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Creating transplant tolerance by taming adverse intragraft innate immunity

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Abstract

Certain forms of inflammation of an allograft are highly detrimental to the induction and maintenance of transplant tolerance as they foster stable commitment to graft-destructive, not graft-protective, forms of T-cell immunity. Hence, a reduction in adverse tissue inflammation may prove crucial in facilitating the induction and maintenance of a long-lasting state of transplant tolerance.

Introduction and context

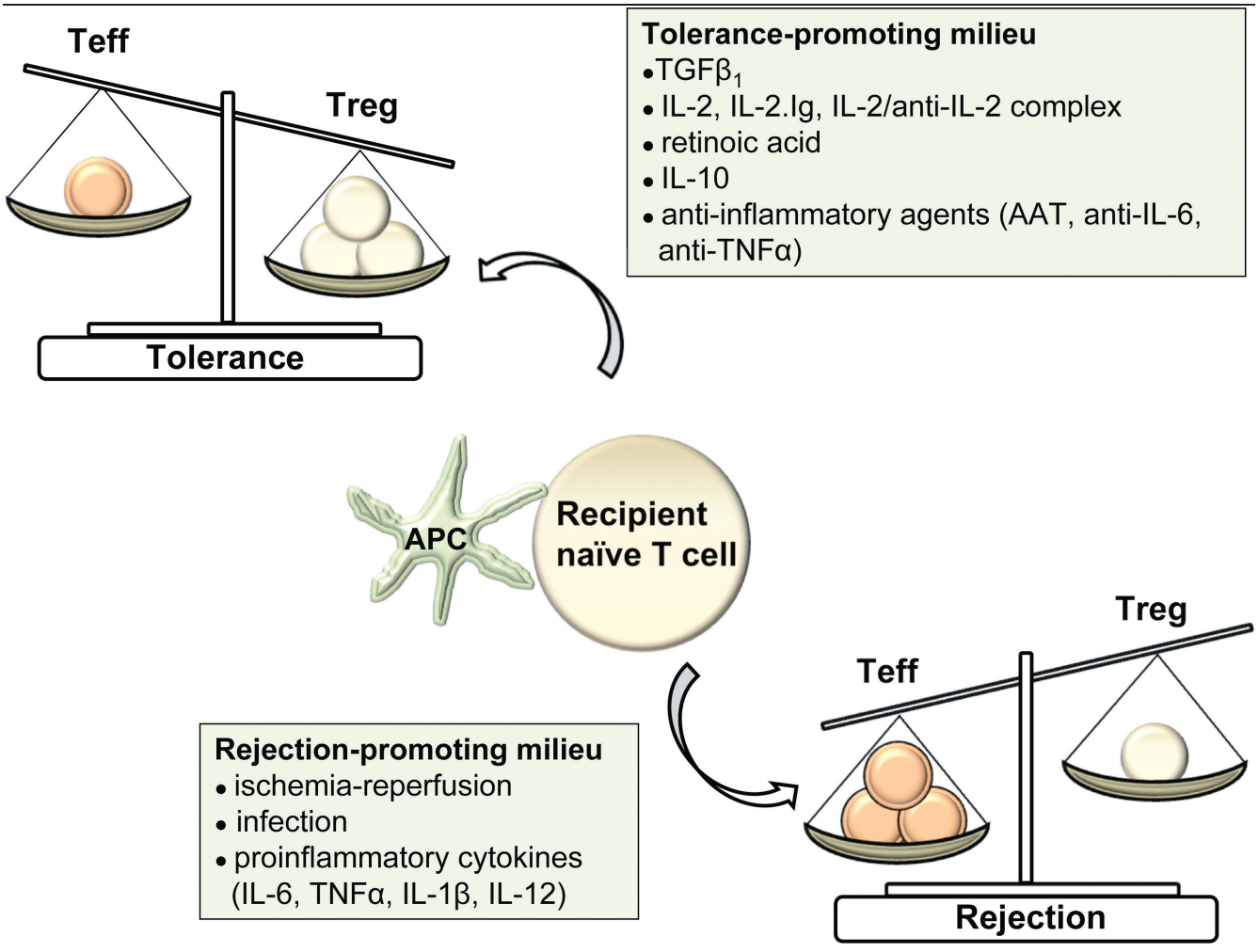
Upon activation by donor alloantigens, recipient naive T cells can differentiate into a variety of graft-destructive, effector T cell or graft-protective, regulatory T cell (Treg) phenotypes. These T-cell commitments are determined largely by the texture of the innate immune milieu in which T-cell activation occurs. Both cytokines and Toll-like receptor agonists are of great importance. A milieu in which transforming growth factor β_1 (TGFβ_1) is expressed in the absence of proinflammatory cytokines promotes the commitment of alloactivated T cells into a tissue-protective, forkhead box P3 (Foxp3)$^+$ Treg phenotype. In contrast, a milieu in which proinflammatory cytokines are abundant prevents the generation of Foxp3$^+$ Tregs and instead directs T-cell commitment into the tissue-destructive T helper 1 (Th1), Th2, or Th17 phenotypes [1-4]. Unfortunately, a robust expression of proinflammatory cytokines is typical for recently engrafted organ transplants. The inflamed state, highly detrimental to the Treg induction and immunoregulatory function [1,3,5], is a consequence of innate immune activation in response to the ischemia and reperfusion injury [5,6]. Although conventionally used agents such as corticosteroids are perhaps useful in restricting the early graft inflammation [7-9], their broad immunosuppressive action, including blocking the expression of TGFβ_1, does not facilitate tolerance [10,11]. Hence, we believe that reducing the adverse forms of inflammation but allowing activity of TGFβ_1 and other inhibitory cytokines within a graft may prove to be a key approach for the induction and maintenance of allograft tolerance. We suspect that the creation of an intra- and peri-graft milieu devoid of proinflammatory cytokines will serve to guide the majority of donor-activated T cells into a TGFβ_1-incited, Treg tissue-protective phenotype. Indeed, our own results and the results of others support this concept [12-15]. Therapies that primarily block adverse inflammation, such as alpha1-antitrypsin, anti-interleukin (IL)-6, or anti-tumor necrosis factor-alpha, have been successfully utilized by our group in tilting the balance of the allograft response toward tolerance, or in restoring tolerance in the non-obese diabetic (NOD) model of type 1 diabetes, even in NOD mice with frank diabetes [14,15]. Given the availability of therapies (many of them approved by the US Food and Drug Administration) that are suitable for blocking the adverse forms of inflammation, rapid translation into the clinic seems possible for the treatment of certain immune-inflammatory disorders. We are aware that a pure prevention or a blockade of inflammation may not be sufficient to achieve lasting transplant tolerance in humans. Additional lymphocyte-depleting measures are required first of all in order to prevent rejection in pre-sensitized recipients [16]. Nonetheless, we believe that favorably tipping the balance of

