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When rubber meets the road: how innate features of adaptive immune cells play critical roles in transplant alloimmunity

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Abstract

Purpose of review: Studies on adaptive cells have largely focused on features that are specific to adaptive immunity. However, adaptive cells utilize innate cell features to modulate their responses, and this area of T and B cell biology is understudied. This review will highlight recent work done to understand how innate features of adaptive immune cells modulate alloimmunity.

Recent findings: Over the past year, research has shown that T cell-expressed DAMPs, TLRs, complement receptors, and Fc receptors regulate T cell alloimmunity in a cell-intrinsic manner. Further, IL-17 and p40 of IL-12 have been implicated in the migration of T cells into allografts. Lastly, innate B cells, specifically B1 cells, have been shown to produce clinically relevant autoantibody associated with poor graft outcome.

Summary: These data provide evidence that innate features are utilized by adaptive immune cells to control adaptive alloimmunity.

Keywords
innate; adaptive; T cells; B cells; TLRs; complement; DAMPs

Introduction

Innate and adaptive immunity play unique yet intersecting roles in alloimmunity that drive rejection. Innate cells are activated following recognition of non-polymorphic danger- or pathogen-associated molecular patterns (DAMPs/PAMPs) through TLRs, the activation of complement, and/or cytokine signaling for their activation; whereas adaptive immune cells require specific TCR/BCR interactions and costimulation for their activation. Although they possess quite distinct mechanisms of activation, there are aspects of the innate and adaptive systems that overlap. Here, we will discuss adaptive cells and their utility of innate features to regulate transplant alloimmunity.

DAMPs directly signal on T cells

Danger associated molecular patterns (DAMPs) contribute to alloimmunity by activating innate immune cells. In particular, there has been recent interest in the potential involvement

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of purinergic P2 receptors that bind ATP in the activation of T cells. Interestingly, work by Ledderose et al. identified that autocrine ATP signaling via the purinergic receptor P2X4 induces naïve T cell activation and migration to APCs (1). They also showed that P2X4 antagonism resulted in the prevention of T cell recruitment into the graft and reduced transplant rejection in a murine lung model. As ATP can be released during damage elicited by ischemia and reperfusion, the P2X4-ATP pathway likely contributes to the recruitment and activation of naïve T cells into the graft. Another study on this topic revealed that use of a purinergic receptor antagonist, oxidized ATP, results in diminished IFN-γ-secreting TH1 CD4+ T cells and prolonged graft survival in a murine corneal transplant model (2). These data suggest that extracellular ATP plays an important role in the activation of alloreactive T cells, whether through decreased maturation of CD11b+ APCs as this group showed, or through T cell intrinsic immunomodulatory mechanisms that have been reported by others (3, 4). Alloreactive T cells, then, are able to utilize graft-elicited ATP as a signal for activation and recruitment.

Another innate feature that T cells have channeled to regulate its adaptive function is the ability to utilize aquaporins as a type of damage sensor during prolonged cold ischemia time. Aquaporins rapidly transfer water in and out of cells, but any function in adaptive immunity, other than cell expression, has been understudied (5). Work by Valujskikh’s group has shown that CD4+ and CD8+ T cells express Aquaporin 4 (AQP4) and that its blockade results in improved survival of heart allografts subjected to prolonged cold ischemia time (6). Specifically, they showed that AQP4 blockade reduced T cell infiltration into the graft, proliferation, and cytokine production and is synergistic with CTLA-4Ig in the prolongation of allograft survival. An additional paper by the group outlined alterations in receptors associated with migration and trafficking, including CCR7 and S1PR1, on CD4+ and CD8+ T cells, and that the reduction of circulating T cells was partially due to T cell-intrinsic functions of AQP4 (7). This work illuminates the transfer of water through AQP4, a rudimentary, “innate” feature of immune cells, as an inducer of T cell-mediated allograft damage. Altogether, evidence emerging over the past year reveals that DAMPs such as ATP and water have both direct and indirect mechanisms on T cell alloreactivity.

