

Regulatory T Cells: Context Matters

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DOI 10.1016/j.immuni.2009.04.007

Regulatory T cells are important for ensuring that the immune system does not attack self and does not over-react to external antigens. Understanding how these cells develop and maintain stable function provides general insights into cellular differentiation in general, as well as new opportunities for therapeutic manipulation.

Introduction

The immune system protects us against infectious pathogens and has evolved to do this while minimizing damage to self-tissues. Self-tolerance is enabled by deletion, within the primary lymphoid organs (marrow and thymus) of lymphocytes with self-reactive receptors. It has become clear in the past 20 years that active regulation mediated by CD4⁺ T cells is also necessary.

Understated experiments some 20 years ago (Hall et al., 1985; Kong et al., 1989; Powrie and Mason, 1990; Qin et al., 1993; Sakaguchi et al., 1982) demonstrated that CD4⁺ T cells could, in certain circumstances, prevent damaging immune reactions. The importance of these early studies was not properly recognized because the putative regulatory cells could not be experimentally separated from CD4⁺ T cells with immune function. The first operational handle on regulatory T (Treg) cells was CD25, a chain of the IL-2 receptor (Sakaguchi et al., 1995), although it proved to be far from perfect, given that this receptor is expressed on activated T cells. A set of thymus-derived “natural” regulators were defined on the basis of expression of CD25 in a resting immune system. Just as for CD4⁺ and CD8⁺ T cell differentiation, the assumption was that these cells should be considered as a CD4⁺ lymphocyte subset. The finding that CD4⁺CD25⁺ T cells could suppress T cell proliferation in vitro (Thornton and Shevach, 1998) led to the widespread adoption of this as an assay to measure functional Treg cells in lymphocyte populations.

The next major breakthrough, effectively the “clincher” for any remaining disbelievers, was the finding that patients with IPEX syndrome, a disease with diverse immunopathologies, carried mutations in

the gene for the transcription factor FoxP3 (Chatila et al., 2000). Murine studies clearly implicated FoxP3 as the transcription factor needed to direct CD4⁺ T cells to become Treg cells (Fontenot et al., 2003; Hori et al., 2003; Khattry et al., 2003).

The last important piece of the regulatory T cell jigsaw came with the finding that Foxp3⁺ Treg cells could also be induced outside of the thymus through signals mediated via the T cell receptor (TCR) in conjunction with an extrinsic source of the cytokine TGF- β (Chen et al., 2003). Moreover, TGF- β was found to be necessary for induction of peripheral tolerance and induction of FoxP3⁺ Treg cells in vivo (Cobbold et al., 2004; Daley et al., 2007). The subsequent demonstration that TGF- β was also needed to convert naive T cells to other functional phenotypes (Th17 and Th9 cells) (Bettelli et al., 2006; Veldhoen et al., 2006; Veldhoen et al., 2008) opened up a much-needed discussion on what factors determined functional polarization of T cells, how stable that differentiated state was, and the extent to which any T cell could exhibit the plasticity to change function. Obviously, the issue of plasticity is a critical one for those wishing to apply in vitro-expanded Treg cells as a personalized form of treatment for autoimmune diseases.

Challenging Questions in the Field

In this issue of *Immunity*, the series of review articles on FoxP3⁺ Treg cells are timely and offer an appraisal of the main issues introduced above. They address the following questions: What are the differences between natural Treg cells and induced ones? How do these Treg cells develop? What factors determine the stability or plasticity of Treg cells? At what stages of an immune response can Treg cells act, and where might they do

this? Are there specialized microenvironments that favor and reinforce their generation and maintenance? How do Treg cells regulate immune function? Can we harness them as therapeutic agents in their own right?

Josefowicz and Rudensky (2009) assess the relative contributions of natural, thymic-derived Treg (nTreg) cells and periphery-induced Treg (iTreg) cells to the functional regulatory cell repertoire and argue for somewhat different signaling needs mediated through the TCR that favor development of each. Their analysis of the influential transcription factors, and other inductive conditions for iTreg cells, draws attention to the need for a supportive microenvironment (e.g., gut-associated lymphoid tissue) for their generation. They also analyze carefully those factors that can determine stability of Treg cell phenotype and Foxp3 expression in vivo, with prospects for pharmacological enhancement of those epigenetic mechanisms to ensure optimal function.

Zhou et al. (2009) focus specifically on the issue of plasticity and the extent to which Treg cells and Th17 cells can change their functional commitment as a result of particular cytokine milieus or through competing transcription factors and other antagonistic mediators. Their analysis of epigenetic mechanisms for fixing cell function indicate that within seemingly polarized cell populations, there still remain cells poised to “turn” if the right conditions are met.

