IJIR. 2019;(3)1:R4 DOI: 10.15305/ijir/v3i1/318

REVIEWS

Multicellular and multimolecular immune interactions in the transplantation tolerance and rejection

Shilpi[1], Girdhari Lal[2]*

[1, 2,]National Centre for Cell Science, Pune, MH-411007, India

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] Parallelly, 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. [6] 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.[7] 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.[8] 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.[9] 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.[10-12] 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.[13] 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.[14] 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.[15] 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.[16] 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.[17] 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.[18] 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.[18] 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.[14, 19] 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.[20]

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.[21] In acute and chronic rejection, macrophages account for about 38-60% of total graft infiltrating leukocytes in the rejecting organs.[21-23] Increased infiltration of macrophages within glomeruli is

associated with reduced survival of renal transplants.[24] Allograft infiltrating macrophages are associated with acute cell-mediated rejection as well as acute antibody-mediated rejection.[21] Depletion of macrophages reduces the allograft injury and pathology of rejecting allografts.[23, 25] 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.[26, 27] NK cells are known to control the survival of hematopoietic as well as solid organ transplantations.[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.[30, 31] NK cells eliminate donor antigen-presenting cells in the graft, which mediates the long-term survival of skin allografts.[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.[33] Mast cells act as antigen-presenting cells, express several co-stimulatory molecules and produce wide varieties of both anti-inflammatory and proinflammatory molecules.[34, 35] Mast cell degranulation breaks the peripheral tolerance in skin and cardiac allograft.[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.[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.[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.[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.[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.[42] Furthermore, IFN-$\gamma^{-/-}$ (GKO) mice showed accelerated rejection of kidney and cardiac allografts.[43-45] IFN-γ expression of alloantigen reactive Tregs was found to be required for their function in controlling skin allograft rejection.[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.[47] In human renal allograft recipients, Th2 cells dominate during chronic rejection.[48] Transfer of *in-vitro* generated Th2 cells induced rejection of H-Y disparate skin graft.[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*.[50]

Th17 cells

The cytokine transforming growth factor-β (TGF-β) with IL-6 drives the expression of RORγt 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.[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.[52, 53] Graft interstitial infiltrates show expression of IL-17 and IL-21, which correlated with reduced allograft survival.[54] In murine lung transplantation, neutralization of IL-6 was shown to control the IL-17-induced severity

of tracheal obliteration.[55] 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.[56] IL-17-mediated recruitment of neutrophils is found to be associated with cardiac allograft rejection.[57]

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).[58] 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 naive $CD4^+$ T cells towards Treg lineage.[59, 60] Tregs themselves are anergic, but their overexpression of CD25 helps them to quench IL-2 and suppress the activation of effector T cells.[58, 61] Expression of granzymes by Tregs, cytotoxic T lymphocytes (CTL) and NK cells induce killing of APCs, which leads to compromised antigen presentation.[62] Cytotoxic T-lymphocyte-associated antigen 4 (CTLA4) expression mediates the tolerogenic activity of Tregs. CTLA4 binding delivers inhibitory signals by counteracting CD28 costimulation, inhibition of TCR immune synapse and increasing the mobility of T cells, which reduces their ability to interact with APCs.[63] TGF-β secretion by Tregs or Foxp3-cells (Tr1 cells) is shown to mediate the generation of induced Tregs (iTregs).[64-66] TGF-β has also been shown to induce the expression of regulatory markers CD39 and CD73 on T cells and DCs.[67, 68] IL-10 secreted by Tregs or Foxp3 negative Tr1 cells have been shown to regulate inflammatory bowel disease (IBD), experimental autoimmune encephalomyelitis (EAE), collagen-induced arthritis, and allergic airway inflammation.[69,70] Adoptive transfer experiments with Tr1 cells were shown to suppress allogenic skin graft rejection.[71] Treatment of recipients with IL-2-IL-2 mAb complex induce the expansion of Tregs *in-vivo*, which in turn known to promote the survival of islet allograft.[72] Tregs generated in the presence of alloantigen and IFN-γ prevents the rejection of islet and skin allograft.[73] Exposure of DCs and macrophages to TGF-β and IL-10 or retinoic acid leads to an augmentation of their ability to induce Tregs and mediate tolerance against allograft.[74]

Apart from Tregs, adoptive transfer of tolerogenic DCs is shown to promote tolerance in recipients to skin allografts.[75] Immature DCs, which are reportedly tolerogenic, are shown to prolong the survival of cardiac allograft.[76] Techniques for the propagation of tolerogenic DCs have been employed as cellular therapy in hematopoietic stem cell transplantation. Infusion of immature DC pulsed with antigen led to the generation of antigen-specific regulatory $CD8^+$ T cells, which efficiently secrete IL-10, but have impaired IFN-γ secretion and cytolytic functions.[77]

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.[78] 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 tolerized in the absence of thymus-derived lymphocytes.[79] Another subset of BMD cells that require thymus-derived lymphocytes cannot be tolerized.[79] 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.[79] 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.[80] 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.[81] In the receptor editing, thymocytes with high-self-reactivity undergo secondary gene-arrangement at the TCRα loci, which leads to altered TCR specificity.[81]

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.[82] 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.[83] 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.[83]

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.[84] 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.[85]

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.[86] 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.[87] 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.[88] 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-

Table 1: Costimulatory-blockade and T cell depletion to prolong the survival of murine allogenic skin allograft