**Targeting T-cell expressed TLRs can induce tolerance**

The concept that TLRs can act as costimulatory signals on T cells is not new (8, 9); for example, TLR signaling has been shown to augment T cell responses, raising the idea that inhibition of TLR expression on T cells can inhibit alloimmunity (10, 11). Interestingly, recent work on T cell-expressed TLRs identify that low, repetitious doses of TLR ligands can result in TLR desensitization, or TLR tolerance (12). Although this concept has been tested in autoimmunity (13, 14), whether it has any effect in alloimmunity was unknown. Remarkably, Zogas et al. have shown that treatment of allogenic splenocytes with the TLR7 agonist R848 prior to bone marrow transplant induces tolerance and reduced GvHD by the induction of functional TREGs (15). Further, they showed that treatment of CD3+ T cells alone with TLR7 agonist induced tolerance and reduced GvHD, describing a particular role of agonizing TLR7 on T cells that mediates transplant tolerance. This is the first study to show TLR7 tolerization results in reduced GvHD. Similarly, another study has shown the impact of TLR-signaling in T cells in GvHD by providing evidence that MyD88 contributes

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to both T cell survival and differentiation in GvHD (16). These studies suggest that TLR and MyD88 signaling are important in the GvHD T cell response, and that tolerization through this pathway represents a potential effective T cell therapeutic in GvHD.

**T cell expressed complement receptor**

Another intriguing and understudied area of investigation is the interaction of T cells with the complement system (17). Recent work in the Heeger group have specifically focused on the involvement of complement receptors on T cell-mediated alloimmunity. They have identified that C5aR2 and C5aR1 on T cells, specifically C5aR2 on T\textsubscript{REG} and T\textsubscript{EFF}, and C5aR1 on T\textsubscript{FH}, can regulate alloimmune responses. Specifically, C5aR2 expression was found to be critical for iT\textsubscript{REG} generation and lacking it resulted in an increased ratio of intragraft CD8:T\textsubscript{REG} and increased numbers of IFN\textgamma-secreting donor-reactive cells in the spleen post murine heart transplantation (18). Data further showed that transgenic overexpression of C5aR2 under the CD2 promotor prolonged heart graft survival of anti-CD154 treated mice and resulted in fewer IFN\textgamma-secreting donor-reactive cells and a greater T\textsubscript{REG}:T\textsubscript{EFF} ratio. Of note, C5aR2, although a complement receptor, does not signal as a typical G-protein coupled receptor, and instead is hypothesized to act as a scavenger receptor of C5a, internalizing and breaking down extracellular C5a and therefore antagonizing the function of C5aR1 (19, 20). The group has also found that C5aR1, on the other hand, is critical for T\textsubscript{FH} differentiation via the amplification of IL-6-induced mTOR and IL-21 (21). They additionally found that blockade of signals through C5aR1 reverses a T\textsubscript{FH}-dependent chronic graft-versus-host disease model of bronchiolitis obliterans, implicating C5aR1 as a critical factor for T\textsubscript{FH}-driven alloimmunity. These data provide evidence that C5aR1 and C5aR2 are counterparts to each other that serve to modulate the T cell response. Along with recent data correlating urinary C5a levels with delayed graft function (22), C5a and its receptors C5aR1 and C5aR2 play pivotal roles in T cell alloimmunity and represent novel targets for immunotherapy in controlling the immune response following transplantation.

In addition to the C5a receptors, the C3aR1 receptor was also shown to critically impact CD8\textsuperscript{+} T cell alloreactivity (23). C3aR1-deficient CD8\textsuperscript{+} T cells exhibited decreased proliferation and mTOR activation following murine cardiac transplantation, implicating C3aR1 as an important modulator of CD8\textsuperscript{+} T cell alloimmunity. Along with the data above, these studies provide evidence that C3 and C5 receptors regulate aspects of both CD4\textsuperscript{+} and CD8\textsuperscript{+} T cell alloimmunity. Moreover, emerging data of the T cell expression of the complement receptor CD46, originally found to be an important modulator of T\textsubscript{H1} responses, has also been implicated as a costimulatory molecule on human CD8\textsuperscript{+} T cells, providing evidence for its potential therapeutic use in transplantation (24). Together, these data suggest that the expression of innate complement receptors on T cells potentially impacts their activation and effector function, and that these pathways can be targeted to modulate transplant rejection.

