

## Pathogen-Sensing and Regulatory T Cells: Integrated Regulators of Immune Responses

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### Abstract

We present the concept that pathogen-sensing and regulatory T cells (Treg) mutually regulate immune responses to conventional and tumor antigens through countervailing effects on dendritic cells (DC). Normally, conventional CD4 T cells recognizing their cognate antigen presented by a DC will respond only if the DC also receives a signal through its pathogen-sensing/danger/adjuvant recognition systems (the pathogen-sensing triad). However, in the absence of Tregs capable of interacting with the same DC, DCs are competent to present antigens, both foreign and self, even without the stimulation provided by the pathogen-sensing triad. Tregs recognizing an antigen presented by the DC that is also presenting antigen to a conventional CD4 T cell will prevent the activation of the CD4 T-cell responses, but a signal delivered by a member of the pathogen-sensing triad will overcome the inhibitory action of Tregs, thus allowing CD4 T-cell responses to go forward. These considerations take on special meaning for responses to "weak antigens" such as many of the antigens displayed by spontaneous human tumors. *Cancer Immunol Res*; 2(6); 503–9. ©2014 AACR.

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### Learning Objectives

Dendritic cells (DC) are principle antigen-presenting cells to T cells for adaptive immune responses, and DCs have receptors for sensing pathogens and danger signals for innate immunity. Understanding the countervailing effects of pathogen-sensing and regulatory T cells (Treg) on DCs is necessary to improve immune responses against weak antigens such as tumor antigens. Upon completion of this activity, the participant should gain a basic knowledge of the roles of Tregs and the pathogen-sensing triad in regulating immune responses.

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### Introduction

Modern immunology can be dated from two discoveries in the 1880s that opened the fields of adaptive and innate immunity, respectively. In 1880, Louis Pasteur reported that inoculation of an attenuated form of a bacterium into chickens protected them against infection with a virulent form of the same organism (1), and in 1882 Élie Metchnikoff discovered the phenomenon of phagocytosis in observing the response of starfish larvae to a foreign body (2).

For more than 100 years, the fields of innate and adaptive immunity developed largely independently of each other. There was, of course, always the recognition that there was some relationship between them, perhaps most clearly demonstrated by the requirement for adjuvants to be used with immunogens to obtain a robust response, although there was little notion of what adjuvants actually did (3). Then, in 1989, Charles Janeway proposed a hypothesis to link these two arms of immunity (4) in a lecture that continues to reverberate even today.

### Pathogen-Sensing

Janeway argued that the occupancy of T-cell receptors (TCR) or B-cell receptors (BCR) by their cognate ligands would not elicit a robust *in vivo* immune response unless an additional signal was provided to the responding lymphocytes. That signal would be generated by another cell that had sensed the presence of a pathogen. The process of pathogen-sensing in the

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Janeway concept depended upon the existence of receptors (Janeway called them pathogen recognition receptors; PRR) that recognized constituents of pathogens so central to the survival or function of the pathogen that they could not be dispensed with; these constituents were designated pathogen-associated molecular patterns (PAMP).

The Janeway model of PAMP/PRR interaction as essential to mounting immune responses was extended in 1994 by Polly Matzinger (5). She proposed that inflammatory responses or certain types of cell death could provide endogenous stimuli that she designated "danger" signals. Such danger signals would cause responses similar to those achieved by the sensing of pathogenic microbes.

While in 1989, these were speculative ideas, beginning in 1996 real molecules were discovered that were PRRs and PAMPs. In 1996, there was only a single sensor (Toll in the fruit fly; ref. 6) and no PAMP as *Drosophila* Toll bound to the endogenous molecule, spaeztle, which is cleaved to achieve its active form by proteases that sense virulence factors, more in keeping with the Danger model. Toll was then shown to have a human homolog (a Toll-like receptor; TLR) by Medzhitov and colleagues, who demonstrated that cross-linking this TLR activated nuclear factor  $\kappa$ B (NF- $\kappa$ B; ref. 7). However, they still did not have a ligand for TLR and thus could not be sure that it was sensing a PAMP. Then, Poltorak and colleagues demonstrated that mutations in the mouse version of the Medzhitov–Janeway TLR, then identified as TLR4, were responsible for the inability of two strains of mice to respond to bacterial lipopolysaccharide (LPS), thus implying that LPS, a component of the outer membrane of gram-negative bacteria, was the PAMP for the PRR TLR4 (8).

