Multicellular and multimolecular immune interactions in the transplantation tolerance and rejection

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Abstract
Transplantation tolerance remains the paramount goal for achieving long-term allograft survival. Several chemotherapeutic drugs are in use to prolong allograft survival, but its side effects and toxicity limits its clinic application. Transplantation tolerance requires complex cellular and molecular interaction between immune cells and stromal cells in the secondary lymphoid tissues. Early interaction of these cells decides the fate of generation and maintenance of tolerance. The role of adaptive immunity (T cells and B cells) in the inflammation and tolerance are well established, and new cellular and molecular interactions are evolving with time. In this review, we discussed the importance of innate and adaptive immune cells and how their interactions contribute to transplantation tolerance or rejection. We also discussed how these cellular and molecular interactions had been explored to control the inflammatory reactions and promote the survival of allogenic grafts in the various transplantation setting.

Keywords: Organ transplantation, Immune tolerance, costimulatory molecules, Immunosuppression, graft rejection

Introduction
Transplantation is a procedure in which a functional cell, tissue or organ is transferred from a donor’s body to the recipient to restore tissue or organ functions. Organ transplantation remains the only option during end-stage liver and heart failure. Continuous research has helped in overcoming the limitations of organ transplantation, as a result of which organs like kidney, liver, heart, trachea, cornea, pancreas, and bone marrow have been used for transplantation in human patients.1,2

The shortage of organ donors and the ultimate rejection of allografts are the two major concerns that limit the widespread use of organ transplantation. Understanding the mechanism of transplant rejection will help in identifying novel targets, which can provide immunosuppression-free lifelong survival.

The rejection of transplanted organ is a consequence of non-immunological (ischemia-reperfusion injury) and immunological events. Ischemia/reperfusion injury (IRI) is an unavoidable condition during organ transplantation, which affects the short-term and long-term survival of the allograft. Due to the high level of vascularity, kidney and liver grafts are most susceptible to IRI. Ischemia is the injury caused to donor organs due to the disturbance in the blood flow after brain death of the donor. Disturbed blood flow leads to an anaerobic environment and acidosis in the tissue. Consequently, the lysosomal membranes become unstable and cause edema due to the accumulation of Na+ ions and water.3,4 Parallely, ischemia also induces activation of innate immune cells in the donor organ, and reperfusion of blood aggravates the damage, leading to the perpetuation of the inflammatory response.

The immunological response against allograft is believed to be the dominant determinant of rejection. The immune response against allograft involves the participation of innate and adaptive immune cells. Following organ transplantation, danger-associated molecular patterns (DAMPS), released due to tissue injury, induces the activation and functional maturation of donor-dendritic cells (DCs). Subsequent migration of donor DCs to the recipient’s lymph nodes and presentation of alloantigen triggers T-cell priming.5 T cells use two distinct pathways for graft rejection, which can be classified as direct and
indirect pathways. In the direct alloresponse, intact donor major histocompatibility complex (MHC) molecules loaded with allogenic peptide is presented to recipient T cells, leading to an acute polyclonal allogenic T cell response. The direct antigen presentation plays an important role in acute allograft rejection. In the indirect alloresponse, alloantigen is taken up by the recipient’s antigen-presenting cells (APCs), processed and presented to recipient T cells leading to oligoclonal T cell response. The indirect response takes time to generate allogenic T cells and is mostly associated with chronic graft rejection. In the present work, we have discussed the important role played by several innate and adaptive immune cells during allograft rejection.

Role of innate immune cells in transplant rejection

The role of adaptive immune cells in allograft rejection is well studied. However, the phenotype and function of innate immune cells in transplantation are not entirely understood. The ability of innate immune cells to recognize allograft, generate an early immune response, prime and alert the adaptive immune system makes them an important cell types in determining the generation of tolerance or rejection of the allograft. Some of the crucial contributions of innate immune cells in transplantation tolerance and rejection are discussed below:

Neutrophils

Transplant injury activates the secretion of several chemokines by vascular endothelium such as CXCL1, CXCL2, and CXCL8. This plays a key role in the recruitment of neutrophils. Neutrophils accumulate in large numbers within a few hours of transplantation and contribute to transplant-induced IRI. Degranulation of neutrophils leads to the release of the tissue-digesting enzymes like metalloproteinases (MMPs) and reactive oxygen species (ROS), which induces damage to the allograft. After infiltration into the donor organ, neutrophils secrete chemokines such as C-C motif ligand 1 (CCL1), CCL2, and CCL5, which further recruits T cells in the allograft. Consequently, inhibition of neutrophil recruitment into the allograft delays the T cell infiltration and chronic rejection. It has also been shown that depletion of neutrophil enhances the costimulatory-blockade induced survival of cardiac allografts and prevents lung and liver IRI. Besides, TNF-α production by neutrophils is shown to stimulate DCs to produce IL-12, which skews the T cell differentiation towards Th1 lineage and promotes allograft rejection. These studies suggested that neutrophil provides an important link between adaptive and innate immune cells in the transplantation.