pro- and anti-inflammatory cytokines in the milieu in which non-depleted T cells re-populate will foster T-cell commitment into a tissue-protective mode and promote tolerance. Our current efforts at blocking TIM4 (T-cell immunoglobulin and mucin domain-containing protein 4), a molecule upregulated by antigen-presenting cells exposed to proinflammatory cytokines and Toll-like receptor agonists [17,18], have proven equally successful in promoting long-term engraftment in preclinical models of transplantation and autoimmunity, although this method is not yet ready for testing in humans. If confirmed that TIM4 blockade leaves T-cell anti-viral responses largely intact, it may be an especially attractive strategy to promote tolerance in patients with chronic viral infections. Collectively, our own studies and the studies of others establish 'adverse' inflammation as a key therapeutic target in the quest for transplant tolerance.

Major recent advances

Induction and maintenance of transplant tolerance require that graft-protective Foxp3$^+$ Tregs efficiently and durably restrain the pool of graft-destructive effector T cells after anti-rejection therapy is withdrawn [19] (Figure 1). For tolerance to be permanent, the Foxp3-dependent Treg immunoregulatory phenotype must be stabilized. Why? A stable expression of Foxp3, the Treg lineage specification transcription factor, is required to maintain Treg function and thereby maintain transplant tolerance [20,21]. Loss of Foxp3 gene expression, such as that occurring in the inflamed, IL-6-rich environments,

Figure 1. Impact of local cytokine milieu on the balance between effector and regulatory T cells



A milieu dominated by anti-inflammatory cytokines or inflammation-dampening agents promotes the commitment of donor-activated T cells into a regulatory T-cell phenotype and thereby fosters transplant tolerance. A milieu dominated by proinflammatory cytokines promotes effector T cell generation and rejection of the allograft. AAT, alpha-1-antitrypsin; APC, antigen-presenting cell; IL, interleukin; Teff, effector T cell (T helper 1 [Th1], Th2, and Th17); TGFβ_1, transforming growth factor β_1; TNFα, tumor necrosis factor-alpha; Treg, regulatory T cell.

can destabilize Treg molecular phenotype and immunoregulatory function, thereby undermining the maintenance of transplant tolerance [22,23]. With the loss of immunoregulatory function among these destabilized Tregs, rejection occurs as the immunoregulatory restraints upon donor-reactive effector T cells are released.

