Published in final edited form as:
Curr Opin Organ Transplant. 2012 February ; 17(1): 1–7. doi:10.1097/MOT.0b013e32834ef52a.

REJECTION AND REGULATION: A TIGHT BALANCE

Isa F. Ashoor[1] and **Nader Najafian**[2,*]

[1]Division of Nephrology, Children's Hospital Boston and Harvard Medical School, Boston, MA, USA

[2]Division of Nephrology, Brigham and Women's Hospital, Children's Hospital Boston and Harvard Medical School, Boston, MA, USA

Abstract

Purpose of review—Achieving allograft tolerance is the holy grail of transplantation. However, tolerance and rejection are two extreme ends of a scale that can be tipped in either direction. We review the novel effector and regulatory mechanisms involved and factors that tip the balance in favor of rejection or regulation.

Recent findings—It is increasingly recognized that established T cell phenotypes could change their commitments. New data point to the plasticity of Th17 cells in vivo with a reciprocal balance of Th17 cells and regulatory T cells driven by the local cytokine environment. Regulatory T cell profiles have been linked to acute and chronic allograft outcomes, and emerging data also indicate a novel role of a regulatory B cell population. Current research efforts are looking into factors that tip the balance towards allograft tolerance by targeting cytokines, novel co-stimulatory pathways such as T cell Ig mucin molecules, and components of innate immunity, particularly dendritic cells.

Summary—The balance of effector and regulatory mechanisms contributing to allograft outcome is very complex. It is likely that targeting multiple pathways will be required to achieve tolerance. Further studies are warranted to define this balance and identify optimal combination of therapeutic interventions.

Keywords

Allograft rejection; allograft tolerance; transplantation; regulatory cells

Introduction

Transplantation tolerance is defined as the absence of pathologic immune response against a graft in an immunocompetent host not using maintenance immunosuppressive drugs while still maintaining protective responses against third party antigens (1). Significant research contributions have been made to the field since the first description of acquired tolerance to allograft antigens was published in a landmark *nature* article by Billingham et al in 1953 (2). It is well recognized now that the fate of the allograft depends on the balance of effector and regulatory immune mechanisms, with allograft tolerance and rejection being two opposite ends of the scale. While most research in the past decade have focused on the balance of effector memory and regulatory T cells and how they are affected by co-stimulation, there is

*Corresponding author: Transplantation Research Center, Renal Division, 221 Longwood Avenue, Boston, MA 02115. Tel: 617-732-7253, Fax: 617-732-5254, nnajafian@rics.bwh.harvard.edu.

Conflicts of interest:
None declared

increasing effort to understand the contribution of newly discovered populations such as T helper 17 and Regulatory B cells. There is also a renewed interest in defining the role of innate immunity in tipping the balance towards allograft rejection through inflammatory monocytes or tolerance through tolerogenic dendritic cells. The purpose of this review is to summarize recently discovered novel effector and regulatory mechanisms and the various over-riding pathways that affect their balance and determine the fate of the allograft.

A. Effector Mechanisms

The role of monocytes in alloimmunity, and memory T cells and their exhaustive differentiation is discussed in detail by Mannon et al. and Li et al. in the accompanying articles. We provide below a brief overview of other new emerging players in rejection and tolerance.