No	Mice strains	Tolerogenic regimen	Median survival time (days)
1	C57BL/6 recipient, BALB/c donor [45, 134]	Untreated	8 days
		DST	7 days
		Anti-CD40L	13 days
		DST + Anti-CD40L	46 days
		Adult thymectomy + DST + Anti-CD40L	> 100 days
2	C57BL/6 recipient, BALB/c donor [135, 136]	CTLA4-Ig	10 days
		CTLA4-Ig + Anti-CD40L	20 days
3	CBA/Ca recipient, B10.BR donor[137, 138]	Adult thymectomy + CD8+ T cell-depletion + Anti-CD40L	Indefinite survival, >100 days for the second donor graft
4	$CD28^{-/-}$ C57BL/6 recipient, BALB/c donor[136]	$CD8^{+}$ T cell-depletion + Anti-CD40L	49 days
		$CD8^{+}$ T cell depletion + Anti-CD40L + CTLA4-Ig	57 days
5	C57BL/6 recipient, BALB/c donor [139, 140]	Anti-CD45RB	Early rejection
		Anti-CD45RB + Anti-CD40L	Prolonged
		Anti-CD45RB + Anti-CD40L + CD4 T cell depletion	Acute rejection
		Anti-CD45RB + Anti-CD40L+ CD8 T cell depletion	90 days
6	C57BL/6 recipient, DBA/2 donor[32]	CTLA4-Ig + Anti-CD40L+ Anti-OX-40L	100 days

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.[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.[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.[92] CTAL4-Ig (abatacept) has been successfully used for the long-term survival of islet, cardiac, and renal allografts in rodents.[93] Belatacept, a second-generation CD28 antagonist, has shown significant potential in phase

III (BENEFIT) trial and showed that it is superior in inducing long-term renal graft function over cyclosporine.[94]

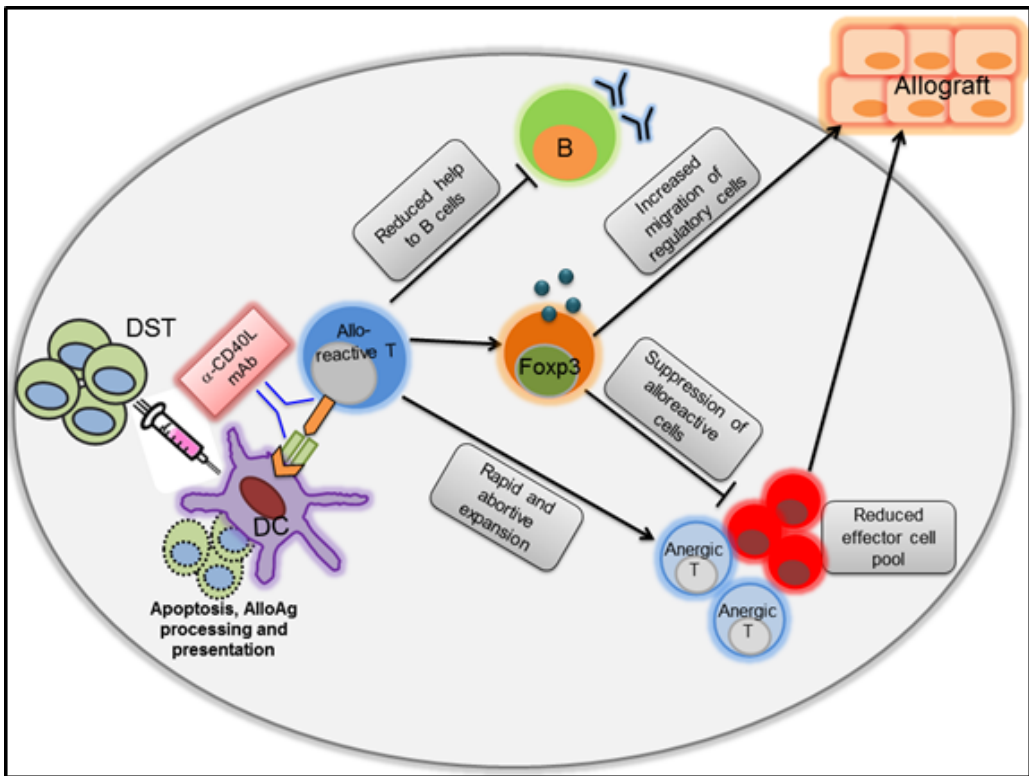
(ii) Blocking CD40L-CD40 interaction

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.[95] 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.[96] 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.[97] 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.[98] 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.[99] 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.[100] 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.[101]

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.[102] 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.[103] 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.[103] Combination of DST and cyclosporine is shown to be effective in reducing acute rejection of the human renal allografts.[104] 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.[105] Blocking of CD40L/CD40 interaction is reported to induce long-term tolerance to cardiac, skin, islets, myoblasts, limbs and bone marrow transplants.[106, 107] 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).[102] 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.[45] Additional strategies of simultaneous but blockade of the CD28 and CD40 pathways effectively aborts the T cell expansion both i*n-vitro* and *in-vivo* and

Fig. 1. Mechanism of induction of tolerance with donor splenocytes transfusion (DST) and anti-CD40L mAb

Upon injection, DST is taken up by the host DCs, leading to the generation and presentation of alloantigenic peptides. Presentation of donor alloantigen bound to host APCs in the absence of CD40/CD40L signals leads to rapid and abortive expansion of T cells. Due to this, the part of alloreactive cells undergoes apoptosis or become hyporesponsive (anergic) to further stimulation. Parallelly, this environment supports the expansion of alloantigen-specific regulatory T cells enter into the allograft and control the function of effector cells. Furthermore, the blockade of CD40/CD40L interaction leads to inhibition of B cells activation and maturation, which hampers the alloantigen-specific alloantibody response.