Today, the microbial sensors and the microbial products they identify are legion. There are both cell membrane and cytosolic PRRs, and the PRRs are members of several distinct families, including the TLRs, the nucleotide-binding oligomerization domain (NOD)–like receptors (NLR), and retinoic acid–inducible gene 1 (RIG-I)–like receptors (RLR; ref. 9). They mediate their functions by a variety of mechanisms, including the direct activation of inflammatory pathways, such as the NF- $\kappa$ B system, as well as the production of mature forms of proinflammatory cytokines such as IL1 $\beta$  and IL18. An important target cell of pathogen-sensing is the dendritic cell (DC), the principal cell that presents antigen to T cells (10).

### Regulatory T Cells

The pathogen-sensing revolution in the study of the immune system is mirrored by a second revolution in modern immunology, the recognition of the key role that regulatory T cells (Treg) play in the immune response. The time frames of the two "revolutions" are quite similar. The modern study of Tregs can be dated to the pioneering work of Sakaguchi and colleagues (11), who recognized that the autoimmune responses that appeared in mice that had been thymectomized at 3 days of age could be explained by the failure of Tregs to gain access to the periphery before day 3 and thus to the largely unopposed action of conventional T cells presumably specific for self-antigens. These 3-day thymectomized mice developed a wide

range of autoimmune phenomena, including thyroiditis, oophoritis, and gastritis. In 1982, Sakaguchi and colleagues (11) showed that transfer of CD4 T cells from adult mice into the 3-day thymectomized animals prevented these autoimmune diseases, implying that there was a suppressive or regulatory population in normal mice that was lacking in the 3-day thymectomized animals. Sakaguchi and colleagues also showed that the Tregs expressed large amounts of CD25, the  $\alpha$  chain of the IL2 receptor, thus providing a tool to identify them (12).

In 2000 and 2001, it was discovered that the human immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome (13, 14) and the scurfy abnormality in mice (15) were due to mutations in the transcription factor forkhead box P3 (Foxp3), and in 2003 that in the absence of Foxp3, Tregs did not appear (16–18). These observations placed the study of the function of Tregs on a sound footing. The immunologic defect in patients with IPEX and in scurfy mice is the absence of Tregs. The consequences of such absence are disastrous. In mice, the absence of Tregs leads to death by week 3 of age; in humans, survival chances are best with bone marrow transplantation.

While the modes of action of Tregs are still hotly debated and are almost certainly multiple, an important pathway is the repression of DC activation (19).

### Integrating Pathogen-Sensing and Tregs

In this article, we argue that pathogen-sensing and Tregs jointly regulate immune responses through countervailing effects on DCs. In the absence of Tregs, DCs are competent to present antigens, both foreign and self, even without the stimulation provided by pathogen-sensing or danger signals or their analogue, adjuvant action (for simplicity, we often refer to this triad as "pathogen-sensing"). However, if Tregs are present, then pathogen-sensing is essential if a productive response is to be achieved. These considerations take on special meaning for responses to "weak antigens" such as many of the antigens displayed by spontaneous human tumors.

As we pointed out, immune responses to purified protein or polypeptide antigens require the use of an adjuvant. Indeed, the founding chief of the National Institute of Allergy and Infectious Diseases (NIAID) Laboratory of Immunology, Jules Freund, pioneered in adjuvant development with his introduction of both complete and incomplete Freund's adjuvants (20). The requirement for adjuvants was the "immunologist's dirty little secret" (21), tacitly referring to the apparent contradiction with the basic theory of immunology, clonal selection, that called for responses whenever mature lymphocytes encounter their cognate antigens (22, 23).

However, there is a circumstance in which adjuvants are not required for robust T-cell responses to foreign antigens, that is, when lymphocytes are introduced into a lymphopenic environment. It is well known that transferring polyclonal T cells into mice that lack T cells, such as recombination-activating gene (Rag)1- or Rag2-deficient mice or mice in which components of the TCR have been disrupted, results in the rapid proliferation of some of the transferred cells (24). This "lymphopenia-induced proliferation" reflects T-cell recognition of

antigens, possibly self-antigens or antigens of the microflora of the recipient individual (25, 26). As we discuss later, only a small proportion of the transferred cells participate in this proliferation, presumably reflecting those cells with the highest affinity for the available antigens.