1. T Helper 17 (Th17) Cells—T helper 1 (Th1) cells have long been considered the classic effector T cell phenotype implicated in allograft rejection (3). However, the development of cardiac allograft rejection in a mouse model lacking the Th1 lineage led to the implication of Th17 cells as a novel T cell subset characterized by the production of Interleukin-17 (IL-17) in allograft rejection (4). Increasingly, IL-17 secreting cells are being identified in human allograft recipients as we continue to understand their significance and contribution to allograft outcomes (5–9). Recently, Hester et al have identified an increase of IL-17A producing cells in kidney transplant recipients treated with sirolimus monotherapy compared to sirolimus and mycophenolate mofetil following alemtuzumab induction. This finding would be concerning except for the fact that it was compensated for by an increased functionally suppressive Treg population in the same subjects (6). It is important to note that Th17 cells likely develop from the same precursors as induced regulatory T cells (iTregs) depending on the cytokine environment. The presence of Interleukin-6 (IL-6) has been shown to inhibit Treg generation and induce Th17 differentiation (4), which underscores the significant influence of innate immunity in tipping the balance towards rejection in the setting of inflammation. Th17 cells also exhibit considerable plasticity *in vitro* and *in vivo* (10). Depending on the cytokine milieu, Th17 cells can pursue various fates: in inflammatory settings, Th17 cells may remain stable, but in some immune diseases, may convert to Th1-like cells producing IFN-γ, shown to be the main pathogenic force in EAE, while, in contrast, Th17 cells can completely cease IL-17 production in anti-inflammatory conditions, both “ex-Th17” cells “invisible” to methods of detection based on cytokine expression. The role of Th17 plasticity, if any, is currently unknown in alloimmunity. In sum, our understanding of the mechanisms underlying both rejection and tolerance has evolved with the discovery of novel T cell subsets such as Tregs and Th17 cells and the concept of plasticity. Th17 cells are reviewed in more detail by Ansari et al. later in this issue.

2. T Helper 9 (Th9) Cells—A unique subset of CD4+ T helper cells secreting interleukin-9 (IL-9) has recently been identified and implicated in allergic and autoimmune disease models (11). Wong et al have shown that TGF-β and IL-4 drive the generation of this subset from human CD4+ T cells (12). This underscores the importance of the local cytokine environment in shaping the immune response given that TGF-β is also involved in the differentiation of both Th17 and Tregs in the presence of other cytokines. The role of this subset in transplantation and allograft rejection remains unclear. Interestingly, Poulin et al observed massive eosinophilic infiltration in rejected MHC class II mismatched mouse cardiac allografts in donor grafts from IL-9 transgenic mice compared to wild type animals, suggesting that overexpression of IL-9 within the graft may influence transplant outcomes (13). We have unpublished preliminary data indicating that blockade of IL-9 signaling abrogates the tolerogenic effects of CTLA4-Ig (Abstract by Boenisch et al, ATC 2011).

These data in aggregate highlight a new regulatory function of IL-9 in solid organ transplantation.

3. T Follicular Helper (Tfh) Cells—Tfh cells provide a link between the cellular and humoral arms of the adaptive immune systems. Those cells can be found in the germinal centers of lymphoid tissue and instruct B cells to generate affinity-matured antibodies through their production of IL-21 (14). Recently, Choi et al have shown that activation of naïve CD4+ cells by dendritic cells via ICOS signals upregulated Bcl-6 expression; the characteristic marker of the Tfh subset. Maintenance of this population was dependent on the presence of B cells also via ICOS signals (15). Targeting these interactions and/or modulating the expression of Tfh specific markers can provide new means for suppressing anti-donor humoral responses following transplantation.

B. Regulatory Mechanisms

Regulatory T cells are the classic population linked to allograft tolerance but increasing emphasis is currently directed towards defining a regulatory B cell population.

1. Regulatory T Cells (Tregs)—In the context of alloimmunity, the balance between effector and regulatory cells dictates whether the outcome of a transplant is rejection or tolerance. New data indicate that Tregs can exhibit considerable plasticity (16); unstable Tregs can play an effector rather than regulatory role, depending on the inflammatory conditions (17). These new insights have led to a re-evaluation of transplantation biology and form the basis for the development of novel therapeutic approaches for preventing rejection.