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.[98] Blocking of CD28/B7 inhibits the primary T cell response, and CD40L/CD40 blocking inhibits the Th1 differentiation and maintenance of alloantigen-specific response.[108] 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.[109] 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.[110] 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.[111]

(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.[112, 113]

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.[114]

(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.[115] Combination of CD44/CD70 blockade along with anti-CD40L/LFA-1 inhibited the expansion of memory $CD4^+$ and $CD8^+$ T cells, and prolonged the survival of cardiac allograft.[116]

(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.[117] 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.[118] 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.[119]

(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.[120]

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.[121]

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.[121] Apart from alloantibody production of B cells, the role of regulatory B cells was also reported in transplantation.[123] 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.[124, 125]. Depletion of B cells during the generation of tolerance inhibits the survival of cardiac allografts and caused acute rejection of allograft.[125] 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.[124] 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.[126] 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.[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.[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.[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.[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.[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.[132] The test of autologous monocyte-derived Tol-DC in kidney transplant patients is a future prospect.[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/BRB/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

Shilpi, Girdhari Lal. Multicellular and multimolecular immune interactions in the transplantation tolerance and rejection IJIR. 2019;(3)1:R4.

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

*Correspondence: Dr. Girdhari Lal
glal@nccs.res.in

References

1. Watson CJ, Dark JH. Organ transplantation: historical perspective and current practice. British journal of anaesthesia 2012; 108 Suppl 1: i29-42.
2. Grinyo JM. Why is organ transplantation clinically important? Cold Spring Harbor perspectives in medicine 2013; 3(6).
3. Salvadori M, Rosso G, Bertoni E. Update on ischemia-reperfusion injury in kidney transplantation: Pathogenesis and treatment. World journal of transplantation 2015; 5(2): 52-67.
4. Kosieradzki M, Rowinski W. Ischemia/reperfusion injury in kidney transplantation: mechanisms and prevention. Transplantation proceedings 2008; 40(10): 3279-3288.
5. Cozzi E, Colpo A, De Silvestro G. The mechanisms of rejection in solid organ transplantation. Transfusion and apheresis science : official journal of the World Apheresis Association : official journal of the European Society for Haemapheresis 2017; 56(4): 498-505.
6. Benichou G. Direct and indirect antigen recognition: the pathways to allograft immune rejection. Frontiers in bioscience : a journal and virtual library 1999; 4: D476-480.
7. Scozzi D, Ibrahim M, Menna C, Krupnick AS, Kreisel D, Gelman AE. The Role of Neutrophils in Transplanted Organs. Am J Transplant 2017; 17(2): 328-335.
8. Li W, Nava RG, Bribriesco AC, Zinselmeyer BH, Spahn JH, Gelman AE et al. Intravital 2-photon imaging of leukocyte trafficking in beating heart. The Journal of clinical investigation 2012; 122(7): 2499-2508.
9. Kish DD, Gorbachev AV, Parameswaran N, Gupta N, Fairchild RL. Neutrophil expression of Fas ligand and perforin directs effector CD8 T cell infiltration into antigen-challenged skin. J Immunol 2012; 189(5): 2191-2202.
10. El-Sawy T, Belperio JA, Strieter RM, Remick DG, Fairchild RL. Inhibition of polymorphonuclear leukocyte-mediated graft damage synergizes with short-term costimulatory blockade to prevent cardiac allograft rejection. Circulation 2005; 112(3): 320-331.
11. Uchida Y, Freitas MC, Zhao D, Busuttil RW, Kupiec-Weglinski JW. The protective function of neutrophil elastase inhibitor in liver ischemia/reperfusion injury. Transplantation 2010; 89(9): 1050-1056.