Dendritic cells (DCs)

DCs are professional antigen-presenting cells, and present alloantigen to T cells, and play a central role in the establishment of allograft-specific immune response. After organ transplantation, DCs present in the donor graft mobilizes into recipient’s secondary lymphoid organs and directly prime the host allogenic T cells (direct antigen presentation) leading to acute rejection of graft. Donor DCs can also transfer the intact allopeptide loaded-MHC molecules to host DCs, which then activate the allogenic T cells (semi-direct antigen presentation) leading to slower rejection. In other scenario, recipient DCs takes up alloantigen from the graft, process and present self-MHC loaded antigens to recipient T cells leading to chronic rejection. Cytokines produced by DCs mediate CD4+ T cells differentiation and also provide help to B cells for alloantibody production. Targeting DC-T cell interaction is believed to be an efficient way to prevent allograft rejection. In contrast, there are DCs that show immunoregulatory function and improve allograft survival. The tolerogenic DCs have low expression of a costimulatory molecule, show resistance to maturation and secrete anti-inflammatory molecules such as IL-10 and TGF-α. and control the T cell proliferation, and promote the differentiation of regulatory CD4+ T cells. The concept of donor-specific transfusion (DST) is well established to induce transplantation tolerance. Pretransplant infusion of immature donor-derived DCs in combination with short-term immunosuppression has shown reproducible results in laboratory animal models, suggesting the importance of DCs in the transplantation. Several methods have been tried to induce tolerogenic DCs and the same has been investigated in phase I/II clinical trials for kidney transplantation. Recently, it has been shown that reprogramming of dendritic cells with CRISPR/Cas9 nanoparticles for CD40 can reduce the skin graft rejection.

Macrophages

Macrophages and its precursor monocytes are shown to perform both pro- and anti-inflammatory functions and adapt to the specific phenotype based on the microenvironmental cues present in the allograft. In acute and chronic rejection, macrophages account for about 38-60% of total graft infiltrating leukocytes in the rejecting organs. Increased infiltration of macrophages within glomeruli is
associated with reduced survival of renal transplants.\textsuperscript{24} Allograft infiltrating macrophages are associated with acute cell-mediated rejection as well as acute antibody-mediated rejection.\textsuperscript{21} Depletion of macrophages reduces the allograft injury and pathology of rejecting allografts.\textsuperscript{23, 26} These studies suggest that macrophages play an important role in acute graft rejection and act as a diagnostic marker for graft rejection.

**Natural killer (NK) cells**

NK cells are known to express several activating and inhibitory receptors that recognize the class I MHC molecules, and, by engaging these receptors, NK cells can distinguish autologous and allogenic cells.\textsuperscript{26, 27} NK cells are known to control the survival of hematopoietic as well as solid organ transplantations.\textsuperscript{28-30} Perforin secretion and NKG2D interaction of NK cells have been shown to be required for the tolerance to islet and cardiac allografts induced by anti-CD40L mAb.\textsuperscript{30, 31} NK cells eliminate donor antigen-presenting cells in the graft, which mediates the long-term survival of skin allografts.\textsuperscript{32}

**Mast cells**

Mast cells are also known to influence the adaptive and innate immune cells and play an important role in the rejection of allograft.\textsuperscript{33} Mast cells act as antigen-presenting cells, express several co-stimulatory molecules and produce wide varieties of both anti-inflammatory and proinflammatory molecules.\textsuperscript{34, 35} Mast cell degranulation breaks the peripheral tolerance in skin and cardiac allograft.\textsuperscript{36} Mast cells are also known to produce several molecules such as histamine, fibroblast growth factor-2 (FGF-2), TGF-α, chymase and cathepsin G. These molecules contribute to the histological changes in the graft and fibrosis.\textsuperscript{3, 37}

**Role of adaptive immune cells in transplant rejection**

**CD4\(^+\) T cells**

Alloantigen presented by the donor or recipient APCs leads to activation and proliferation of allogenic CD4\(^+\) T cells, which are known to play a dominant role during allograft rejection.\textsuperscript{38, 39} Depending upon their cytokine secretion and functions, CD4\(^+\) T cells are characterized into various subsets, and their functions during allograft rejection are discussed below:

**Th1 cells**

Exposure of naive CD4\(^+\) T cells to the microenvironment enriched with IL-12 and IFN-γ leads to the activation of transcription factor T-bet, which induces the differentiation towards Th1 lineage. These Th1 cells primarily show secretion of IL-2, IFN-γ and TNF-α. CD4\(^+\) T cell clones in the human kidney allografts. Production of a high level of IFN-γ suggests that Th1 cells play an important role in the acute allograft rejection.\textsuperscript{40} IL-2 produced by Th1 cells mediate secretion of IFN-γ by CD8\(^+\) T cells, which further boost Th1 response forming a positive feedback loop to amplify allogenic Th1 response. Th1 cells also activate B cells, which produce alloreactive antibodies, and cause acute rejection of the allograft.\textsuperscript{41} In contrary, IFN-γ is also believed to regulate alloimmune response and help in the induction of transplantation tolerance. On a similar line, IL-12 or IFN-γ deficient recipient mice showed the faster rejection of rat cardiac xenograft then wild-type mice.\textsuperscript{42} Furthermore, IFN-γ\(^-\)(GKO) mice showed accelerated rejection of kidney and cardiac allografts.\textsuperscript{43-45} IFN-γ expression of alloantigen reactive Tregs was found to be required for their function in controlling skin allograft rejection.\textsuperscript{46}

**Th2 cells**

Exposure of naive CD4\(^+\) T cells to IL-4 or IL-33 drives Th2 differentiation through activation of Signal Transducer and Activator of Transcription 6 (STAT6) and GATA3. Th2 cells were shown to mediate allograft rejection by inducing activation of eosinophils.\textsuperscript{47} In human renal allograft recipients, Th2 cells dominate during chronic rejection.\textsuperscript{48} Transfer of in-vitro generated Th2 cells induced rejection of H-Y disparate skin graft.\textsuperscript{49} In contrast, immunosuppressive Th2 cytokine IL-10 secreted by alloantigen-reactive CD4\(^+\) Tregs is shown to inhibit proliferation and Th1 differentiation of naive CD4\(^+\) T cells, which was found to be responsible for tolerance to alloantigen in-vivo.\textsuperscript{50}

**Th17 cells**

The cytokine transforming growth factor-β (TGF-β) with IL-6 drives the expression of RORyt leading to the differentiation of CD4\(^+\) T-cell to Th17 lineage. Renal biopsy of rejected kidney graft showed the presence of CD4 and IL-17, suggesting the role of Th17 cells in transplantation rejection.\textsuperscript{51} Alloantigen-specific CD4\(^+\) T cell lines derived from human patients of chronic allograft rejection show high levels of cytokines such as IL-2, IL-17, and IFN-γ, which suggest the role of Th1 and Th17 in chronic allograft rejection.\textsuperscript{52, 53} Graft interstitial infiltrates show expression of IL-17 and IL-21, which correlated with reduced allograft survival.\textsuperscript{54} In murine lung transplantation, neutralization of IL-6 was shown to control the IL-17-induced severity
of tracheal obliteration. Furthermore, IL-17 production by CD8+ T cells was found to be responsible for rejection of cardiac allograft in T-bet−/− recipient in the presence of CD40-CD40L blockade. IL-17-mediated recruitment of neutrophils is found to be associated with cardiac allograft rejection.

Regulatory T cells (Tregs)

Regulatory cells are recognized to promote and maintain donor antigen-specific tolerance. Sakaguchi et al. showed that IL-2 receptor alpha-chain (CD25) expressing CD4 T cells are suppressive CD4+ T cells that help in maintaining self-tolerance and are known as regulatory CD4 T cells (Tregs). Drugs like rapamycin directly inhibit the mTOR pathway and promote Treg differentiation. Tregs are already being used in the clinic as a successful cellular therapy. Literature supports that rather than independent functions, the interaction of Tregs with APCs and microenvironment mediates immunosuppression. IL-2 and TGF-β induce Foxp3 expression and differentiation of naïve CD4+ T cells towards Treg lineage. Tregs themselves are anergic, but their overexpression of CD25 helps them to quench IL-2 and suppress the activation of effector T cells.Expression of granzymes by Tregs, cytotoxic T lymphocytes (CTL) and NK cells induce killing of APCs, which leads to compromised antigen presentation. Cytotoxic T-lymphocyte-associated antigen 4 (CTLA4) expression mediates the tolerogenic activity of Tregs. CTLA4 binding delivers inhibitory signals by counteracting CD80 costimulation, inhibition of TCR immune synapse and increasing the mobility of T cells, which reduces their ability to interact with APCs. TGF-β secretion by Tregs or Foxp3-cells (Tr1 cells) is shown to mediate the generation of induced Tregs (iTregs).