**Fc receptor signaling on T cells**

Fc receptors are an additional innate pathway that adaptive cells employ to modulate their function. Specifically, B cell expression of the inhibitory Fc receptor Fc\gammaRIIB has been
shown to modulate chronic antibody mediated rejection (25). Although CD8+ T cell-expressed FcγRIIB was recently defined in the LCMV model (26), whether it functioned in a cell-intrinsic manner to modify CD8+ T cell responses was unknown. Moreover, any role it might have in the modulation of T cell alloimmunity is unknown. Our work has thus endeavored to understand the function of T cell-expressed FcγRIIB in transplantation, and surprisingly, our data demonstrate that FcγRIIB modulates apoptosis of donor-specific CD8+ T cells in a cell-intrinsic manner (Morris and Ford, unpublished data). Furthermore, animals containing donor-specific CD8+ T cells that lack the ability to express FcγRIIB following transplantation exhibit accelerated graft rejection. Interestingly, this is also true when memory CD8+ T cells lack FcγRIIB, implicating this pathway as a modulator of both primary and secondary CD8+ T cell responses. Moreover, different infections (i.e. acute, latent, chronic), elicit various frequencies of FcγRIIB+ CD8+ T cells, therefore implying that infection history could influence graft outcome based on CD8+ T cell expression of FcγRIIB. These data suggest that FcγRIIB functions intrinsically on CD8+ T cells and is beneficial for graft survival, and, further, its deficiency might represent a patient population at increased risk for allograft rejection.

Cytokine signaling on T cells

Cytokines have long been understood to play integral roles in T cell activation and their close interactions reflect how innate mediators can modulate adaptive immune responses. In addition to their role in modulating T cell activation and differentiation, they can interact with T cells independent of the TCR in a non-cognate manner.

For example, IL-17 has been shown to be implicated in the recruitment of CD8+ T cells in airway epithelial injury and allograft rejection following transplantation (27). Interestingly, CD8+ T cell depletion decreased the intragraft level of IL-17, indicating that CD8+ T cells are an early producer of IL-17. Moreover, IL-17 neutralization resulted in decreased accumulation of CD8+ T cells in the graft, correlating with downregulation of CXCL9, 10 and 11 and CCL20, the ligands for the CXCR3 and CCR6 receptor, respectively, that are expressed by CD8+ T cells. These data suggest that CD8+ T cell-produced IL-17 induces changes in the environment that recruits CD8+ T cells following transplantation, offering a positive feedback mechanism for the recruitment of CD8+ T cells.

In addition to IL-17, p40 homodimers, a protein in the IL-12 and IL-23 receptor complexes, have also recently been implicated in allograft T cell responses (28). Interestingly, prolonged cold ischemia time led to increased proliferation of endogenous memory CD8+ T cells in allografts that was dependent on DC and CD4+ T cell interactions. The group then identified that p40 homodimers were required for this memory CD8+ T cell proliferation in the graft as both knock out and blockade studies of p40 showed drastic decreases in the frequencies of BrdU+ CD8+ T cells. Lastly, the numbers of infiltrating IL-12Rβ1+ CD8+ T cells and IL-12Rβ2+ CD8+ T cells into the graft were higher following prolonged cold ischemia time. These data suggest that CD4+ T cell-elicited p40 homodimers interact with CD8+ T cells via the IL-12R and increase the mobilization and expansion of memory CD8+ T cells in allografts exposed to prolonged cold ischemia time, representing a unique role of cytokines on T cell responses outside of TCR interactions.
As evidenced by these studies, cytokines are important modulators of T cell responses in transplantation outside of their typical functions in activation and differentiation and targeting one or a few of these pathways can alter transplant outcomes. Interestingly, Mesaki et al. provide evidence that the overexpression of suppressor of cytokine signaling 3 (SOCS3) in T cells results in reduced epithelial damage and obstruction of murine tracheal allografts (29). Combined, these studies suggest that altering cytokine signaling in T cells in transplantation represents a promising avenue of research to improve transplant outcome, even beyond the typical roles of cytokines in altering T cell activation and differentiation.