12. Kim SY, Moon KA, Jo HY, Jeong S, Seon SH, Jung E et al. Anti-inflammatory effects of apocynin, an inhibitor of NADPH oxidase, in airway inflammation. Immunol Cell Biol 2012; 90(4): 441-448.
13. Kreisel D, Sugimoto S, Zhu J, Nava R, Li W, Okazaki M et al. Emergency granulopoiesis promotes neutrophil-dendritic cell encounters that prevent mouse lung allograft acceptance. Blood 2011; 118(23): 6172-6182.
14. Schlichting CL, Schareck WD, Kofler S, Weis M. Involvement of dendritic cells in allograft rejection new implications of dendritic cell-endothelial cell interactions. Mini reviews in medicinal chemistry 2007; 7(4): 423-428.
15. Morelli AE. Dendritic cells of myeloid lineage: the masterminds behind acute allograft rejection. Curr Opin Organ Transplant 2014; 19(1): 20-27.
16. Sivaganesh S, Harper SJ, Conlon TM, Callaghan CJ, Saeb-Parsy K, Negus MC et al. Copresentation of intact and processed MHC alloantigen by recipient dendritic cells enables delivery of linked help to alloreactive CD8 T cells by indirect-pathway CD4 T cells. J Immunol 2013; 190(11): 5829-5838.
17. Zhuang Q, Liu Q, Divito SJ, Zeng Q, Yatim KM, Hughes AD et al. Graft-infiltrating host dendritic cells play a key role in organ transplant rejection. Nature communications 2016; 7: 12623.
18. Marin E, Cuturi MC, Moreau A. Tolerogenic Dendritic Cells in Solid Organ Transplantation: Where Do We Stand? Frontiers in immunology 2018; 9: 274.
19. Raimondi G, Thomson AW. Dendritic cells,tolerance and therapy of organ allograft rejection. Contributions to nephrology 2005; 146: 105-120.
20. Zhang Y, Shen S, Zhao G, Xu CF, Zhang HB, Luo YL et al. In situ repurposing of dendritic cells with CRISPR/Cas9-based nanomedicine to induce transplant tolerance. Biomaterials 2019; 217: 119302.
21. Salehi S, Reed EF. The divergent roles of macrophages in solid organ transplantation. Curr Opin Organ Transplant 2015; 20(4): 446-453.
22. Schmidt A, Sucke J, Fuchs-Moll G, Freitag P, Hirschburger M, Kaufmann A et al. Macrophages in experimental rat lung isografts and allografts: infiltration and proliferation in situ. J Leukoc Biol 2007; 81(1): 186-194.
23. Jose MD, Ikezumi Y, van Rooijen N, Atkins RC, Chadban SJ. Macrophages act as effectors of tissue damage in acute renal allograft rejection. Transplantation 2003; 76(7): 1015-1022.
24. dos Santos DC, de Andrade LG, de Carvalho MF, Moraes Neto FA, Viero RM. Mononuclear inflammatory infiltrate and microcirculation injury in acute rejection: role in renal allograft survival. Renal failure 2013; 35(5): 601-606.
25. Qi F, Adair A, Ferenbach D, Vass DG, Mylonas KJ, Kipari T et al. Depletion of cells of monocyte lineage prevents loss of renal microvasculature in murine kidney transplantation. Transplantation 2008; 86(9): 1267-1274.
26. Paul S, Lal G. The Molecular Mechanism of Natural Killer Cells Function and Its Importance in Cancer Immunotherapy. Front Immunol 2017; 8: 1124.
27. Rueff J, Medinger M, Heim D, Passweg J, Stern M. Lymphocyte subset recovery and outcome after autologous hematopoietic stem cell transplantation for plasma cell myeloma. Biol Blood Marrow Transplant 2014; 20(6): 896-899.
28. van der Touw W, Bromberg JS. Natural killer cells and the immune response in solid organ transplantation. Am J Transplant 2010; 10(6): 1354-1358.
29. Benichou G, Yamada Y, Aoyama A, Madsen JC. Natural killer cells in rejection and tolerance of solid organ allografts. Curr Opin Organ Transplant 2011; 16(1): 47-53.