Transplantation tolerance

Immunologic tolerance came into light when Ray Owen observed that dizygotic bovine twins are tolerant to each other’s blood due to placental interchange during gestation. In transplantation, operational tolerance is defined as well-functioning of the graft with no rejection sign in the absence of any immunosuppressive drug. Achieving transplant tolerance remains elusive beyond the liver transplantation in human patients. Studies with non-human primates have helped in identifying potential tolerogenic approaches. Deliberately establishing tolerance to the donor tissues by reprogramming the recipient’s immune system holds great promise for the success of organ transplantation. In the 1970s, Gerson and Kondo used thymectomized, lethally irradiated, bone marrow-reconstituted mice and showed that a subset of bone marrow-derived (BMD) lymphocytes makes antibody. The antibody production was independent of thymic lymphocyte interaction and can be tolerated in the absence of thymus-derived lymphocytes. Another subset of BMD cells that require thymus-derived lymphocytes cannot be tolerated. However, newly emerging cells from the bone-marrow can break the antigenic tolerance to the primary antigen and proposed that induction of tolerance as well as immune response in thymus is dependent on BMD cell population and they require co-operation of thymic lymphocytes. Various protocols have been used for successful induction of tolerance to alloantigen in the rodent model. Majority of the strategies used for tolerance induction relies on regulating T-cells functions by either intensive T cell depletion or costimulatory-blockade.

Central and peripheral tolerance

Recombination of TCR genes in immature thymocytes gives rise to some cells with potential self-reactivity. Central tolerance mechanisms operate in thymus due to which thymocytes which have a TCR with low affinity for the self-peptide-MHC complex are positively selected, while the unwanted ones die due to neglect. Central
tolerance comprises of four processes-clonal deletions, clonal diversion, anergy, and receptor editing. Clonal deletion mediates apoptosis of the cells which have high affinity TCR-peptide and MHC interaction leading to the elimination of self-reactive clones. During the clonal diversion, cytokines TGF-β and IL-2 suppress the apoptosis of T cells, which show high-affinity interaction with self-MHC molecules and mediates their conversion to Tregs. In the receptor editing, thymocytes with high-self-reactivity undergo secondary gene-arrangement at the TCRα loci, which leads to altered TCR specificity.

Peripheral tolerance takes care of the self-reactive cells that have escaped the central tolerance. Peripheral tolerance mediates unresponsiveness (anergy) or deletion of self-reactive T cells upon their encounter of self-antigen outside of the thymus. T cells activation, in the absence of costimulation, induces their long-term hypo-responsiveness and anergy in the T cells. Tregs express CD39 and CD73, which promote a hypoxic environment and regulate T-cells activation by inducing anergy. Negative costimulatory signals through CTLA4 and PD-1 play an important role in mediating peripheral tolerance. For peripheral deletion of self-reactive lymphocytes, Fas and Bim are recognized as important contributors. Binding of Fas-FasL mediates activation-induced cell death (AICD) in the T cells that had received repeated stimulation by self or foreign antigen. Furthermore, Bim helps in restoring homeostasis by killing activated T cells at the end of an immune response.

Regimens to induce transplantation tolerance

A. Chemotherapeutic drugs: Immunosuppressants
Immunosuppressive drugs are the mandatory treatment given after organ transplantation in human patients. Advances in immunosuppressive therapies have helped in controlling the acute rejection of the allograft. Currently used immunosuppressive drugs mainly fall under the category of glucocorticoids and cytostatics.

Glucocorticoids are the most crucial treatment required after organ transplantation. They act by inhibiting the expression of proinflammatory cytokines such as IL-2, IL-4, IL-6, and TNF-α. As a result of such immunosuppression, a general cell-mediated immune response is compromised. They also suppress humoral immunity by affecting the B cells expansion and antibody synthesis.

Calcineurin inhibitors are one of the widely used immunosuppressive drugs taken to prevent acute rejection. Cyclosporine and tacrolimus (also known as fujimycin or FK506) inhibit calcineurin, thereby inhibiting transcription of IL-2. Calcineurin also enhances the expression of TGF-β, which inhibits the proliferation of alloantigen-specific T cells.

Antiproliferative drugs like methotrexate and azathioprine are milder and given during the maintenance phase of the treatment. Azathioprine is a purine analog that blocks nucleic acid synthesis. Methotrexate is a competitive inhibitor of dihydrofolate reductase (DHFR; a folate analog), which inhibits the synthesis of nucleic acid. Advances in the understanding of immune system functioning have resulted in effective use of immunosuppressive drugs. However, generalized immunosuppression leads to a compromised immune system and a high risk of infection and cancer. Hence, the future of organ transplantation relies on the implementation of strategies to generate alloantigen-specific tolerance.

B. Biologics
Various components of the immune system have been targeted to attenuate the immune response to the allograft. Activation of naive CD4 or CD8 T cells requires three distinct signals. The first signal is the basis of the specificity of T cell response and is provided by the interaction of TCR on a T-cell with peptide bound to MHC molecules on APC. Costimulation, the second signal is provided by the interaction of cell surface molecules between T cells and APCs. Cytokines provide the third signal and determine the differentiation status of the T cell. It has been shown that, in the absence of the costimulatory signals, T cells become anergic, and obstinate to further stimulation by the same alloantigen. Due to the significant contribution of costimulatory signals in T cells activation and function, blocking of costimulatory signals stand out to be a potential target for suppressing the allograft-specific immunity.