Erythropoietin (Epo), although not a cytokine, is a hormone predominantly made by the kidney that regulates red blood cell (RBC) production (30) and has recently gained interest in the transplant community as a potential therapeutic option because of its ability to protect against anemia-induced graft damage. A review this year outlined the role of Epo in transplantation, citing evidence that Epo treatment in a rat model of transplantation possesses additional immune-modulating properties independent of anemia correction (31). Evidence from studies over the past few years has described T cell-intrinsic immunoregulation by Epo in that it can induce T_{REG} proliferation and inhibit conventional (T_{conv}) proliferation via binding to T cell-expressed Epo-R, and that the addition of Epo to CTLA-4Ig treatment can significantly prolong graft rejection (32). New evidence this year describes that Epo treatment inhibits T_{H17} driven autoimmunity and induces Foxp3+CD4+ T cells (33). These data are suggestive that Epo modulates T cell immunity intrinsically and has specific implications for alloimmunity in addition to its role as an RBC-inducing hormone. Further human studies are needed to determine whether these mechanisms of Epo activity in alloimmunity are clinically relevant.

**Innate features of B cells**

Surprisingly, B cells can also contribute to inflammation outside of the production of antibody and activation of T cells as an APC. Panzer et al. show that B cells control macrophage infiltration and overall inflammatory phenotype; deficiency of B cells led to decreased macrophage infiltration, increased T_{REG} accumulation and splenic IL-10, and decreased number of T_{FH} cells (34). These data suggest that B cells have the ability to regulate cell infiltration, cytokine levels, and inflammation in the graft, making them an orchestrator of innate and adaptive cell infiltrate.

Furthermore, new research has explored how innate B cells and their effector molecules contribute to allograft rejection, and a recent review outlines some of the ongoing research in this area (35). Innate B cells are known for their ability to make pre-existing natural antibodies (Nabs) of the IgM class against the ABO blood group and xenoantigens. Interestingly, IgM Nabs can class-switch to IgG in inflammation and potentially have reactivity against graft antigens, i.e. HLA (36). In addition to reactivity against HLA, Nabs from innate B cells can also be autoreactive, such as the anti-LG3 autoantibody recently shown to be made predominantly by memory B1 cells (37). Non-HLA, natural antibodies such as these have been associated with increased allograft injury and poor graft outcomes, and the ability for innate-like B cells to contribute to this reveals a novel mechanism of alloimmunity.
Concluding Remarks

Here, we summarize recently-described innate features of adaptive immune cells that regulate their function. Research over the past year has identified the regulation of alloreactive T cells by T cell-expressed DAMPs, TLRs, complement receptors, and Fc receptors. Furthermore, several cytokines, like IL-17 and p40 of IL-12, have been implicated in “non-cognate” functions outside of activation and differentiation of T cells, such as migration. Lastly, in addition to their large role in secreting class-switched IgG and contributing to ABMR, innate B cells, like B1 cells, have been shown to produce clinically relevant autoantibody associated with poor graft outcome, implicating these cells as important modulators of transplant rejection. These data support the concept that adaptive immune cells utilize certain innate features to control adaptive immunity.

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through IL-17. Importantly, this data describe the potential to manipulate intragraft T cell numbers through cytokine blockade.

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Key Points

- Innate features, such as DAMPs, TLRs, complement receptors, and Fc receptors, contribute to alloimmunity by altering T cell responses in a cell-intrinsic manner.

- Cytokines, like IL-17 and p40 of IL-12, can alter the function of T cells outside of their typical roles in directing T cell differentiation by affecting the migration of T cells into allografts.

- Innate B cells, although conceptually innate, can produce clinically relevant autoantibody in transplantation.