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New Insights into T Cell Cosignaling in Allograft Rejection and Survival

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Abstract

Purpose of Review—Stimulatory and inhibitory receptor signaling (cosignaling) on T cells is a critical component of T cell responses that mediate graft rejection. The blockade of cosignaling pathways is an attractive strategy for preventing allogeneic T cell responses. Here we review new studies that provide critical insight into well-studied CD28/CTLA-4 and CD40/CD40L cosignaling pathways, as well as the identification of novel cosignaling receptors that play a role in allogeneic T cell responses.

Recent Findings—Recently it has been appreciated that the CD28/CTLA-4 pathway has unique roles on specific T cell subsets, particularly on FoxP3⁺ Treg and Th17 cells. New insight has been provided into the mechanism by which CD40:CD154 blockade to elicits FoxP3⁺ Treg conversion and memory T cells to elicit CD40-independent alloantibody responses. Finally, several novel cosignaling pathways have been demonstrated to be important to graft-specific T cells, including CD160, SLAM family member 2B4, TIM-4, and the Notch receptor.

Summary—Recent work has provided more granular understanding of the CD28/CTLA-4 and CD40/CD154 pathways on T cell subsets and provided important insight into the generation and maintenance of FoxP3⁺ Treg. This information, as well as the characterization of novel transplantation-relevant cosignaling pathways, has implications for modulation of alloreactive T cell responses.

Keywords

cosignaling; cytotoxic lymphocyte antigen 4 (CTLA-4); regulatory T cells

Introduction

The importance of cosignaling during in alloreactive T cell responses is widely accepted, perhaps most convincingly through the salutatory effects of blocking these signals during transplantation. Indeed, blockade of many costimulatory pathways has been investigated in pre-clinical models of solid organ and hematopoietic cell transplantation, and recently this work was translated to the clinic with the approval of CTLA-4 Ig derivative belatacept for use in renal transplantation. Here we review exciting recent developments in our

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understanding of prototypic cosignaling pathways as well as promising discoveries of novel cosignaling pathways that play critical roles during transplantation.

New Insights into the CD28/CTLA-4 Pathway in Transplantation

While the CD28/CTLA-4 pathway is perhaps the most well-known cosignaling pathway on T cells, its functional salience in graft rejection was recently confirmed in an elegant study by Venner et al (1). Taking advantage of a recently modified histological criteria to identify pure T cell mediated renal allograft rejection, the investigators used expression microarrays in a discovery set-validation set approach to specifically investigate genes associated with T cell mediated rejection in 704 patients (1). This investigation found that T cell costimulation pathways, particularly the CD28/CTLA-4 and PD-1 families, were highly associated with T cell mediated rejection. This unbiased investigation offers validation that manipulation of these “classic” cosignaling pathways has tremendous potential to attenuate allogeneic responses and prevent graft rejection. Recently a number of studies have significantly advanced our understanding of the CD28/CTLA-4 pathway on alloreactive T cells.

CD28 Costimulation on FoxP3⁺ Treg

Targeting of the CD28/CTLA-4 pathway with CTLA-4 Ig and its derivatives has proven efficacious in pre-clinical models of transplantation, was recently approved for post-renal transplant rejection prophylaxis, and is effective in the treatment of several autoimmune diseases (2, 3). However, CTLA-4 Ig has paradoxical effect of inhibiting effector T cells while simultaneously diminishing numbers of suppressive FoxP3⁺ Treg, which has made dissecting the specific effects of CD28 cosignaling on CD4⁺FoxP3⁺ Treg difficult (2, 4).

Zhang and colleagues made a major advancement in this field by generating a Treg-specific CD28 conditional knockout (CD28 cKO) mouse (5). They found that CD28 is required for optimal Treg suppressive function and homeostasis of Treg numbers in the periphery. CD28 cKO mice developed severe skin and lung immunopathology, despite similar number of Treg in CD28 cKO and WT mice. The most profound defect identified in CD28 cKO Treg is their diminished ability of to proliferate *in vivo*, as demonstrated in a fully allogeneic skin graft model in which CD28 cKO Treg expanded less than WT Treg. CD28 cKO Treg also express diminished amounts of classical Treg markers, including mildly less of the coinhibitor CTLA-4. Overall, this study demonstrates that CD28 is critically important for Treg homeostasis, and suggests that the diminished numbers of Treg observed in CTLA-4 Ig treated patients might be due largely to blockade of CD28 signals.