Costimulatory molecules are categorized based on their structural and functional properties. Structurally, costimulatory molecules fall under four categories, immunoglobulin (Ig) superfamily (CD28 and ICOS), tumor necrosis factor receptor family (e.g., CD27, OX40, 4-1BB), cell adhesion receptors and integrins (LFA-1), and T cells Ig-domain and mucin domain (TIM) molecules. Functionally, positive costimulation promotes T cell activation, survival, and differentiation, whereas negative costimulation inhibits T cells activation and function. Different strategies employed for blocking the costimulatory signals for inducing long-
Term survival or tolerance to allograft are given in Table 1 and further described below:

(i) Blocking CD28-CD80/CD86 interaction
CD28 remains constitutively expressed on naive and activated CD4 and CD8 T cells, while its ligand CD80/CD86 are expressed on activated DCs.\textsuperscript{89,90} The importance of CD28 is evident, as CD28 knockout mice show significantly decreased IL-2 production resulting in reduced T cell activation, T cell differentiation, and defective B cell response.\textsuperscript{91} Upon activation, T cells upregulate CTLA4, which is a structural homolog of CD28, but have ~20-fold higher affinity for CD80/CD86. CTLA4 provides negative costimulation by inhibiting IL-2 synthesis as well as promoting the expansion of Tregs. Engagement of CD80/86 with CTLA4-Ig leads to upregulation of indoleamine 2, 3-dioxygenase (IDO), which degrades tryptophan, an essential amino acid required for T cell proliferation.\textsuperscript{92} CTLA4-Ig (abatacept) has been successfully used for the long-term survival of islet, cardiac, and renal allografts in rodents.\textsuperscript{93} Belatacept, a second-generation CD28 antagonist, has shown significant potential in phase

<table>
<thead>
<tr>
<th>No</th>
<th>Mice strains</th>
<th>Tolerogenic regimen</th>
<th>Median survival time (days)</th>
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<tbody>
<tr>
<td>1</td>
<td>C57BL/6 recipient, BALB/c donor\textsuperscript{45, 134}</td>
<td>Untreated</td>
<td>8 days</td>
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<td></td>
<td></td>
<td>DST</td>
<td>7 days</td>
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<td></td>
<td></td>
<td>Anti-CD40L</td>
<td>13 days</td>
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<td></td>
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<td>DST + Anti-CD40L</td>
<td>46 days</td>
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<td></td>
<td></td>
<td>Adult thymectomy + DST + Anti-CD40L</td>
<td>&gt; 100 days</td>
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<tr>
<td>2</td>
<td>C57BL/6 recipient, BALB/c donor\textsuperscript{135, 136}</td>
<td>CTLA4-Ig</td>
<td>10 days</td>
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<tr>
<td></td>
<td></td>
<td>CTLA4-Ig + Anti-CD40L</td>
<td>20 days</td>
</tr>
<tr>
<td>3</td>
<td>CBA/Ca recipient, B10.BR donor\textsuperscript{137, 138}</td>
<td>Adult thymectomy + CD8+ T cell-depletion + Anti-CD40L</td>
<td>Indefinite survival, &gt;100 days for the second donor graft</td>
</tr>
<tr>
<td>4</td>
<td>CD28\textsuperscript{-} C57BL/6 recipient, BALB/c donor\textsuperscript{136}</td>
<td>CD8\textsuperscript{+} T cell-depletion + Anti-CD40L</td>
<td>49 days</td>
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<tr>
<td></td>
<td></td>
<td>CD8\textsuperscript{+} T cell depletion + Anti-CD40L + CTLA4-Ig</td>
<td>57 days</td>
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<tr>
<td>5</td>
<td>C57BL/6 recipient, BALB/c donor\textsuperscript{139, 140}</td>
<td>Anti-CD45RB</td>
<td>Early rejection</td>
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<td>Anti-CD45RB + Anti-CD40L + CD4 T cell depletion</td>
<td>Acute rejection</td>
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<td>Anti-CD45RB + Anti-CD40L + CD8 T cell depletion</td>
<td>90 days</td>
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<tr>
<td>6</td>
<td>C57BL/6 recipient, DBA/2 donor\textsuperscript{52}</td>
<td>CTLA4-Ig + Anti-CD40L + Anti-OX-40L</td>
<td>100 days</td>
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</table>
(ii) Blocking CD40L-CD40 interaction