A more direct way of demonstrating the capacity of T cells to proliferate in response to antigen without requirements for adjuvants is to transfer T cells from TCR-transgenic donors into lymphopenic recipients. In such a transfer model, most CD4 TCR-transgenic T cells do not undergo lymphopenia-induced proliferation, that is, proliferation without the introduction of their cognate antigen. This presumably reflects the requirement that lymphopenia-induced proliferation is a property only of cells with high affinity for self- or microflora-antigens, and most TCR-transgenic T cells do not have such affinity for resident antigens. However, the introduction of a synthetic peptide representing the epitope for which the TCR-transgenic cells are specific induces the transferred cells to undergo robust expansion. Indeed, in a model in which TCR-transgenic T cells specific for a cytochrome C peptide (5C.C7 cells) are transferred into Rag1<sup>-/-</sup> B10.A recipients that are then immunized with the synthetic cytochrome C peptide, a response occurs that far exceeds that of the same number of cells transferred into an intact recipient immunized with the same peptide in the presence of LPS. Furthermore, providing LPS to the lymphopenic recipient of 5C.C7 cells only modestly increases their response to the cytochrome C peptide (J. Quiel, J. Milner, and W.E. Paul; unpublished data).

What property of the lymphopenic environment allows this adjuvant-independent response to occur? That is not completely clear, but we have shown that if a large number of polyclonal Tregs are present in the lymphopenic recipient, then conventional CD4 T cells (Tconv) do not undergo lymphopenia-induced proliferation when they are introduced into the same animals (27). This strongly implies that it is the absence of Tregs in the lymphopenic animal that allows the antigen-responsive CD4 T cells to proliferate in response to their cognate antigen.

On this basis, we have proposed the following model (28). Naïve CD4 T cells encountering DCs that express a peptide/MHC complex for which the CD4 TCR have high affinity will respond to that stimulation. Among their responses is the upregulation of the CD40 ligand, which, interacting with the costimulatory protein CD40 on the DC, results in DC activation. The continued interaction between the now activated DC and the responding CD4 T cell leads to a robust T-cell proliferative response and the differentiation of the responding T cells to an effector or a memory state; this response is symbolized by the designation "On" for activation of the DC and for a T-cell response in Fig. 1A, which illustrates the interaction of a Tconv and a DC, in which the DC presents an antigen for which the TCR of the Tconv is specific, and the curved arrow indicates the activation of the DC by the Tconv.

The presence of a Treg that also recognizes a peptide/MHC complex on the same DC will prevent the DC from becoming activated and thus restrain T-cell activation, designated as "Off" for no DC activation or T-cell response in Fig. 1B. Thus, little or no response occurs in a system in which a full range of

Tregs exists. However, if the DC receives a signal through its pathogen-sensing receptors, it will then become activated even in the presence of Tregs. The activated DC will then stimulate the specific Tconv to undergo its full range of responses, again designated as "On" in Fig. 1C, in which the PAMP is LPS and thus the PRR would be TLR4.

Thus, the DC acts as the determinant of whether responses go forward. Its activation status depends upon three factors: a Tconv specific for a peptide/MHC complex expressed by the DC; a Treg specific for a peptide/MHC complex expressed by the DC; and a signal delivered through pathogen/danger/adjuvant sensing. Given the primary recognition event, depending on which of the other two factors is also engaged, the DC will either become activated or remain quiescent, and correspondingly a response of the Tconvs will occur or will fail.

Before exploring the implications of these proposals, it is necessary to point out that Tregs are not limited in their functionality to blocking initial T-cell responses in secondary lymphoid organs. They have been shown to mediate a wide variety of functions both in sites in which T cells are primed and in tissue sites where effector functions are mediated (19). However, we argue that the evolutionary pressure that led to their initial development was the need to control unwarranted primary T-cell responses and that, once evolved, these Treg cells took on additional very valuable functions.