Despite much effort, the roles of CD28 and CTLA-4 cosignaling on Treg have remained poorly defined. However, in conjunction with a previous study of Treg CTLA-4 cKO mice (6), Zhang et al reveals important features of CD28 and CTLA-4 on Treg. CTLA-4 cKO mice have a fatal autoimmune phenotype in multiple tissues and a profound defect in suppressive function in multiple models, including an inability to diminish CD80/CD86 expression on APCs (6). However, in sharp contrast to CD28 cKO Treg, CTLA-4 cKO Treg exhibited similar homeostasis compared to WT Treg. In effector T cells, a large portion of CTLA-4 expression is dependent on CD28 signals (7), it is worth noting that this appears not be the case on Treg, as CD28 cKO Treg had only a mild diminution of CTLA-4

expression (5). Thus, these two elegant studies draw important contrasts in the function of CD28 and CTLA-4 on Treg: CD28 is critical for Treg development in the thymus and peripheral homeostasis, while CTLA-4 is a potent effector molecule on Treg.

CD28/CTLA-4 on Th17 cells

Within the CD4⁺ compartment, IFN- γ producing Th1 cells have been shown to be sensitive to CD28/CTLA-4 blockade with CTLA-4 Ig (8). Th17 cells are a recently described CD4⁺ T helper subset that mediates protective immunity against fungi and bacteria, and can be pathogenic in autoimmune disease (9, 10). While the role of Th17 cells in allogeneic T cell responses has not been widely studied, IL-17 has been reported to be associated with graft rejection (11, 12). Studies have demonstrated that Th17 cell differentiation is resistant to costimulation blockade with CTLA-4 Ig (13), and that CTLA-4 Ig treatment results in enhanced IL-17 production (14). Our group recently found that Th17 memory cell frequencies are elevated in renal transplant recipients treated with the CTLA-4 Ig derivative belatacept during rejection (15). In an antigen-specific murine model of graft rejection, mice with Th17-polarized CD4⁺ T cell populations underwent costimulation blockade resistant rejection (16).

The mechanism underlying these observations of the relative resistance of Th17 cells to CTLA-4 Ig compared to Th1 cells is not completely understood. However, it has been demonstrated that Th1 and Th17 cells express similar levels of CD28, while Th17 cells express significantly greater levels of coinhibitory CTLA-4 (15, 16). Thus, the blockade of CD28/CTLA-4 signals alters the cosignaling balance on these two CD4⁺ T cell subsets, thus augmenting Th17 proliferative responses. Multiple groups have reported that Th17 cells rely heavily on non-CD28 cosignaling pathways (such as ICOS, SLAMF3, and SLAMF6) for optimal function (17, 18). It remains to be seen whether the resistance of Th17 cells to CTLA-4 Ig is intrinsic to this pathway or the result of compensatory cosignaling.

Advances in Understanding of the CD40/CD40L Cosignaling in Transplantation

Blockade of the CD40:CD154 pathway has been shown in multiple rodent and non-human primate models to prolong graft survival (3). Recently, blockade of the CD40:CD154 pathway was shown to induce antigen-specific CD4⁺CD25⁺FoxP3⁺ Tregs (iTreg) (19). Using an CD154 blockade-based tolerance induction strategy, Warren et al provided mechanistic insight into the function of these iTreg by demonstrating that Treg have distinct localization within the draining lymph node of heart allografts (20). In contrast to immune mice (given allogeneic DST but no ant-CD154), antigen-specific CD4⁺ T cells, plasmacytoid dendritic cells, and Tregs preferentially migrated to the cortical ridge of lymph nodes in tolerance mice, suggesting that iTreg localization plays a key role in determining the outcome following transplant.

Deeper insight into the signals necessary for conversion to Treg in the context of CD154 blockade has been generated by two recent studies. The conventional model for CD40-mediated CD4⁺ T cell help is that CD154 expressed on CD4⁺ T cells engages CD40

expressed by antigen presenting cells (APCs). However, it is also known that CD40 can be expressed by CD8⁺ T cells. Using an antigen-specific model of graft rejection, Liu et al showed that CD40 signals delivered by CD8⁺ T cells play a significant role in mediating graft-specific responses (21). Interestingly, loss of CD40 on either APCs or CD8⁺ T cells alone did not enable the conversion of antigen-specific FoxP3⁺ iTregs. However, loss of CD40 signals on both APCs and CD8⁺ T cells did result in the conversion of FoxP3⁺ iTreg, demonstrating a critical role for CD8⁺ T cell CD40 expression in the differentiation program of CD4⁺ T cells. In a model of antigen-specific mixed chimerism in which CD154 blockade prevents chimerism rejection, Ferrer et al found that CD154 blockade attenuates donor-reactive T cell responses by potentially inhibiting the expression of pro-inflammatory cytokines IL-6, IL-1b, TNF, and IL-12 by APCs (22). In this study, the expression of MHC and costimulatory molecules on APCs was not affected by CD154 blockade. Thus, this study reveals a previously unappreciated role of CD154 blockade to suppress signal three inflammatory cytokine responses by APCs.