Debate continues regarding the use of Tregs as biomarkers for tolerance in transplantation. Recent human data from renal transplant recipient biopsies reported by Kollins et al failed to correlate the level of intragraft FoxP3+ cells with the severity of graft rejection or renal function at 1 or 2 years (18). Batsford et al had similar findings when they showed that even though FoxP3+ Treg cells within the infiltrating T-cell population can increase transiently during episodes of acute rejection, the phenomenon was not consistently seen in acute cellular rejection and the information did not correlate with serum creatinine at the time of biopsy, at 3 months or 1 year later (19). On the contrary, when Iwase et al utilized real time PCR to detect peripheral blood FoxP3 mRNA in renal transplant recipients up to 1-year post transplantation they noted significantly lower levels of FoxP3 in those with chronic rejection compared to patients with stable graft function (20). Similarly Bhorade et al found that a threshold of 3.2% CD4+FoxP3+ cells in the broncho-alveolar lavage of lung transplant recipients distinguished stable recipients from those subsequently developing Bronchiolitis Obliterans within the first 2 years post transplantation (21). These observations highlight the complexities of using Tregs as a biomarker for acute rejection in human allograft recipients but show promising results in correlating FoxP3+ Treg levels to chronic allograft outcomes. This correlation may differ between different organ types and sampling sources used to detect Tregs (22, 23)

Recent research efforts have focused on identifying novel medications or combinations of current immunosuppressants to expand regulatory T cells in vivo and increase the Treg/T effector ratio to tip the allograft outcome towards tolerance (24–28). Bestard et al used a combination of rATG, belatacept (Bela) and sirolimus (SRL) in a group of renal transplant recipients and compared them to other groups treated with a CNI-based (rATG/tacrolimus/MMF), and two other Bela-based regimens (rATG/Bela/MMF and basiliximab/Bela/MMF/steroids). Their results were highlighted by an increased ratio of Tregs to memory T cells in the rATG/Bela/SRL arm of the study during the first year of follow up (29). Our group has

also shown in vitro that low dose rATG is able to expand human Tregs (30). While we still await mechanistic results from low dose rATG induction clinical trials, Gurkan et al described the immune profile in pediatric and adult renal transplant recipients following rATG induction therapy and demonstrated an expansion of circulating regulatory T cells in vivo (31).

Further studies in this area are warranted to identify the safest and most effective combination strategy, however, given the complexity of immune pathways, it is likely that individually tailored regimens guided by accurate biomarkers of rejection and tolerance will be the ultimate goal.

2. Regulatory B Cells (Bregs)—B cell deficiency or depletion worsens disease in mouse models of EAE, IBD, and contact hypersensitivity, suggesting that B cells can exhibit immunomodulatory function (32–34). The importance of B cells in human transplantation tolerance is becoming a subject of increasing research efforts. This is highlighted by Newell et al when they identified, using gene expression profiles and peripheral blood lymphocyte subset analysis, a characteristic B cell “signature” in a cohort of tolerant renal transplant recipients who had stable graft function and received no immunosuppression for more than 1 year (35).

Harnessing the tolerance inducing properties of regulatory B cells requires the identification of their unique properties and surface markers to facilitate their isolation and expansion for therapeutic purposes. Carter et al recently reported their findings in an IL-$10^{-/-}$ B cell deficient mouse model where they noted exacerbated arthritis compared to the wild type control. They found a decrease in the absolute numbers of FoxP3+ Tregs and increased IFN-γ and IL-17A producing CD4+ T cells in the IL-$10^{-/-}$ B cell deficient mice that was reset by adoptive transfer of wild type Bregs confirming a role of this subset in modulating the balance of effector and regulatory T cell subsets (36). Rothstein et al (37) have recently shown that the surface marker TIM-1 identifies a large majority of B cells capable of IL-4 and IL-10 expression. Those TIM-1^{+} Bregs are induced by TIM-1 ligation and can transfer long-term acceptance of islet allografts to untreated recipients in an IL-10 dependent fashion. Therefore, both the surface expression of TIM-1 and cytokine production of IL-10 provides attractive options for future studies of Bregs.