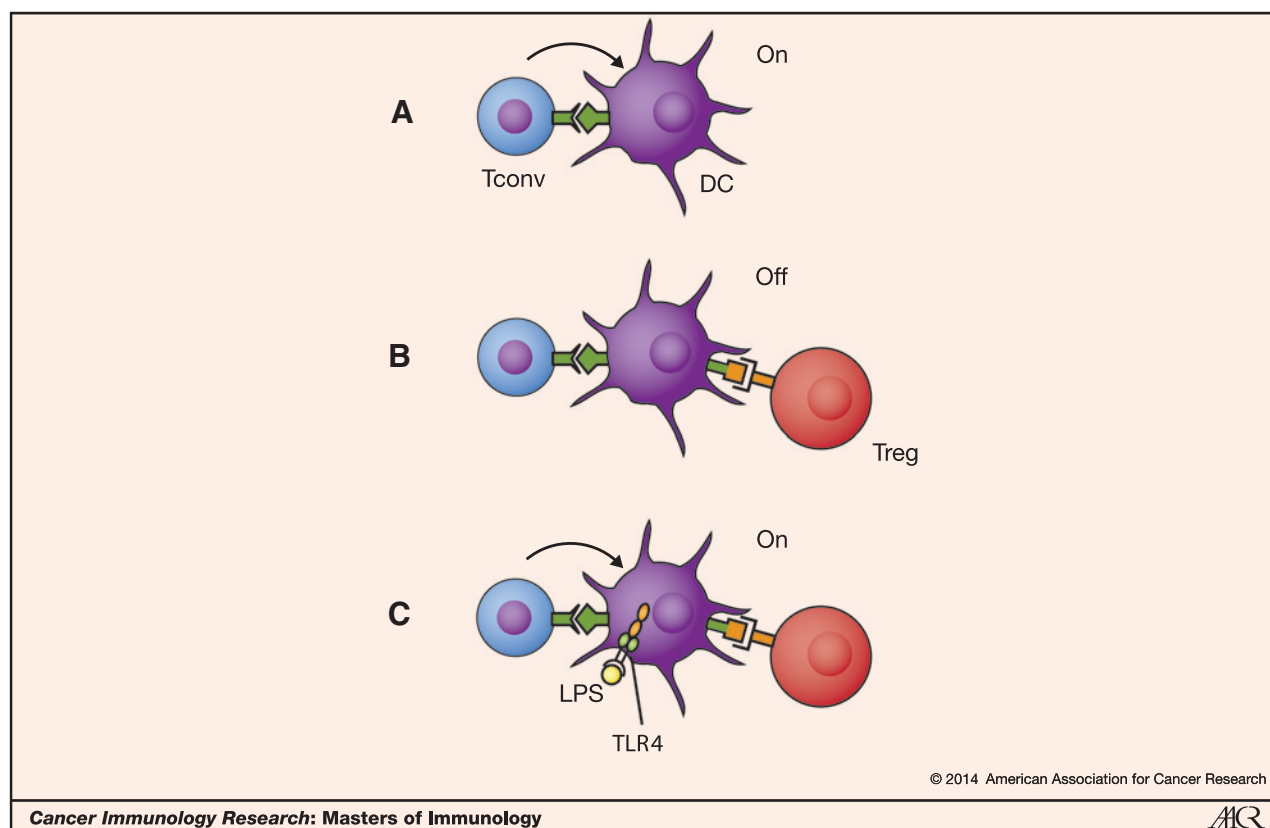
## Effect of TCR Repertoire on Immune Responses

### Consequences of a limited TCR repertoire

Humans who have limited TCR repertoires often display severe autoimmune/autoinflammatory responses. The best examples are patients with Omenn syndrome, which occurs in individuals that produce limited numbers of T cells (29). The most common cause of Omenn syndrome is mutations that impair the recombination mechanism through which TCRs and BCRs are generated, such as hypomorphic mutations in Rag1 or Rag2 (30). Individuals bearing such mutations produce relatively few T cells in the thymus, and those T cells that escape to the periphery express an activated phenotype. Infants born with this disorder display a severe eosinophilic inflammation that affects many organs. Patients with Omenn syndrome require hematopoietic stem cell transplantation to survive.

One interpretation of the abnormal proliferation/activation of these patients' peripheral T cells is that their TCRs are of limited heterogeneity. We argue that this is the major reason for the autoimmunity of these infants. However, it should be noted that in many patients with Omenn syndrome, there is a second important abnormality, the failure of the thymus to develop normally (31). Thymic development requires an interaction between the epithelial cells of the thymus and the lymphoid compartment. When T-cell development is impaired, the thymus forms abnormally. This is important because negative selection in the thymus requires the participation of medullary thymic epithelial cells (32); if they are absent or diminished in number, then autoreactive cells may escape to the periphery.