CD40L and CD40 interaction have been shown to play a central role in adaptive immunity. It has been shown that mutation in the CD40L gene leads to the X-linked hyper-IgM syndrome and reduced class switching to IgG, IgA, and IgE. Also due to the non-functional activity of CD40L molecules in X-linked hyper-IgM syndrome patients, thymus-dependent (TD) response, and generation of GCs, memory B cells, and plasma cells, is severely impaired. Activated CD4+ T cells and CD8+ T cells upregulate CD40L, whereas its ligand CD40 is shown to express on APCs. CD40L-CD40 ligation induces B cell maturation, class switching, plasma cell differentiation, and enhanced expression of CD80/CD86, which mediates the generation of T cell-dependent humoral immune response. Ligation of CD40 on DCs with CD40L expressed on activated CD4+ T cells results in increased expression of MHC, enhanced expression of costimulatory molecules, augmented production of proinflammatory cytokines such as IL-1, IL-6, IL-12, and increase the survival of DCs. These events lead to the generation of effective T cells response. CD40L-CD40 signaling is shown to augment inflammatory response by inducing the production of various chemokines (e.g., macrophage-inflammatory protein-1α MIP-1α, MIP1β, MCP-1 [monocyte chemoattractant protein-1] and RANTES [regulated upon activation, normal T cell expressed and secreted]), which mediate the recruitment of immune cells at the site of inflammation. The use of blocking monoclonal antibody against CD40L has shown success in prolonging the survival of skin, cardiac, and islet allograft in rodents as well as in non-human primates. However, anti-CD40L mAb therapy provides limited survival due to the activity of CD40L blockade resistant primed or memory CD8+ T cells. Hence, combined therapy of CD8+ T cells depletion along with anti-CD40L blockade was proven to be a better treatment to achieve long-term graft survival in the sensitized recipients. Although monoclonal anti-CD40L therapy showed promising results in murine models, however, clinical trials with anti-CD40L mAbs in patients with autoimmune disorders led to unexpected thromboembolic complications. Newer antibodies targeting the CD40/CD40L pathway have been shown to avoid platelet activation and consequent thromboembolic complications, but are yet to be translated into clinical trials. It has been shown that a glycosylated form of anti-CD154 possesses reduced ability to bind Fc receptors and activate complement and has been proven to be equally effective in prolonging allograft survival.

It has been shown that treatment with donor-specific transfusion (DST) along with antibody against CD40 ligand permits long-term survival of highly antigenic donor skin allografts, despite the presence of functionally intact alloreactive lymphocytes. Infusion of DST and anti-CD40L leads to pre-emptive induction of tolerance by inducing the mass reduction of alloreactive effector T cells and generating anergic/regulatory cells days before the placement of allograft.

Sensitization of host to a wide range of donor MHC antigens, which arises due to blood transfusions or previously failed grafts, remains one of the critical issues in clinical transplantation. Pre-exposure to DST leads to broad alloantigen-induced tolerance, which helps in subsequent engraftment of the allograft. Combination of DST and cyclosporine is shown to be effective in reducing acute rejection of the human renal allografts. The infused DST is taken up and processed by APCs, which present the alloantigen to recipient alloreactive T cells in the presence of anti-CD40L antibody. This leads to rapid abortive expansion of alloreactive T cells, which results in anergy as shown in Figure 1. Besides, anti-CD40L antibody prevents the maturation of host APCs inducing them for the tolerogenic presentation of DST-derived allopeptides. CD40L blockade inhibits the generation of growth factors like IL-2, IL-7, and IL-15. This also impacts the T cells activation and function. Blocking with anti-CD40L antibody is shown to enhance iTregs development, which in-turn suppresses the alloreactive T cells response and induce prolonged allograft survival.

Blocking of CD40L/CD40 interaction is reported to induce long-term tolerance to cardiac, skin, islets, myoblasts, limbs and bone marrow transplants. Anti-CD40L antibody inhibits maturation of APCs, downmodulate CD28-B7 interaction, resulting in the lack of costimulatory signals and T cell anergy. Combined treatment with an antibody against CD40 ligand, along with the one transfusion of donor splenocytes, prolonged survival of fully mismatched BALB/c skin allografts on C57BL/6 recipients (~20% of grafts survived more than 100 days). However, indefinite allograft tolerance is not possible with DST plus anti-CD40L therapy, especially during highly allogenic grafts like skin. Later, it was shown that thymectomy in allograft recipients treated with DST plus anti-CD40L mAb induced indefinite allograft survival. Additional strategies of simultaneous but blockade of the CD28 and CD40 pathways effectively aborts the T cell expansion both in-vitro and in-vivo and...
promotes long-term survival of fully allogenic skin grafts and inhibit the development of chronic vascular rejection of cardiac allografts. Simultaneous blockade of CD28 and CD40 effectively promotes skin allograft survival in C3H/HeJ mice extending the MST beyond 100 days, but with the same treatment C57BL/6 mice rejected allografts with MSTs ranging between 20 to 30 days. However, CD28 and CD40 pathways are critically independent regulators of T-cell-dependent immune responses. Blocking of CD28/B7 inhibits the primary T cell response, and CD40L/CD40 blocking inhibits the Th1 differentiation and maintenance of alloantigen-specific response. Conclusively, CD40/CD40L blocking leads to prolonged allograft survival and show a synergistic effect when used in combination with the blocking of other members of costimulatory molecules.