30. van der Touw W, Burrell B, Lal G, Bromberg JS. NK cells are required for costimulatory blockade induced tolerance to vascularized allografts. Transplantation 2012; 94(6): 575-584.
31. Beilke JN, Kuhl NR, Van Kaer L, Gill RG. NK cells promote islet allograft tolerance via a perforin-dependent mechanism. Nature medicine 2005; 11(10): 1059-1065.
32. Yu G, Xu X, Vu MD, Kilpatrick ED, Li XC. NK cells promote transplant tolerance by killing donor antigen-presenting cells. The Journal of experimental medicine 2006; 203(8): 1851-1858.
33. Elieh Ali Komi D, Ribatti D. Mast cell-mediated mechanistic pathways in organ transplantation. Eur J Pharmacol 2019; 857: 172458.
34. Mortaz E, Amani S, Mumby S, Adcock IM, Movassaghi M, Folkerts J et al. Role of Mast Cells and Type 2 Innate Lymphoid (ILC2) Cells in Lung Transplantation. Journal of immunology research 2018; 2018: 2785971.
35. de Vries VC, Pino-Lagos K, Nowak EC, Bennett KA, Oliva C, Noelle RJ. Mast cells condition dendritic cells to mediate allograft tolerance. Immunity 2011; 35(4): 550-561.
36. de Vries VC, Wasiuk A, Bennett KA, Benson MJ, Elgueta R, Waldschmidt TJ et al. Mast cell degranulation breaks peripheral tolerance. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2009; 9(10): 2270-2280.
37. Ngo Nyekel F, Pacreau E, Benadda S, Msallam R, Abrink M, Pejler G et al. Mast Cell Degranulation Exacerbates Skin Rejection by Enhancing Neutrophil Recruitment. Front Immunol 2018; 9: 2690.
38. Bolton EM, Gracie JA, Briggs JD, Kampinga J, Bradley JA. Cellular requirements for renal allograft rejection in the athymic nude rat. The Journal of experimental medicine 1989; 169(6): 1931-1946.
39. Eteghadi A, Pak F, Ahmadpoor P, Jamali S, Karimi M, Yekaninejad MS et al. Th1, Th2, Th17 cell subsets in two different immunosuppressive protocols in renal allograft recipients (Sirolimus vs mycophenolate mofetil): A cohort study. International immunopharmacology 2019; 67: 319-325.
40. D'Elios MM, Josien R, Manghetti M, Amedei A, de Carli M, Cuturi MC et al. Predominant Th1 cell infiltration in acute rejection episodes of human kidney grafts. Kidney international 1997; 51(6): 1876-1884.
41. Ingulli E. Mechanism of cellular rejection in transplantation. Pediatric nephrology (Berlin, Germany) 2010; 25(1): 61-74.
42. Wang H, DeVries ME, Deng S, Khandaker MH, Pickering JG, Chow LH et al. The axis of interleukin 12 and gamma interferon regulates acute vascular xenogeneic rejection. Nature medicine 2000; 6(5): 549-555.
43. Konieczny BT, Dai Z, Elwood ET, Saleem S, Linsley PS, Baddoura FK et al. IFN-gamma is critical for long-term allograft survival induced by blocking the CD28 and CD40 ligand T cell costimulation pathways. Journal of immunology (Baltimore, Md : 1950) 1998; 160(5): 2059-2064.
44. Halloran PF, Afrouzian M, Ramassar V, Urmson J, Zhu LF, Helms LM et al. Interferon-gamma acts directly on rejecting renal allografts to prevent graft necrosis. The American journal of pathology 2001; 158(1): 215-226.
45. Markees TG, Phillips NE, Gordon EJ, Noelle RJ, Shultz LD, Mordes JP et al. Long-term survival of skin allografts induced by donor splenocytes and anti-CD154 antibody in thymectomized mice requires CD4(+) T cells, interferon-gamma, and CTLA4. The Journal of clinical investigation 1998; 101(11): 2446-2455.
46. Sawitzki B, Kingsley CI, Oliveira V, Karim M, Herber M, Wood KJ. IFN-gamma production by alloantigen-reactive regulatory T cells is important for their regulatory function in vivo. The Journal of experimental medicine 2005; 201(12): 1925-1935.