The concept that limited repertoires might play an important role in the activation of T cells in the periphery led



**Figure 1.** A, in the absence of Tregs, a Tconv interacting with a DC that presents an antigen for which the TCR of the Tconv is specific will undergo a response. This response is mediated by activation of the DC by the conventional CD4 T cell (indicated by the curved arrow) and the reciprocal activation of the CD4 T cells by the activated DC. In such a situation, the activation of the DC is designated by its being "On." B, in the presence of a Treg that also recognizes a peptide/MHC complex on the same DC, the DC is prevented from becoming activated, thus restraining T-cell activation. The DC is designated as "Off" and no DC activation or T-cell response occurs. C, if the DC receives a signal through its pathogen-sensing receptors (here LPS stimulating TLR4), it will then become activated even in the presence of Tregs. The activated DC will then stimulate the specific conventional CD4 T cell to undergo its full range of responses. Thus, the DC is "On." Adapted from Paul et al. (28).

Booki Min and Josh Milner (see refs. 27 and 33), working with us, to ask whether comparable abnormalities occurred in mice whose T cells had a limited repertoire but in which these T cells underwent normal negative selection. The first experiments were done by Min and colleagues (33). They transferred varying numbers of polyclonal CD4 T cells into lymphopenic recipients. The animals received from  $10^5$  to  $10^7$  cells. After the transferred cells had been in residence for several weeks, they measured the number of T cells and their TCR diversity. No matter how many cells were initially transferred, the recipients had essentially the same number of cells at equilibrium, and the vast majority had a memory/effector phenotype. The finding that steady-state cell number was largely independent of the numbers of transferred cells is consistent with the concept that there is a fixed "niche" for cells of given types (in this case, memory phenotype CD4 T cells) and that expansion will cease when the niche is filled (24). However, when we estimated the diversity of the receptors on the T cells, using spectratype analysis (34) to evaluate the distribution of lengths of the hypervariable complementary-determining region 3 and thus the complexity of the TCR $\beta$  chains, we observed a major difference between mice that received differing numbers of

transferred cells. Mice that received  $10^7$  cells had a TCR diversity that was far greater than those that received  $10^5$  cells (33). Thus, we had created mice whose TCR repertoires were very different in their degree of complexity, but in which the problem of the failure to have undergone negative selection was avoided.

We then transferred a fixed number of polyclonal CD4 T cells into each of the recipients. We observed that mice that had initially received  $10^7$  cells and had a broad repertoire did not support the proliferation of the newly transferred cells, whereas mice that had initially received  $10^5$  cells and had a narrow repertoire allowed the newly transferred CD4 cells to undergo lymphopenia-induced proliferation. Because the steady-state number of CD4 T cells in recipients that had received  $10^7$  or  $10^5$  cells was the same, the critical difference between the two sets of recipients was not the numbers of CD4 T cells they possessed but the repertoire complexity of these cells.

Milner and colleagues followed up these experiments with an extended analysis of lymphopenic mice that received either  $3 \times 10^4$  or  $2 \times 10^6$  polyclonal CD4 T cells (27). They found that mice that had received  $2 \times 10^6$  CD4 T cells remained healthy,

but those that had received  $3 \times 10^4$  cells developed a severe pneumonitis dominated by the presence in the lung of eosinophils and type II macrophages. These animals succumbed to lung infiltration with immune cells. Thus, limited TCR repertoires are, by themselves, potentially causative of severe autoimmune and/or autoinflammatory responses.

In view of our demonstration that Tregs could prevent lymphopenia-induced proliferation of Tconvs, we asked whether it was the repertoire complexity of the Tregs that determined whether the conventional T cells would undergo lymphopenia-induced proliferation.

For this study, we prepared mice that differed in the complexity of their Treg population (27). This was done by transferring  $3 \times 10^4$  or  $3 \times 10^5$  Tregs into lymphopenic recipients. The transferred Tregs expanded to reach the same number in both sets of recipients but, as we showed in the transfer of Tconvs, recipients of  $3 \times 10^4$  Tregs would have a more limited Treg repertoire than that in the recipients of  $3 \times 10^5$  Tregs. Both sets of mice were then challenged by the transfer of polyclonal Tconvs, and their capacities to allow the transferred Tconvs to proliferate were compared with each other, and also to expansion of Tconvs in lymphopenic mice that had not received any Tregs.