(iii) Blocking OX40-OX40L interaction
OX40 (CD134) and its ligand OX40L (CD252) belong to tumor necrosis factor superfamily of costimulatory signaling molecules and are expressed primarily on activated CD4+ and CD8+ T cells. Signals from OX40 promote T cell survival, clonal expansion of effector and memory population. Also, OX40 suppresses the differentiation and function of Tregs. The importance of OX40/OX40L pathway came into light upon demonstration of rejection of allografts in CD28 and CD40L double knockout (DKO) mice. The sole blockade of OX40/OX40L pathway leads to the prolonged survival of skin allograft in CD28 and CD40L DKO mice. The blockade of OX40/OX40L signaling along with CD28/CD40L blockade leads to prolonged skin allograft survival. It was shown that memory T cells express a higher level of OX40 and mediate rejection of allograft in the absence of CD28 and CD40L signaling.

(iv) Blocking ICOS-ICOSL interaction
Inducible T cell costimulator (ICOS) expression is induced on the surface of activated T cells. Its ligand ICOSL is upregulated on activated APCs. ICOS-ICOSL signaling promotes T cells activation and differentiation, and enhances T cell-dependent B cell response.
CD28-CD80/CD86 interaction is shown to optimize ICOS expression. Stimulation of ICOS in activated T cells induces production of IFN-γ, IL-4 and IL-10. Blocking of ICOS and CD40L has been shown to prevent chronic rejection in mouse cardiac transplantation model.  

(v) Blocking CD27-CD70 interaction
CD27 belongs to TNF superfamily and is expressed on NK cells, and naive T and B cells. Its ligand CD70 is expressed on APCs. Signaling through CD27 induces positive costimulatory signals leading to T cell proliferation and survival. Blocking of the CD27-CD70 pathway has been shown to prolong the murine cardiac allograft survival. Combination of CD44/CD70 blockade along with anti-CD40/LFA-1 inhibited the expansion of memory CD4+ and CD8+ T cells, and prolonged the survival of cardiac allograft.

(vi) Blocking 41BB-41BBL interaction
41BB (CD137) is a member of TNFR superfamily, which is shown to promote CD8+ T cells proliferation by binding to its ligand 41BBL expressed on mature APCs. Anti-4-1BB mAb augmented the generation of alloantigen-specific CD8 T cells in graft vs. host disease and enhanced the rate of cardiac and skin allograft rejection. Thus 41BB-41BBL pathway serves as an important target in CD8+ T cells-dependent rejection.

(vii) Blocking LFA-1: ICAM interaction
Lymphocyte function-associated antigen-1 (LFA-1) is a member of β2 integrin family mediating the adhesion of leukocytes to the endothelium. The ligand for LFA-1, intercellular adhesion molecule-1 (ICAM-1) is shown to be expressed on mononuclear cells, B cells and vascular endothelium. The survival of allogenic islet and cardiac grafts were prolonged in the presence of anti-LFA-1 mAb, which blocks LFA-1-ICAM-1 interaction. Furthermore, when combined with costimulatory blockade (CTLA4-Ig plus anti-CD40L mAb), treatment with blocking anti-VLA4 and anti-LFA-1 mAb, controlled the CD8 memory T cells trafficking and functions leading to the enhanced survival of murine skin allograft.

(viii) Promoting PD-1-PD-L1/L2 interaction
Similar to CTLA-4, PD-1 (CD279) is another member of Ig superfamily that shows co-inhibitory functions by suppressing T cells activation and maintaining peripheral tolerance to self-antigens. Blocking antibodies targeting PD-L1 prevents T cell apoptosis, increase T cell proliferation and Th1 cell differentiation, which result in the faster rejection of MHC class II-mismatched skin grafts.

C. Inducing chimerism for transplantation tolerance
Chimerism can be categorized into mixed chimerism and full chimerism. Mixed chimerism, as the name suggests, is defined when both donor and recipient cells co-exist in the recipient body. Whereas, full chimerism implies complete elimination of recipient hematopoietic lineages and the existence of 100 percent donor cells in the recipient bone marrow. Reports suggested that partial irradiation of the recipient bone marrow combined with deletion of recipient T cells in peripheral organs leads to mixed chimerism, which supports the induction of tolerance towards donor tissue. Mixed chimerism has been shown to promote tolerance generation towards kidney allograft.