47. Goldman M, Le Moine A, Braun M, Flamand V, Abramowicz D. A role for eosinophils in transplant rejection. Trends in immunology 2001; 22(5): 247-251.
48. Nocera A, Tagliamacco A, De Palma R, Del Galdo F, Ferrante A, Fontana I et al. Cytokine mRNA expression in chronically rejected human renal allografts. Clinical transplantation 2004; 18(5): 564-570.
49. Zelenika D, Adams E, Mellor A, Simpson E, Chandler P, Stockinger B et al. Rejection of H-Y disparate skin grafts by monospecific CD4+ Th1 and Th2 cells: no requirement for CD8+ T cells or B cells. Journal of immunology (Baltimore, Md : 1950) 1998; 161(4): 1868-1874.
50. Hara M, Kingsley CI, Niimi M, Read S, Turvey SE, Bushell AR et al. IL-10 is required for regulatory T cells to mediate tolerance to alloantigens in vivo. Journal of immunology (Baltimore, Md : 1950) 2001; 166(6): 3789-3796.
51. Van Kooten C, Boonstra JG, Paape ME, Fossiez F, Banchereau J, Lebecque S et al. Interleukin-17 activates human renal epithelial cells in vitro and is expressed during renal allograft rejection. Journal of the American Society of Nephrology : JASN 1998; 9(8): 1526-1534.
52. Tsaur I, Gasser M, Aviles B, Lutz J, Lutz L, Grimm M et al. Donor antigen-specific regulatory T-cell function affects outcome in kidney transplant recipients. Kidney international 2011; 79(9): 1005-1012.
53. van Besouw NM, Yan L, de Kuiper R, Klepper M, Reijerkerk D, Dieterich M et al. The Number of Donor-Specific IL-21 Producing Cells Before and After Transplantation Predicts Kidney Graft Rejection. Front Immunol 2019; 10: 748.
54. Deteix C, Attuil-Audenis V, Duthey A, Patey N, McGregor B, Dubois V et al. Intragraft Th17 infiltrate promotes lymphoid neogenesis and hastens clinical chronic rejection. J Immunol 2010; 184(9): 5344-5351.
55. Nakagiri T, Inoue M, Morii E, Minami M, Sawabata N, Utsumi T et al. Local IL-17 production and a decrease in peripheral blood regulatory T cells in an animal model of bronchiolitis obliterans. Transplantation 2010; 89(11): 1312-1319.
56. Burrell BE, Csencsits K, Lu G, Grabauskiene S, Bishop DK. CD8+ Th17 mediate costimulation blockade-resistant allograft rejection in T-bet-deficient mice. Journal of immunology (Baltimore, Md : 1950) 2008; 181(6): 3906-3914.
57. Healy DG, Watson RW, O'Keane C, Egan JJ, McCarthy JF, Hurley J et al. Neutrophil transendothelial migration potential predicts rejection severity in human cardiac transplantation. European journal of cardio-thoracic surgery : official journal of the European Association for Cardio-thoracic Surgery 2006; 29(5): 760-766.
58. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol 1995; 155(3): 1151-1164.
59. Lal G, Bromberg JS. Epigenetic mechanisms of regulation of Foxp3 expression. Blood 2009; 114(18): 3727-3735.
60. Lal G, Zhang N, van der Touw W, Ding Y, Ju W, Bottinger EP et al. Epigenetic regulation of Foxp3 expression in regulatory T cells by DNA methylation. J Immunol 2009; 182(1): 259-273.
61. Corthay A. How do regulatory T cells work? Scandinavian journal of immunology 2009; 70(4): 326-336.
62. Shevach EM, DiPaolo RA, Andersson J, Zhao DM, Stephens GL, Thornton AM. The lifestyle of naturally occurring CD4+ CD25+ Foxp3+ regulatory T cells. Immunol Rev 2006; 212: 60-73.
63. Buchbinder EI, Desai A. CTLA-4 and PD-1 Pathways: Similarities, Differences, and Implications of Their Inhibition. American journal of clinical oncology 2016; 39(1): 98-106.
64. Nakamura K, Kitani A, Strober W. Cell contact-dependent immunosuppression by CD4(+)CD25(+) regulatory T cells is mediated by cell surface-bound transforming growth factor beta. The Journal of experimental medicine 2001; 194(5): 629-644.
65. Regateiro FS, Chen Y, Kendal AR, Hilbrands R, Adams E, Cobbold SP et al. Foxp3 expression is required for the induction of therapeutic tissue tolerance. Journal of immunology (Baltimore, Md : 1950) 2012; 189(8): 3947-3956.
66. Kulkarni N, Sonar SA, Lal G. Plasticity of Th17 and Tregs and its clinical importance as therapeutic target in inflammatory bowel disease. Indian Journal of Inflammation Research 2017; 1(1): R2.
67. Regateiro FS, Howie D, Nolan KF, Agorogiannis EI, Greaves DR, Cobbold SP et al. Generation of anti-inflammatory adenosine by leukocytes is regulated by TGF-beta. European journal of immunology 2011; 41(10): 2955-2965.
68. Regateiro FS, Cobbold SP, Waldmann H. CD73 and adenosine generation in the creation of regulatory microenvironments. Clinical and experimental immunology 2013; 171(1): 1-7.
69. Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. Annual review of immunology 2001; 19: 683-765.
70. Asadullah K, Sterry W, Volk HD. Interleukin-10 therapy--review of a new approach. Pharmacological reviews 2003; 55(2): 241-269.
71. Zelenika D, Adams E, Humm S, Graca L, Thompson S, Cobbold SP et al. Regulatory T cells overexpress a subset of Th2 gene transcripts. Journal of immunology (Baltimore, Md : 1950) 2002; 168(3): 1069-1079.
72. Webster KE, Walters S, Kohler RE, Mrkvan T, Boyman O, Surh CD et al. In vivo expansion of T reg cells with IL-2-mAb complexes: induction of resistance to EAE and long-term acceptance of islet allografts without immunosuppression. The Journal of experimental medicine 2009; 206(4): 751-760.
73. Feng G, Wood KJ, Bushell A. Interferon-gamma conditioning ex vivo generates CD25+CD62L+Foxp3+ regulatory T cells that prevent allograft rejection: potential avenues for cellular therapy. Transplantation 2008; 86(4): 578-589.
74. Vlad G, Suciu-Foca N. Tolerogenic dendritic cells and induction of T suppressor cells in transplant recipients. Methods in molecular biology (Clifton, NJ) 2013; 1034: 359-371.
75. Peche H, Trinite B, Martinet B, Cuturi MC. Prolongation of heart allograft survival by immature dendritic cells generated from recipient type bone marrow progenitors. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2005; 5(2): 255-267.
76. Fu F, Li Y, Qian S, Lu L, Chambers F, Starzl TE et al. Costimulatory molecule-deficient dendritic cell progenitors (MHC class II+, CD80dim, CD86-) prolong cardiac allograft survival in nonimmunosuppressed recipients. Transplantation 1996; 62(5): 659-665.
77. Dhodapkar MV, Steinman RM. Antigen-bearing immature dendritic cells induce peptide-specific CD8(+) regulatory T cells in vivo in humans. Blood 2002; 100(1): 174-177.
78. Owen RD. IMMUNOGENETIC CONSEQUENCES OF VASCULAR ANASTOMOSES BETWEEN BOVINE TWINS. Science (New York, NY) 1945; 102(2651): 400-401.
79. Gershon RK, Kondo K. Cell interactions in the induction of tolerance: the role of thymic lymphocytes. Immunology 1970; 18(5): 723-737.
80. Palmer E, Naeher D. Affinity threshold for thymic selection through a T-cell receptor-co-receptor zipper. Nature reviews Immunology 2009; 9(3): 207-213.
81. Xing Y, Hogquist KA. T-cell tolerance: central and peripheral. Cold