Mice that had received no Tregs were permissive for a robust proliferation of the newly transferred Tconvs, whereas mice that had earlier received either  $3 \times 10^4$  or  $3 \times 10^5$  Tregs severely limited the expansion of the transferred Tconvs. However, a comparison of those that had received the larger number of Tregs with those that had received the smaller number showed that, although the total number of Tregs at the time of the second transfer was the same, there was much more proliferation of Tconvs in the recipients of  $3 \times 10^4$  Tregs than in the recipients of  $3 \times 10^5$  Tregs. We interpreted these results to indicate that the complexity of the Treg repertoire determined the effectiveness of those Tregs in limiting the expansion of the transferred Tconvs in a lymphopenic setting.

#### **Do Tregs and Tconvs have similar repertoires?**

Although relatively little direct data exist on this issue, there are some experimental results and theoretical considerations to argue that the repertoires of Tconvs and Tregs are different. The classical view is that Tconvs undergo two types of selection in the thymus. In the cortex, in response to antigens presented by cortical thymic epithelial cells, CD4 thymocytes must be able to bind to self-peptide/MHC complexes with some basal affinity if they are to survive. Thus, those T cells that have little or no self-peptide/MHC specificity are filtered out and do not go forward to the next stage, which involves migration to the thymic medulla. Here, T cells with high affinity for self-peptide/MHC complexes presented by medullary thymic epithelial cells undergo programmed cell death, purging the conventional T-cell pool of many autoreactive cells (32). Tregs, while probably requiring cortical positive selection, are able to tolerate a higher degree of self-reactivity in the medulla (35). Indeed, it may be that they are generated in response to high-affinity interactions, as an alternative to programmed cell death.

Thus, Tconvs are selected to have a low-to-moderate degree of self-reactivity, whereas Tregs are selected to have substantial self-reactivity. This would imply that Tregs mediate their function in the periphery not by responding to foreign-peptide/MHC complexes, as Tconvs do, but rather by being activated by self-peptide/MHC complexes. Indeed, in an elegant experiment, Hsieh and colleagues provided evidence that the range of TCR $\alpha$  chains used by Tregs and Tconvs was not identical (36).

The notion that the repertoires of the two cell types are different leads to the concept that for inhibition of DC activation to occur, the conventional T cell and the Treg need not recognize the same peptide/MHC complex on the DC, but rather that the Treg must recognize a peptide/MHC complex displayed by the same DC that presents a peptide/MHC complex to a conventional T cell (see Fig. 1B).

#### **Why should limited Treg repertoire diminish their efficacy?**

Why should the complexity of the TCR repertoire of Tregs matter? If each DC presented the full range of self-peptide/MHC complexes, it might be expected that even when the repertoire of Tregs was limited, they should still recognize their "cognate" peptide/MHC complex on all DCs and thus should prevent the DC from becoming activated.

In our initial presentation of this concept, we argued that the simplest explanation for the failure of Tregs of limited repertoire to fully control the expansion of Tconvs was that Tregs of limited repertoire would fail to block the activation of a subset of DCs, that is, DCs that did not present a peptide/MHC complex that any of the limited repertoire Tregs could recognize. This notion, therefore, suggested that DCs were not uniform in their antigen-presenting function. Each DC would display on its surface peptide/MHC complexes at sufficient concentration to effectively activate Tregs specific for only some of the full set of self-peptide/MHC complexes. Probably, and for purely stochastic reasons, peptides derived from highly expressed self-antigens would be expressed by all DCs, but peptides derived from self-antigens that were expressed at substantially lower levels might be effectively presented by only a subset of DCs.

There are alternative explanations that need consideration. For example, as Treg repertoires become limited, but the total number of Tregs remains similar, the frequency of Tregs expressing any particular TCR will be greater. These Tregs may compete with one another and therefore may be less active on a per-cell basis. Alternatively, Tregs that recognize a peptide/MHC complex expressed at high levels may be absent when repertoires are limited, and thus a strong Treg-activating signal may be lost. In these models, the key element would not be the variability in antigen presentation by the DCs but rather the lower degree of activity of the Treg population as a whole and thus a proportionate reduction in their efficacy of inhibition of DCs.

Direct tests of whether DCs vary in their ability to activate Tregs need to be carried out. Although this is a challenging problem, there are approaches that promise to test this concept.

### Why do only some Tconvs participate in lymphopenia-induced proliferation?

The concept that all Tconvs are self-reactive (i.e., they had to be positively selected in the thymus) and that in the absence of Tregs the interaction of DCs and T cells specific for a peptide/MHC complex presented by the DC will lead to DC and T-cell activation might imply that in the absence of Tregs all Tconvs would undergo proliferative/differentiation responses. We argued earlier that this was not the case. We indicated that only those CD4 T cells with relatively high affinity for a self-peptide/MHC complex will respond. Can we estimate the frequency of cells with sufficient self-reactivity to respond in the absence of Tregs?