Curotto de Lafaille and Lafaille (2009) examine the division of labor between nTreg and iTreg cells. They highlight examples in which optimal outcomes require both and describe situations in which one suffices. iTreg cells, for example, can sometimes be sufficient to establish

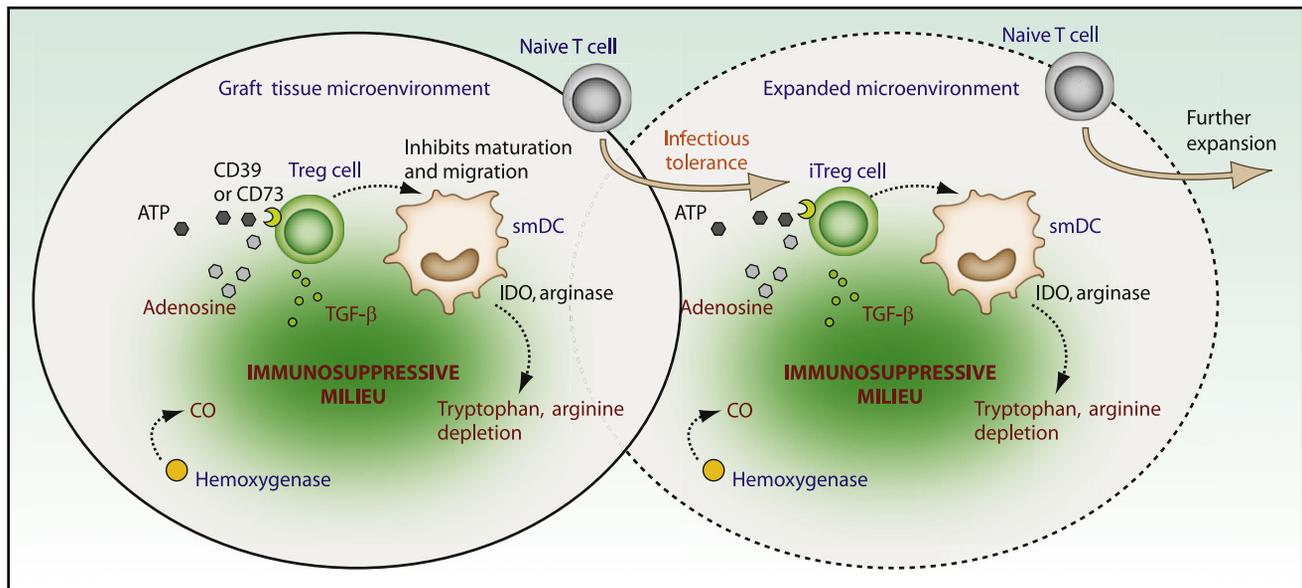


Figure 1. Amplification of the Regulatory Microenvironment

Treg cells migrate to sites within the grafted tissue. Activated Treg cells can convert ATP released by inflamed tissues to adenosine via the ectoenzymes CD39 and CD73 (Deaglio et al., 2007). Local adenosine contributes to the initial immunosuppressive milieu. Treg cells also inhibit the maturation and migration of dendritic cells (DCs), and these “semi-mature” (sm) DCs express Indoleamine 2,3-dioxygenase (IDO) and arginase, which locally deplete tryptophan and arginine, and further amplify the tolerogenic microenvironment (Mellor et al., 2004). The induction of hemoxygenase (HO) by the induction of tissue-protective responses also generates locally immunosuppressive carbon monoxide (CO) (Soares et al., 2001). Each of these components within the microenvironment, some in synergy with TGF- β , further reinforce the local anti-inflammatory state such that any naive T cell that migrates into this area is converted, via infectious tolerance, to an induced (iTreg) cell. The iTreg cell then acts to expand and yet further amplify the tolerogenic milieu.

tolerance in their own right and can reduce inflammation and allow remodeling of tissues. They can also be an impediment to vaccination and cancer therapy. Possible explanations for differential function between the two types are discussed and the issue of context of antigen exposure (tissue microenvironments) is raised as a missing factor in understanding how these cells function.

Shevach (2009) discusses how Treg cells exert their suppressive function. Although an initial proponent of direct T-T suppression at the inductive stage, Shevach now considers decommissioning of antigen-presenting cells as a realistic alternative and offers a cautious assessment of in vitro systems that not adequately reflect the function of Treg cells in vivo.

Finally, Riley et al. (2009) assess the merits and risks of using adoptive transfer of expanded regulatory T cells for the treatment of autoimmune diseases and in hemopoietic transplantation. Even if Treg cells have failed to prevent an autoimmune disease, the processes involved in their selective in vitro expansion may correct any deficit. Progress in defining good manufacturing practice (GMP) conditions

for such therapies and in minimizing possible risks is outlined.

