs that were unique to either CD4$^+$CD25$^+$ or CD4$^+$CD25$^-$ subsets and re-expressed them in recombination-activating gene (RAG)-deficient TCR transgenic CD4$^+$ T cells by retroviral transduction. The respective pools of transduced T cells were then transferred into lymphopenic hosts or cocultured with autologous splenocytes so that their respective expansion abilities could be assessed. The T cells that had been transduced with receptors from CD4$^+$CD25$^+$, but not CD4$^+$CD25$^-$ T cells, conveyed an

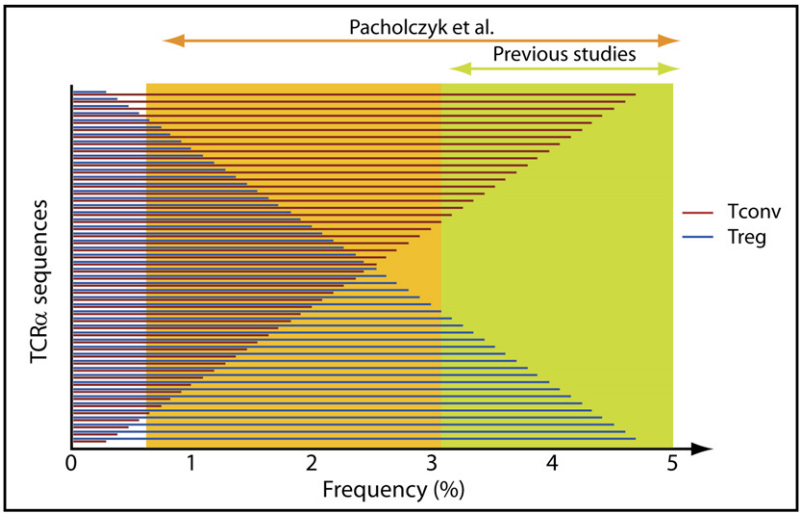

Figure 1. Examining Conventional and Regulatory TCR Repertoire
Previous attempts to compare TCR repertoires of Treg (blue bars) and Tconv (red bars) cells focused exclusively on the most frequently found receptors (green shading) in each subset and thus excluded large numbers of shared TCRs. Pacholczyk et al. (2007) have carried out a more detailed analysis with a mouse that expresses a highly restricted but still polyclonal repertoire. Analysis with this model permitted a much more complete picture of both repertoires to emerge because TCRs present at very low frequencies in both subsets could now be studied (orange shading).

enhanced ability to expand, consistent with a higher avidity for self-ligands.

One important caveat to these studies was that by focusing exclusively on the most frequently used TCRs in each group, both studies might have markedly underestimated the degree of repertoire overlap by excluding many infrequently used TCRs from their analyses. With this in mind, Pacholczyk et al. (2007) re-examined the repertoires of Treg and Tconv cell subsets, employing a mouse model in which the T cells express a single transgenic TCRβ chain and a variety of TCRα chains derived from an unrearranged TCRα minilocus. In contrast to their previous work, the authors included all TCRs present above a defined threshold frequency (Figure 1). By including these less frequent TCRs, a substantial degree of overlap was found in the two repertoires, although the degree of usage of any particular TCR varied considerably between the subsets. This observation held true in mice expressing a diverse array of peptide-MHC class II complexes and in mice whose MHC class II complexes were exclusively occupied by a single peptide. The finding of extensive overlap between the Treg and Tconv cell repertoires argues against a model of Treg cell development in which the specific TCR expressed dictates commitment to the Foxp3$^+$ lineage. These data are also consistent with a recent report that found a surprising degree of repertoire overlap by using an analogous model (Wong et al., 2007).