We have conducted two types of experiments that give some information on this subject. In the first, we transferred  $10^4$  to  $10^7$  mainly naïve CD4 T cells into lymphopenic recipients and measured the complexity of the TCR $\beta$  chain response using spectratype analysis (33). We observed that when  $10^4$  cells were transferred, among most V $\beta$  types, all the TCR $\beta$  chains had the same TCR length. This strongly implies that there was but one clone per V $\beta$  type and thus approximately 20 clones total. Because only approximately 10% of transferred cells established themselves functionally within the recipients, when  $10^4$  cells were transferred, the "functional" 1,000 cells gave rise to 20 clones, a frequency of approximately 2%.

A second approach that we used to test the frequency of memory phenotype cells undergoing proliferation in lymphopenic recipients was to compare cells that had not divided and cells that had divided approximately 7 times for the complexity of their TCR V $\beta$  repertoires among the set of cells that expressed a V $\beta$ 2/J $\beta$ 1.1 TCR $\beta$  chain (37). We chose to limit ourselves to that group of T cells so that we could sample a substantial proportion of all expressed V $\beta$  chains with a relatively small number of sequences. Our result was that the complexity of the V $\beta$  pool among the proliferating cells was approximately 1/10 that of the cells that had not divided, implying that approximately 10% of memory phenotype cells could undergo lymphopenia-induced proliferation. Although the two estimates do vary, this can probably be accounted for by the fact that one is an estimate of "self-reactivity" mainly among naïve cells and the other among memory phenotype cells.

One possibility for the low frequency of Tconvs that proliferate in the absence of Tregs is that only those cells that have sufficiently high affinity for self-peptide/MHC complexes displayed by DCs respond. An alternative, that we favor, is that self-reactivity is controlled in the periphery by responsiveness tuning (38), a phenomenon that we believe underlies T-cell anergy. Peripheral Tconvs all have a degree of self-reactivity, that being a condition for their positive selection in the thymus.

Furthermore, survival of naïve CD4 T cells in the periphery depends upon their periodic interaction with the selecting self-peptide/MHC complex. These repetitive interactions "tune" the TCR signaling complex so that such interactions are subthreshold, sufficient to send a survival signal but not to lead to full activation, thus explaining why most CD4 T cells are unresponsive upon transfer into lymphopenic settings. The approximately 2% of naïve CD4 T cells that can respond may do so either because of incomplete tuning or because of abrupt stimulation of DCs by Tconvs and vice versa, leading to an increased expression of class II MHC or of costimulatory molecules, resulting in a super-threshold signal. The generally low affinity of autoreactive interactions contributes to the low frequency of such events.

Thus, the CD4 T-cell response is a finely controlled one, in which Tregs of a broad repertoire restrain DC activation. Even when Treg function is incomplete, most T cells will still not undergo activation because their tuned state renders the stimuli subthreshold. When pathogen/danger/adjuvant-induced signals are provided, CD4 T cells respond strongly to their cognate foreign antigen, but only a subset of self-reactive cells respond and, even for these, the response probably ends when the signal generated by pathogen-sensing terminates.

### Relevance to Antitumor Responses

It is now clear that tumors have evolved a variety of strategies to blunt the force of immune responses and thus to protect themselves against immune attack. The discussion of the variety of mechanisms that exist within the tumor microenvironment to achieve this blunting is well beyond the scope of this article. However, to the extent that many tumor antigens represent normal antigens presented at high levels or relatively minor mutational modifications of self-antigens, the concepts of mutual regulation of responses by the action of Tconvs, Tregs, and pathogen/danger/adjuvant-sensing applies with special force to many antitumor responses. The insights that derive from understanding this finely balanced system may contribute significantly to a more effective utilization of the developing set of new approaches to enhance the robustness and efficacy of antitumor immunity.

Our discussion is particularly relevant because of accumulating knowledge about the high frequency of Tregs in the tumor bed of many malignancies (39). The importance of the ratio of Tregs and effector T cells in patients receiving immunotherapy emphasizes the need to understand how Treg function integrates with that of pathogen-sensing in determining whether a robust T-cell response will go forward (40).

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