D. B-cell therapy for transplantation tolerance
Tolerant renal transplant patients show an increased percentage of naive B cells and transitional B cells. Transitional B cells are known to produce immunoregulatory cytokine IL-10.122 Long-term islet allograft survival was achieved in recipients that received a combination of rabbit anti-thymocyte globulin (ATG) and rituximab, a CD20+ B cell-depleting mAb. Blockade of BAFF (B cell-activating factor) using belimumab promoted tolerance of cardiac and islet allografts in murine models. Belimumab induces the depletion of alloreactive B cells, and promote transitional B cells, and abrogate alloantibody response. Bortezomib is a proteasome inhibitor that causes apoptosis of plasma cells and helps in the regulation of alloantibody synthesis and shown to improve allograft survival. Apart from alloantibody production of B cells, the role of regulatory B cells was also reported in transplantation. We have shown that CD40-CD40L costimulatory blockade induces IL-10-producing marginal zone B cells (MZP Bregs) and it acts as regulatory B cells and contributes to the generation of tolerance to cardiac allograft in mouse. Depletion of B cells during the generation of tolerance inhibits the survival of cardiac allografts and caused acute rejection of allograft. It has been shown that adoptive transfer of IL-10-producing MZP Bregs promotes the follicular regulatory CD4+ T cells (Tfr) cells and inhibit the differentiation of inflammatory follicular CD4+ T cells (Tfr) in the secondary lymphoid organs. These studies suggest that B cells play an important role in transplantation tolerance. Recently, a clinical study on kidney transplantation showed that presence of donor-specific HLA antibodies (DSA) in the circulation has direct link with the survival of allograft and monitoring the DSA in serum before transplant may help in risk stratification of patient. Similarly, another study from
Netherlands showed that the presence of autoantibodies against Rho-GDP dissociation inhibitor 2 (ARHGDIB) is significantly associated with loss of renal transplants and monitoring ARHGDIB antibody in recipients may help in the pretransplant risk assessment.\textsuperscript{127}

E. Role of innate immune cells in transplantation tolerance

Macrophages acquire regulatory phenotype (Mreg) in response to macrophage colony-stimulating factor (M-CSF) and IFN-γ and help in allograft survival. Mregs are reported to enhance cardiac allograft survival by directly eliminating allogenic T cells through phagocytosis. Besides, the production of iNOS, Mregs decreases IL-2 and IFN-γ production and suppress the proliferation of alloreactive T cells.\textsuperscript{128} Production of indoleamine 2,3-dioxygenase (IDO) by Kupffer cells leads to degradation of tryptophan which is a crucial molecule required for efficient T-cell proliferation.\textsuperscript{129} Apart from this, macrophages are shown to play an important role in wound healing, which helps in resolving the injury caused during transplantation surgery. In the lymphopenic hosts, NK cells compete for IL-15 and suppress the homeostatic proliferation of memory CD8 T cells, which help in inducing the prolonged allograft survival.\textsuperscript{130} DCs are known to mediate tolerance vs. rejection, depending upon their maturation status. The immunosuppressive potential of these immature/tolerogenic DCs arises due to their deficient expression of class II MHC and costimulatory molecules.\textsuperscript{131} Cognate interaction of immature DCs with Ag-specific T cells leads to anergy or apoptosis. In support, infusion of immature DCs in cardiac allograft recipients treated with anti-ICAM-1 and CTLA-4-Ig led to prolonged allograft survival.\textsuperscript{132} The test of autologous monocyte-derived Tol-DC in kidney transplant patients is a future prospect.\textsuperscript{133}

Conclusion

It has been well established that transplantation tolerance requires very complex, multi-cellular, and three-dimensional interaction in the allograft and in the secondary lymphoid tissues. The cumulative interaction of various cells and its molecular signaling dictates the clinical advantage of the establishment of tolerance. Several of these interactions were discovered and also under investigation, which will guide us in developing the new strategies to control the inflammation and promote the allogenic tolerance in the clinic. Discovery of new regulatory immune cells for adoptive cellular therapy, neutralizing the inflammatory cytokines, blocking/antagonizing the activating receptors or disrupting the migration of inflammatory immune cells into the allograft may provide the new leads in the transplantation immunology.

Funding

Shilpi received JRF/SRF from Indian Council of Medical Research (ICMR), Government of India. GL received grants from the Department of Biotechnology (Grants numbers, BT/PR15533/MED/30/1616/2015; BT/PR14156/BRR/10/1515/2016), Science Education and Research Board (EMR/2016/007108), and Department of Science and Technology (DST/SJF/LSA-01/2017-18), Government of India.

Competing interests

The authors declare that they have no competing interests.

Citation


Submitted: 30 July 2019, Accepted: 22 August 2019 Published: 9 September 2019

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