Spring Harbor perspectives in biology 2012; 4(6).
82. Sitkovsky MV. T regulatory cells: hypoxia-adenosinergic suppression and re-direction of the immune response. Trends in immunology 2009; 30(3): 102-108.
83. Hildeman DA, Zhu Y, Mitchell TC, Bouillet P, Strasser A, Kappler J et al. Activated T cell death in vivo mediated by proapoptotic bcl-2 family member bim. Immunity 2002; 16(6): 759-767.
84. Barnes PJ. Corticosteroids: the drugs to beat. European journal of pharmacology 2006; 533(1-3): 2-14.
85. Hartono C, Muthukumar T, Suthanthiran M. Immunosuppressive drug therapy. Cold Spring Harbor perspectives in medicine 2013; 3(9): a015487.
86. Wood KJ, Goto R. Mechanisms of rejection: current perspectives. Transplantation 2012; 93(1): 1-10.
87. Bretscher P, Cohn M. A theory of self-nonself discrimination. Science (New York, NY) 1970; 169(3950): 1042-1049.
88. Kinnear G, Jones ND, Wood KJ. Costimulation blockade: current perspectives and implications for therapy. Transplantation 2013; 95(4): 527-535.
89. Lee KP, Taylor C, Petryniak B, Turka LA, June CH, Thompson CB. The genomic organization of the CD28 gene. Implications for the regulation of CD28 mRNA expression and heterogeneity. Journal of immunology (Baltimore, Md : 1950) 1990; 145(1): 344-352.
90. Hathcock KS, Laszlo G, Pucillo C, Linsley P, Hodes RJ. Comparative analysis of B7-1 and B7-2 costimulatory ligands: expression and function. The Journal of experimental medicine 1994; 180(2): 631-640.
91. Shahinian A, Pfeffer K, Lee KP, Kundig TM, Kishihara K, Wakeham A et al. Differential T cell costimulatory requirements in CD28-deficient mice. Science (New York, NY) 1993; 261(5121): 609-612.
92. Grohmann U, Orabona C, Fallarino F, Vacca C, Calcinaro F, Falorni A et al. CTLA-4-Ig regulates tryptophan catabolism in vivo. Nature immunology 2002; 3(11): 1097-1101.
93. Baliga P, Chavin KD, Qin L, Woodward J, Lin J, Linsley PS et al. CTLA4Ig prolongs allograft survival while suppressing cell-mediated immunity. Transplantation 1994; 58(10): 1082-1090.
94. Vincenti F, Charpentier B, Vanrenterghem Y, Rostaing L, Bresnahan B, Darji P et al. A phase III study of belatacept-based immunosuppression regimens versus cyclosporine in renal transplant recipients (BENEFIT study). American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2010; 10(3): 535-546.
95. Ochs HD, Hollenbaugh D, Aruffo A. The role of CD40L (gp39)/CD40 in T/B cell interaction and primary immunodeficiency. Seminars in immunology 1994; 6(5): 337-341.
96. Howard LM, Miller SD. Immunotherapy targeting the CD40/CD154 costimulatory pathway for treatment of autoimmune disease. Autoimmunity 2004; 37(5): 411-418.
97. Ferrer IR, Liu D, Pinelli DF, Koehn BH, Stempora LL, Ford ML. CD40/CD154 blockade inhibits dendritic cell expression of inflammatory cytokines but not costimulatory molecules. Journal of immunology (Baltimore, Md : 1950) 2012; 189(9): 4387-4395.
98. Larsen CP, Elwood ET, Alexander DZ, Ritchie SC, Hendrix R, Tucker-Burden C et al. Long-term acceptance of skin and cardiac allografts after blocking CD40 and CD28 pathways. Nature 1996; 381(6581): 434-438.
99. Zhai Y, Meng L, Gao F, Busuttil RW, Kupiec-Weglinski JW. Allograft rejection by primed/memory CD8+ T cells is CD154 blockade resistant: therapeutic implications for sensitized transplant recipients. Journal of immunology (Baltimore, Md : 1950) 2002; 169(8): 4667-4673.
100. Kawai T, Andrews D, Colvin RB, Sachs DH, Cosimi AB. Thromboembolic complications after treatment with monoclonal antibody against CD40 ligand. Nature medicine 2000; 6(2): 114.
101. Pinelli DF, Wagener ME, Liu D, Yamniuk A, Tamura J, Grant S et al. An anti-CD154 domain antibody prolongs graft survival and induces Foxp3(+) iTreg in the absence and presence of CTLA-4 Ig. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2013; 13(11): 3021-3030.
102. Markees TG, Phillips NE, Noelle RJ, Shultz LD, Mordes JP, Greiner DL et al. Prolonged survival of mouse skin allografts in recipients treated with donor splenocytes and antibody to CD40 ligand. Transplantation 1997; 64(2): 329-335.
103. Singh A, Ramachandran S, Graham ML, Daneshmandi S, Heller D, Suarez-Pinzon WL et al. Long-term tolerance of islet allografts in nonhuman primates induced by apoptotic donor leukocytes. Nat Commun 2019; 10(1): 3495.
104. Sharma RK, Rai PK, Kumar A, Kumar P, Gupta A, Kher V et al. Role of preoperative donor-specific transfusion and cyclosporine in haplo-identical living related renal transplant recipients. Nephron 1997; 75(1): 20-24.
105. Ferrer IR, Wagener ME, Song M, Kirk AD, Larsen CP, Ford ML. Antigen-specific induced Foxp3+ regulatory T cells are generated following CD40/CD154 blockade. Proceedings of the National Academy of Sciences of the United States of America 2011; 108(51): 20701-20706.
106. Benda B, Ljunggren HG, Peach R, Sandberg JO, Korsgren O. Co-stimulatory molecules in islet xenotransplantation: CTLA4Ig treatment in CD40 ligand-deficient mice. Cell transplantation 2002; 11(7): 715-720.
107. Elgueta R, Benson MJ, de Vries VC, Wasiuk A, Guo Y, Noelle RJ. Molecular mechanism and function of CD40/CD40L engagement in the immune system. Immunological reviews 2009; 229(1): 152-172.
108. Howland KC, Ausubel LJ, London CA, Abbas AK. The roles of CD28 and CD40 ligand in T cell activation and tolerance. Journal of immunology (Baltimore, Md : 1950) 2000; 164(9): 4465-4470.
109. Croft M, So T, Duan W, Soroosh P. The significance of OX40 and OX40L to T-cell biology and immune disease. Immunological reviews 2009; 229(1): 173-191.
110. Demirci G, Amanullah F, Kewalaramani R, Yagita H, Strom TB, Sayegh MH et al. Critical role of OX40 in CD28 and CD154-independent rejection. Journal of immunology (Baltimore, Md : 1950) 2004; 172(3): 1691-1698.
111. Vu MD, Clarkson MR, Yagita H, Turka LA, Sayegh MH, Li XC. Critical, but conditional, role of OX40 in memory T cell-mediated rejection. Journal of immunology (Baltimore, Md : 1950) 2006; 176(3): 1394-1401.
112. Yoshinaga SK, Whoriskey JS, Khare SD, Sarmiento U, Guo J, Horan T et al. T-cell co-stimulation through B7RP-1 and ICOS. Nature 1999; 402(6763): 827-832.
113. McAdam AJ, Chang TT, Lumelsky AE, Greenfield EA, Boussiotis VA, Duke-Cohan JS et al. Mouse inducible costimulatory molecule (ICOS) expression is enhanced by CD28 costimulation and regulates differentiation of CD4+ T cells. Journal of immunology (Baltimore, Md : 1950) 2000; 165(9): 5035-5040.
114. Harada H, Salama AD, Sho M, Izawa A, Sandner SE, Ito T et al. The role of the ICOS-B7h T cell costimulatory pathway in transplantation immunity. The Journal of clinical investigation 2003; 112(2): 234-243.
115. Shariff H, Greenlaw RE, Meader L, Gardner N, Yagita H, Coccia M et al. Role of the Fc region in CD70-specific antibody effects on cardiac transplant survival. Transplantation 2011; 92(11): 1194-1201.
116. Shao W, Yan G, Lin Y, Chen J, Dai H, Wang F et al. CD44/CD70