Pacholczyk et al. (2007) employed T cell hybridoma technology to generate large numbers of cells bearing receptors derived from Treg cells. Because these hybridomas lost the expression of Foxp3, confirmation of their Treg cell origin was accomplished by comparing hybridoma TCR sequences with the database of Treg cell sequences obtained from polymerase chain reaction (PCR) products of single-cell-sorted Foxp3$^+$ T cells. In contrast to the anergy exhibited by Treg cells, the hybridomas secreted IL-2 in response to TCR stimulation. Previous studies demonstrated that the majority of T cells selected in mice whose MHC class II molecules are exclusively occupied with a single peptide react with wild-type MHC class II molecules of the same MHC haplotype because these T cells have not been rendered tolerant to the normal spectrum of MHC class II-peptide complexes found in wild-type mice. Applying this logic, Pacholczyk et al. (2007) compared the reactivity of hybridomas derived from Tconv and Treg cells selected in TCR minilocus mice expressing single-peptide-loaded MHC class II complexes. A substantial portion of Treg and Tconv cell-derived hybridomas demonstrated reactivity toward wild-type antigen-presenting cells (APCs) displaying foreign peptides, whereas none reacted with autologous APCs. To exclude the possibility that abundant expression of MHC class II complexes loaded with a single peptide led to the deletion of Treg cells bearing self-specific receptors, the authors also examined the self-reactivity of Treg cell-derived hybridomas from TCR minilocus and wild-type mice selected on the normal array of MHC-peptide complexes. In both cases, none of the over 250 Treg hybridomas tested reacted with MHC class II complexes loaded with self-peptides. A comparable frequency of Tconv and Treg cell-derived hybridomas from wild-type mice reacted with allogeneic APCs, supporting the concept that the specificities of the both repertoires are biased toward nonself- rather than self-MHC reactivity. In contrast to the claim of Hsieh et al. (2004) that the self-specificity of TCRs from Treg cells resulted in wasting disease upon transfer to lymphopenic recipients, Pacholczyk et al. (2007) demonstrate that nonself-antigens rather than self-antigens are responsible for wasting disease. Disease was only observed when CD4$^+$CD45^{hi} T cells from TCR^{mini} or TCR^{mini} mice expressing single-peptide-MHC class II complexes were transferred into lymphopenic animals expressing wild-type peptide-MHC class II complexes, but not into mice that expressed single-peptide-MHC class II complexes that lack the ability to present exogenous antigens.

Although the results of Pacholczyk et al. (2007) challenge the hypothesis that Treg cell development in the thymus is determined by the affinity of their TCR for self, these data still do not exclude the possibility for a moderately enhanced avidity of Treg cell for self-antigens unrelated to their TCR repertoire. Two recent studies (Lin et al., 2007; Gavin et al., 2007) provide strong evidence that Foxp3 is not responsible for the early development

of the Treg lineage because Treg cells derived from mice with a disabled Foxp3 gene express many, but not all, of the phenotypic and functional properties of normal Treg cells. Thus, the signal(s) responsible for the initiation of Treg cell development remains unknown, and it remains possible that one result of commitment to the Treg cell lineage is an increased resistance to negative selection in the thymus resulting in a population of Treg cells with a TCR repertoire similar to Tconv cells but with slightly higher affinity to self. The self-reactivity might be below the threshold detectable in a T cell hybridoma. Although the studies of Pacholczyk et al. (2007) indicate that the T cells that initiate wasting disease recognize nonself, it is premature to conclude that the Treg cells that protect from wasting disease also exclusively recognize nonself. Self-specific Treg cells might play a critical early role in preventing disease by reacting to enhanced amounts of self-peptide MHC class II and mediating bystander suppression. This model does not exclude the possibility that foreign antigen-specific Treg cells play a complementary role in protection.

Lastly, one must also consider the implications of these studies of TCR repertoire to vaccination. In contrast to the well-described in vitro anergic state of Foxp3$^+$ Treg cells, they proliferate and expand as efficiently as do Tconv cells after in vivo priming. Because the TCR repertoires of Treg and Tconv cells are certainly capable of recognizing any foreign or pathogen-derived antigen, it is likely that vaccination will result in proliferation of both populations. Because the marked proliferation of Treg cells in the tumor-bearing host can be secondary to their higher affinity for tumor (self-) antigens, tumor vaccines can result in further proliferation of the tumor-specific Treg cells. Manipulation of the balance between Treg and Tconv cells in response to vaccination remains a challenge for the future.

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Crosspresentation: Plasmacytoid Dendritic Cells Are in the Business

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DOI 10.1016/j.immuni.2007.08.006

Crosspriming and crosspresentation are performed by specialized subsets of dendritic cells. In this issue, Hoeffel et al. (2007) show that human plasmacytoid dendritic cells can crosspresent HIV-derived peptides conjugated to a lipopeptide or HIV-infected cells undergoing apoptosis.

"Exogenous antigens are loaded onto major histocompatibility complex (MHC) class II molecules, whereas endogenous antigens are loaded onto MHC class I molecules" is a golden rule with notable exceptions. Pioneering work showed that antigen-specific CD8$^+$ T cells expand in vivo when small amounts of exogenous antigen are delivered together with dead or dying cells, a phenomenon designated crosspriming or crosspresentation for memory responses. A few years later, several groups demonstrated that a small fraction of dedicated dendritic cells (DCs) within the DEC205$^+$CD8α^+ subset performs crosspriming (Bevan, 2006). Since then, the relevance of crosspriming in immune responses has been the subject of extensive investigation. Certainly, extending our knowledge of the key players and molecular mechanisms involved in crosspriming and crosspresentation might allow the experimental manipulation of this pathway for therapeutic intervention in cancer and autoimmunity. In this issue of *Immunity*, Hoeffel et al. (2007) show that human plasmacytoid DCs (pDCs) can crosspresent HIV-derived antigens.

Under steady-state conditions, crosspresentation provides tolerance