C. Tipping the Balance

The balance between effector and regulatory mechanisms is strongly influenced by the cytokine milieu and the pattern of co-stimulation. APCs, particularly DCs, are the fulcrum upon which the balance between tolerance and immunity pivots (38). DCs play a key role in antigen presentation, and, especially in the context of endogenous or exogenous “danger signals” from pattern recognition receptors, are capable of orchestrating $CD4^{+}$ T helper cell differentiation through costimulatory interaction and/or soluble cytokines (39, 40).

1. Cytokines—Naïve T cells commitment to an effector or regulatory phenotype as well as the “plasticity” of Tregs/Th17 cells are strongly influenced by the local cytokine environment (41). Park et al exploited this plasticity to promote regulatory T cell expansion by directed cytokine delivery using nanoparticles (42). Inflammatory cytokines of the innate system (IL-6, TNF-α, and IL1-β) have been associated with both chronic renal and cardiac allograft rejection (43, 44). Data from our group has revealed that the pro-inflammatory cytokine IL-6 plays a critical role in allograft rejection in a fully mismatched mouse cardiac transplant model. CTLA4Ig treatment led to allograft acceptance in IL-6 $^{-/-}$ mice while wild type recipients rejected the graft suggesting that intact IL-6 production contributed to rejection (45). It remains to be seen whether blocking humanized IL-6 receptor antibodies

such as tocilizumab, which has recently been used for treatment in rheumatoid arthritis (46), will be effective in human transplantation. Finally, there are emerging data about the role of IL-23 in allograft rejection (47). IL-12 and IL-23 share the p40 subunit and are crucial for the development of Th1 and Th17 cell responses in acute allograft rejection. A recent work demonstrated that treatment with anti-p40 inhibited Th1- and Th17-cell responses and prolonged survival of cardiac allograft in mice (48). There are also new data indicating IL23/IL17 axis in bronchiolitis obliterans syndrome, the leading cause of death after lung transplantation(49).

2. Co-stimulatory molecules—Costimulatory molecules play an important role in determining the balance between effector and regulatory mechanisms and ultimately the fate of the allograft. This topic was extensively reviewed in an excellent article by Li et al (40).

Novel ligands such as T cell Ig mucin (TIM) molecules are increasingly recognized as promising co-stimulatory targets in transplantation, as there is compelling evidence that molecules in the TIM family may broadly regulate Treg/Breg and Th1/Th2/Th17 activity (37, 40, 50, 51). TIM molecules are type I transmembrane glycoproteins, that can be expressed on antigen presenting cells (52), T cells (53, 54) and B cells (37). Our group demonstrated prolonged survival of fully MHC-mismatched vascularized mouse cardiac allografts using a short course of antagonistic TIM-1-specific antibody monotherapy. The prolongation was associated with inhibition of alloreactive Th1 responses and preservation of Th2 responses and the effect was abrogated by depletion of natural Tregs (51). Targeting the same TIM-1 molecule with a specific mAb with agonist properties (3B3) prevented allograft tolerance as shown by Degauque et al in diabetic C57BL/6 mice transplanted with DBA/2 pancreatic islet and treated with an anti-CD154 mAb. The authors performed in vitro experiments where the use of TIM-1 agonist 3B3 prevented the induction of CD4+FoxP3+ Tregs from FoxP3− precursors and was associated with a shift towards Th17 differentiation (53). TIM-3: galectin-9 pathway has been shown to affect Th1/Th17 effector T cells. Recent studies from one group of investigators demonstrated that administration of recombinant mouse or human gal-9 can prolong MHC-mismatched skin and heart transplant survival (55–58). Graft prolongation was accompanied by a decrease in the overall number of TIM-3 expressing, $CD4^+$ and $CD8^+$ T cells in the draining lymph nodes, as well as a decline of INF-γ and IL-17 mRNA in the grafts. Based on pro-apoptotic effects of recombinant gal-9 seen *in vitro*, the authors propose the possibility that recombinant gal-9 may selectively induce the death of TIM-3-expressing Th1/Th17 cells *in vivo.* Recently, our group studied the physiological role of TIM-3: gal-9 pathway in cardiac allograft rejection, by interrupting the interaction of TIM-3 with endogenous gal-9 using a blocking anti-TIM-3 monoclonal antibody (RMT3–23) (50). We demonstrated that blockade of TIM-3 accelerates allograft rejection by increasing IL-6 production in recipient $CD4^+$ T cells. Accelerated rate of rejection is also accompanied by increased frequencies of alloreactive splenic Th1/Th17 T cells, a rise in Th17 lymphocytes and a decline in the frequency of allograft Tregs. These data point to TIM-3 as a central master switch, with the unique ability to broadly modulate $CD4^+$ effector T cell differentiation, possibly by its ability to regulate IL-6 production by $CD4^+$ T cells. Activation of innate immunity and tissue inflammation has an important impact on the differentiation of naïve T cells into Th1/Th17/Tregs. Targeting costimulatory pathways that could control innate immune cells might therefore be important in Treg induction and/or effector T cell differentiation *in vivo*, and consequently critical for tolerance induction. TIM-3 is expressed by some innate immune cells and may play a role in their function, thus indirectly affecting adaptive immunity (59). The reader is directed to this review by Yeung et al for more information on the role of TIM molecules in transplantation (60).