A clear message emerging from all these articles is that Treg cells play an important role in the immune system and have the ability to exert very powerful control of innate and adaptive immune functions. That potency is no better exemplified than in the arena of transplantation tolerance.

How Do Treg Cells Operate in Transplantation Tolerance?

The capacity of Treg cells to prevent graft rejection has not only demonstrated the enormous potency of Treg cells but has also provided convenient experimental readouts to test mechanism and to manipulate Treg cells for therapeutic purposes. Short pulses of therapeutic nonablative antibodies directed to the T cell coreceptors CD4 and CD8 can produce long-term acceptance (Cobbold et al., 2004; Qin et al., 1993) and tolerance of genetically mismatched grafts. Tolerance is dependent on the activities of both natural and induced Treg cells and is dependent on TGF- β signaling to T cells (Cobbold et al., 2004; Daley et al., 2007). These Treg cells are potent enough to mediate

“linked suppression” toward third-party antigens, in the same tissue as the tolerated set. “Linked suppression” implied that Treg cells mediated suppression within the context of the antigen-bearing tissues and led to the discovery of Treg cells in tolerated tissues (Graca et al., 2002). The impact of the cohort of Treg cells brought into action by therapy goes well beyond those cells, given that new cohorts of Treg cells are continuously recruited over time (Qin et al., 1993). This process that we have coined “infectious tolerance” has not yet been fully explained mechanistically. Lafaille draws attention to the fact that there are clear examples in which the infectiousness of tolerance is limited (Curotto de Lafaille and Lafaille, 2009) and speculates that this may relate to the context (microenvironment) wherein antigen is presented.

The lymphoid tissues of tolerant animals continue to harbor alloreactive cells that can proliferate and generate inflammatory cytokines in response to antigen in vitro. This, and the finding of linked suppression, suggests that the lymphoid tissue may not be the major site of regulation and that the tissue context or “microenvironment” may be critical. A tissue

“influenced” by Treg cells may take on a reinforcing role to stabilize T cells, demonstrating a tendency for regulation, as long as the Treg cells can spend quality time there. Much of the discussion in the accompanying reviews has focused on the gut as a very permissive and stabilizing environment for Treg cells. There is no reason to assume that other tissues under attack could not provide a similar reinforcing milieu.

Shevach (2009) gives ample discussion to “in vitro” readouts of Treg cell-suppressive function. Is it conceivable that these have been red herrings that have somewhat misled us? The prospect that Treg cells might act to decommission antigen-presenting cells is getting increasing credence and is compatible with “linked suppression.” However, fixation on T cells or dendritic cells (DCs) as the key final targets assumes that Treg cells only operate at the inductive stage of an immune response. Transplant models teach us that this need not be the case (Lin et al., 2002). Perhaps we should not look for Treg cell molecules with direct suppressive activity, but rather molecules that initiate amplification-cascade mechanisms in synergy with tissue contributions (e.g., TGF- β) (Figure 1). These might only manifest in a tissue microenvironment and could be missed in vitro. If we know how tissue and Treg cells co-operated to limit immune damage, then we might be better able to harness those tissue reactions for therapeutic purposes. Curotto de Lafaille and Lafaille (2009) discuss infectious tolerance and speculate that issues of context or “microenvironment” may explain why some model systems see it and others do not. Shevach offers a role for TGF- β in the process (Andersson et al., 2008), whereas we (unpublished data) favor the notion that Treg cells create, within tissues through various amplification cascades, microenvironments permissive for further iTreg cell conversion (Figure 1).

The therapeutic application of Treg cells (Riley et al., 2009) should not be underestimated. Even if we ignore commercial

issues related to personalized cell therapeutics, there are genuine practical concerns. For example, what would be the quality-control criteria for cells that can be reinfused into the patient? What level of contamination with other T cells is acceptable? What of the plasticity issues (Josefowicz and Rudensky, 2009; Zhou et al., 2009) with cells “poised” to change function if they find themselves in the wrong context? Perhaps more conventional approaches to physician-guided expansion of Treg cell in vivo predicated on conventional drugs (Tao et al., 2007) and proteins will be possible and preferred. After all, antibodies to T cell receptors, coreceptors, and costimulatory molecules are all capable of recruiting and favoring regulatory mechanisms in vivo. Notwithstanding this, Riley et al. (2009) correctly identify the control of graft-versus-host disease (GVHD) as an area of unmet medical need in which regulatory cell therapy would be valuable. Perhaps GVHD control provides the appropriate testing ground to assess the risks and benefits of this form of treatment.

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