CD40/CD154 signals are recognized to be critical for sustained antibody responses *in vivo*. Using a fully allogeneic heart graft model, Rabant et al modify this paradigm by demonstrating that memory, but not naïve, CD4⁺ T cells can deliver help to CD40-deficient B cells and induce alloantibodies that mediate heart allograft rejection (23). Although the CD40-independent antibody response was not long-lived, this CD40-independent memory CD4⁺ T cell help occurred in the absence of germinal center formation and was independent of ICOS cosignaling. This work suggests that additional (as yet undefined) cosignaling pathway on memory CD4⁺ T cells can help B cells and induce antibody responses.

Together, these studies modify the model of CD40/CD154 signaling on T and B cells by providing new mechanistic insight into the specific role of these molecules on T cell subsets. Blockade of CD154 is clinically attractive because of the diminution of both allogeneic CD8⁺ T cell and antibody responses, as well as the conversion of CD4⁺ T cells to Treg. In particular, Treg conversion is a clinically advantageous phenomenon because it offers the potential to provide antigen-specific regulation that is kinetically associated with transplantation and long-lasting.

Despite its promise, a major barrier to translation of CD40:CD40L blockade to the clinic has been thromboembolic complications associated with the use of monoclonal antibodies (mAb), presumably due to Fc-mediated cross-linking and platelet activation (24). Using a novel Fc-silent domain antibody (dAb), Pinelli et al establish a potential avenue for clinical blockade of the CD154 pathway (25). In an antigen-specific model of graft rejection, CD154 dAbs had similar efficacy to an anti-CD154 mAb at inhibiting graft rejection and attenuating donor-reactive CD4⁺ and CD8⁺ T cell responses (25). Importantly, conversion of antigen-specific FoxP3⁺ Treg was similar between mAb and dAb treated animals. Surprisingly, the conversion of iTreg in the presence of CD154 dAbs was preserved with the addition of CTLA-4 Ig, which has been demonstrated to inhibit Treg conversion and function (14, 26). Thus, this study suggests that dAb therapy might provide a viable clinical means of CD40:CD40L pathway blockade and that CD154 blockade can override the absence CD28/CTLA-4 signals for the conversion of CD4⁺ T cells into FoxP3⁺ Treg.

Non-traditional Cosignaling Pathways in Transplantation

While the CD28/CTLA-4 and CD40/CD40L cosignaling pathways have received a great deal of attention, particularly in the field of transplant immunology, our understanding of the way in which cosignaling modifies T cell function has expanded tremendously in recent years. In addition to these prototypic pathways, many other receptors and ligands have been demonstrated to provide critical signals that modify T cell differentiation and function. Perhaps the most relevant model, put forth by Chen and colleagues, is that T cells are affected by a constellation of multiple families of cosignaling receptors (27). Immediately following activation, the balance of this signaling is costimulatory (the rising tide), followed by the rise of coinhibitory receptors to extinguish T cell activation (the receding tide). Central to this concept is that multiple receptors play an important role in T cell cosignaling. While several “ancillary” T cell cosignaling molecules – such as OX-40, 4-1BBL, CD2, and LFA-1 – have been studied in the context of transplantation, recent studies have discovered important roles for additional cosignaling receptors within alloreactive T cell responses.

Cosignaling in the absence of CD28 costimulation

Two recent studies have demonstrated important cosignaling pathways that are uncovered in the absence of CD28 signals. Liu et al recently elucidated a role for a network of cosignaling receptors that play a role in donor-reactive CD8⁺ T cell responses downstream of CD28/CTLA-4 (28). The authors found that selective CD28 blockade with a domain antibody resulted in prolonged graft survival and diminished donor-reactive CD4⁺ and CD8⁺ T cell responses that corresponded with diminished expression of ICOS and upregulation of the coinhibitory SLAM-family member 2B4. Genetic loss of 2B4 restored donor-reactive T cell responses and accelerated graft rejection in the presence of the CD28 dAbs. Interestingly, upregulation of 2B4 was dependent on CTLA-4 signals but independent of PD-L1. Diminished ICOS expression, however, was dependent on 2B4 signaling (28). This study demonstrates that upregulation of inhibitory 2B4 expression is required for the maximal effect of CD28 blockade in the context of transplantation.