How is Foxp3 expression stabilized? Foxp3 expression in Tregs is regulated, in part, by epigenetic modifications of the Foxp3 chromosomal locus [24]. The methylation status of CpG-sensitive residues upstream of the transcriptional start site (Exon1) is an important regulator of Foxp3 expression. Methylation of these residues represses Foxp3 gene expression while complete demethylation is required for optimal Foxp3 gene expression [25,26]. Other epigenetic mechanisms, such as histone methylation and acetylation, also modulate Foxp3 stability [26,27]. Of note, therapeutic agents that directly foster maintenance of demethylated Foxp3 promote robust expression of Foxp3-sensitive genes, immunoregulatory T-cell function, and tolerance [26,28]. For this purpose, some inhibitors of DNA methyltransferases, such as nucleoside analog 5-azacytidine, have been successfully used in experimental studies [28]. Perhaps owing to the known toxicity of demethylating agents, formal clinical trials in transplantation have not yet been undertaken. Why are these observations potentially important for clinical application? That Treg infusions have the potential to prolong allograft survival and induce transplant tolerance is widely acknowledged [29]. However, the clinical use of Tregs for the adoptive transfer into transplant recipients is hindered by the inherent instability of the Foxp3-dependent Treg phenotype upon exposure of Tregs to inflamed environments. As noted, in the proinflammatory milieu, Foxp3 expression is diminished, inhibitory function is compromised, and some Tregs even convert to T effector-like phenotypes [22,30-32]. Such Treg instability has been associated with the activation of DNA methyltransferases and the consequent remethylation of CpG residues [26]. It therefore seems intuitive that therapeutic strategies able to maintain Foxp3 in a demethylated state may be essential for the effective application of Treg therapy in the clinic. While inhibitors of DNA methyltransferases and of histone deacetylase are not without toxicity, the addition of safe agents that synergize or supplant these drugs as a means to regulate epigenetic expression of Foxp3 should prove extremely valuable in the long-standing quest to induce tolerance.

Tregs and conventional effector T cells take important cues from their microenvironment, which influences their commitment into regulatory or effector phenotypes. Among extracellular stimuli, IL-2-triggered expression of STAT5 (signal transducer and activator of transcription 5) likely plays a major role in stabilizing Tregs [33]. STAT5, activated downstream of IL-2 receptor complex signaling, binds to the promoter region of the Foxp3 gene and thereby activates its transcription [34]. By enhancing transcription of Foxp3, the STAT5 pathway serves to maintain a stable Treg phenotype. We and others have utilized IL-2, IL-2 anti-IL-2 complexes, or IL-2.Ig either alone or in conjunction with rapamycin and other agents to promote tolerance induction [14,35,36]. Such therapies have successfully induced transplant tolerance or restored self-tolerance in the NOD model of type 1 diabetes. In addition to activating the STAT5 pathway and expression of Foxp3, IL-2 signal, if delivered by a long-lived IL-2 anti-IL-2 complex or IL-2.Ig, also causes apoptosis of repeatedly activated effector (but not regulatory) T cells [37]. The fact that calcineurin inhibitors such as cyclosporin negatively impact Treg function and activation-related effector T-cell apoptosis [19] may indeed be a result of suppressed IL-2 signals. We have previously shown that cyclosporin does prevent tolerance induced by co-stimulation blockade in a cardiac transplant model. Rapamycin, on the other hand, did not impede the induction of such achieved tolerance and, as we showed later, does promote the generation of induced Tregs [38,39]. The imbalance of tissue-protective Tregs and tissue-destructive Th17 cells was recently implicated in the pathogenesis of autoimmunity and certain types of rejection [1]. Of note, the commitment of naive T cells to the Th17 phenotype (a subset with aggressive cytodestructive properties) is inhibited by IL-2, and the expression of STAT5 negatively regulates Th17 cells [40]. It is therefore reasonable to posit that the activation of the IL-2/STAT5 pathway promotes tolerance by favorably tilting the balance between Th17 cells and Tregs toward Treg dominance.

Future directions

Strategies used to obtain transplant tolerance have primarily targeted T cells. These strategies have centered upon attempts to delete or at least deplete donor-reactive T cells or alter the early events of T-cell activation such as through co-stimulation blockade. The notion that graft inflammation is the major impediment to transplant tolerance prompts us to further investigate the means of its most efficient therapeutic targeting. It may not be possible to create transplant tolerance unless the inflammatory milieu in which donor-reactive T cells perceive donor antigen is modified. We believe that blocking 'adverse' inflammation in the peri-transplant period can guide the majority of donor-activated naive T cells into the graft-protective Treg mode. In addition, strategies that foster the expression of Treg Foxp3 by epigenetic modification may be utilized in conjunction

with anti-inflammatory agents to further stabilize Tregs and tolerance.

Abbreviations

Foxp3, forkhead box P3; IL, interleukin; NOD, non-obese diabetic; STAT5, signal transducer and activator of transcription 5; TGFβ_1, transforming growth factor β_1; Th, T helper; TIM4, T-cell immunoglobulin and mucin domain-containing protein 4; Treg, regulatory T cell.

Competing interests

The authors declare that they have no competing interests.

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