3. Dendritic cells (DCs)—Dendritic cells are the prototypical antigen presenting cells and have the capacity to direct the adaptive immune responses towards an inflammatory or tolerant state (61). The development of recipient DCs to an inflammatory or tolerogenic phenotype in vitro was related to HLA-C genotype as shown by Hanvesakul et al (62). In their study of 760 renal transplant recipients, those with HLA-C2 genotype had significantly better long term graft outcome compared to those with HLA-C1 genotype. In vitro, the HLA-C2 genotype was associated with less mature "tolerogenic" DCs secreting anti-inflammatory cytokines while the HLA-C1 genotype was associated with pro-inflammatory cytokine production and enhanced DCs maturation.

It comes as no surprise then that investigators are attempting to modulate DCs into a tolerogenic phenotype for clinical application. Lan and Ge et al utilized soluble CD83 as a DC modulating agent capable of attenuating DC maturation. Those immature DCs were successful in inducing donor-specific allograft tolerance in both a cardiac and renal mouse transplant models (63, 64). Another approach involving an adenovirus encoding PD-L1 viral vector was used by Peng et al to transfect DCs and later transfer them into an MHC-mismatched rat kidney transplant model (65). The approach was successful in suppressing the proliferation and cytokine secretion of CD8+ T cells in recipient rats and also slowed the development of proteinuria and prolonged their survival.

Conclusion

Despite the identification of new regulatory cell subsets and better understanding of co-stimulatory pathways and the contribution of innate immunity to allograft outcomes, achieving tolerance still remains an elusive goal. Future research efforts should focus on harnessing the benefits of regulatory T and B cells, and tolerogenic dendritic cells while selectively depleting effector memory T cells. To further maintain established tolerance, efforts should be directed at engaging tolerogenic costimulatory pathways and minimizing the negative influence of a pro-inflammatory cytokine environment as seen in infection and ischemia-reperfusion injury.

Acknowledgments

This work was supported by NIH grant RO1AI070820 and R56AI089777.

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Key points

1. Allograft outcome (rejection vs. tolerance) is determined by the balance of effector and regulatory mechanisms.
2. Both naïve and established T cell phenotypes can change their commitment providing a potential therapeutic target to expand regulatory T cells but also posing a challenge to establishing long-term tolerance.
3. The balance of positive and negative co-stimulatory molecule activation is critical to the fate of the allograft and new promising targets such as the TIM molecules are currently being investigated.
4. The innate immune system is intricately linked to the adaptive immune system and can determine allograft outcomes through inflammatory cytokines and tolerogenic dendritic cells.