A role for CD160 was also recently demonstrated in the absence of CD28 costimulation. CD160 is an Ig-like family receptor associated with cytolytic activity in NK, NKT, and CD8⁺ T cells. Using a novel CD160 Ig to inhibit CD160:CD160L interactions, D’Addio et al showed that CD160 blockade inhibits proliferation *ex vivo* in activated CD4⁺ and CD8⁺ T cells (29). CD160 Ig prolonged graft survival significantly in a heart allograft model in CD28^{-/-} hosts or with the administration of CTLA-4 Ig, demonstrating that this pathway is particularly relevant in the absence of CD28 signaling. CD160 blockade in the absence of CD28 signals prevented CD8⁺ effector activation, diminished production of multiple cytokines, including IFN- γ , TNF, IL-6, and IL-17, and enhanced production of IL-5 and IL-4. Thus, CD160 represents an important pathway for allogeneic T cell responses in the absence of CD28 signals, such as in conjunction CTLA-4 Ig therapy.

TIM family cosignaling in transplantation

Recent work has demonstrated a role for the T cell Ig mucin (TIM) family of proteins in allogeneic cosignaling. Previous work has identified a role for TIM-1 and TIM-3. TIM-3

restrains allogeneic Th1-type responses and can be used to identify short-lived graft-infiltrating FoxP3⁺ Treg population (30, 31). Blockade of TIM-1 enhances heart allograft survival in a CD4⁺CD25⁺FoxP3⁺ Treg-dependant manner (32), and it has also been identified as a functional marker of regulatory B cells (33). Thus, previous work on the TIM family in transplantation has focused primarily on their role as T cell-expressed cosignaling receptors during allogeneic responses.

In contrast, Yueng et al recently established the important role for TIM-4, a TIM family member that is expressed solely by APCs, in allogeneic T cell responses (34). TIM-4 expression on CD11c⁺ DCs was enhanced following heart allograft challenge, and blockade diminished expression of Th2 cytokines IL-4, IL-5, and IL-13, but not Th1 or Th17 cytokines. Blockade of TIM-4 resulted in enhanced CD4⁺FoxP3⁺ Treg development, which is dependent on diminished signaling through IL-4/STAT6/GATA-3. Prolongation of graft survival by TIM-4 mAbs was dependent on FoxP3⁺, as experiments in FOXP3^{-/-} mice TIM-4 blockade failed to prolong graft survival. Interestingly, TIM-4 blockade was effective at inducing Tregs by activated DCs following allogeneic stimulation. This study establishes a mechanism by which TIM-4 cosignaling induced Th2 skewing and inhibits FoxP3⁺ T reg development.

Notch family signaling in transplantation

A role for the Notch pathway in T cell cosignaling has been established in a model of transplantation. Upon engagement of their ligands expressed on APCs, the intracellular tail of the Notch receptor is cleaved and mediates gene expression in the nucleus. The first demonstration of the utility of this pathway during graft rejection was that blockade of the Notch ligand Delta 1 prolonged graft survival in a fully allogeneic heart graft model in combination with CTLA-4 Ig (35). Recently, Riella et al established a role for the Notch ligand Jagged 2 in allogeneic T cell responses and graft rejection (36). Using a Jagged 2 mAb, rejection was significantly accelerated in fully allogeneic heart graft model in CD28^{-/-} hosts, suggesting that Jagged 2 signaling is enhanced in the absence of CD28. The effect of Jagged mAb engagement on T cell responses was shown to be dependent on IL-6. Thus, the Notch pathway represents an important pathway in alloreactive T cell responses.

Conclusion

Cosignaling is central to alloreactive T cell responses and remains a viable target for inhibiting graft rejection. Recent work has provided insight into traditional as well as novel cosignaling molecules in the context of transplantation. Interrogation of the CD28/CTLA-4 and CD40/CD154 pathways has demonstrated previously unappreciated T cell subset-specific roles for these receptors. As both pathways have been shown to affect both alloreactive and regulatory T cells, an important goal for the future development of blockade of each of these pathways clinically is how to utilize them in a manner that optimizes the attenuation of effector cells while preserving the suppressive function of Treg.

In conclusion, the emerging model of T cell cosignaling proposes that T cells receive a constellation of cosignals during activation, and that the regulation of these signals may be interdependent. In particular, important work has identified novel pathways that are critical

in the absence of CD28 costimulation, a state that is particularly relevant in light of the clinical use of CTLA-4 Ig in transplantation. Finally, new studies have uncovered important functions for novel cosignaling pathways on alloreactive T cells. Future efforts to block these pathways in pre-clinical models will determine if they hold clinical potential for preventing graft rejection and improving outcomes following transplantation.

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Key points

- CD28 is critical for FoxP3⁺ Treg homeostasis *in vivo*
- Th17 cells are resistant to CD28/CTLA-4 blockade with CTLA-4 Ig
- Blockade of the CD40/CD154 pathway has many salutatory effects in the inhibition of alloreactive T cell responses and the generation of FoxP3⁺ Treg
- Blockade of CD28 signals uncovers roles for coinhibitory 2B4 and costimulation via CD160 on donor-reactive T cells
- Cosignaling through the TIM and Notch family of receptors is important for alloreactive T cell responses