blockade and anti-CD154/LFA-1 treatment synergistically suppress accelerated rejection and prolong cardiac allograft survival in mice. Scandinavian journal of immunology 2011; 74(5): 430-437.
117. Shuford WW, Klussman K, Tritchler DD, Loo DT, Chalupny J, Siadak AW et al. 4-1BB costimulatory signals preferentially induce CD8+ T cell proliferation and lead to the amplification in vivo of cytotoxic T cell responses. The Journal of experimental medicine 1997; 186(1): 47-55.
118. Nicolls MR, Coulombe M, Yang H, Bolwerk A, Gill RG. Anti-LFA-1 therapy induces long-term islet allograft acceptance in the absence of IFN-gamma or IL-4. Journal of immunology (Baltimore, Md : 1950) 2000; 164(7): 3627-3634.
119. Kitchens WH, Haridas D, Wagener ME, Song M, Kirk AD, Larsen CP et al. Integrin antagonists prevent costimulatory blockade-resistant transplant rejection by CD8(+) memory T cells. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2012; 12(1): 69-80.
120. Sandner SE, Clarkson MR, Salama AD, Sanchez-Fueyo A, Domenig C, Habicht A et al. Role of the programmed death-1 pathway in regulation of alloimmune responses in vivo. Journal of immunology (Baltimore, Md : 1950) 2005; 174(6): 3408-3415.
121. Page EK, Dar WA, Knechtle SJ. Tolerogenic therapies in transplantation. Frontiers in immunology 2012; 3: 198.
122. Newell KA, Asare A, Kirk AD, Gisler TD, Bourcier K, Suthanthiran M et al. Identification of a B cell signature associated with renal transplant tolerance in humans. The Journal of clinical investigation 2010; 120(6): 1836-1847.
123. Chesneau M, Michel L, Degauque N, Brouard S. Regulatory B cells and tolerance in transplantation: from animal models to human. Front Immunol 2013; 4: 497
124. Lal G, Kulkarni N, Nakayama Y, Singh AK, Sethi A, Burrell BE et al. IL-10 from marginal zone precursor B cells controls the differentiation of Th17, Tfh and Tfr cells in transplantation tolerance. Immunology letters 2016; 170: 52-63.
125. Lal G, Nakayama Y, Sethi A, Singh AK, Burrell BE, Kulkarni N et al. Interleukin-10 From Marginal Zone Precursor B-Cell Subset Is Required for Costimulatory Blockade-Induced Transplantation Tolerance. Transplantation 2015; 99(9): 1817-1828.
126. Wehmeier C, Karahan GE, Krop J, de Vaal Y, Langerak-Langerak J, Binet I et al. Donor-specific B cell memory in alloimmunized kidney transplant recipients - first clinical application of a novel method. Transplantation 2019.
127. Kamburova EG, Gruijters ML, Kardol-Hoefnagel T, Wisse BW, Joosten I, Allebes WA et al. Antibodies against ARHGDIB are associated with long-term kidney graft loss. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2019.
128. Riquelme P, Tomiuk S, Kammler A, Fandrich F, Schlitt HJ, Geissler EK et al. IFN-gamma-induced iNOS expression in mouse regulatory macrophages prolongs allograft survival in fully immunocompetent recipients. Molecular therapy : the journal of the American Society of Gene Therapy 2013; 21(2): 409-422.
129. Mellor AL, Munn DH. IDO expression by dendritic cells: tolerance and tryptophan catabolism. Nature reviews Immunology 2004; 4(10): 762-774.
130. Zecher D, Li Q, Oberbarnscheidt MH, Demetris AJ, Shlomchik WD, Rothstein DM et al. NK cells delay allograft rejection in lymphopenic hosts by downregulating the homeostatic proliferation of CD8+ T cells. Journal of immunology (Baltimore, Md : 1950) 2010; 184(12): 6649-6657.
131. Thomson AW, Ezzelarab MB. Regulatory dendritic cells: profiling, targeting, and therapeutic application. Current opinion in organ transplantation 2018; 23(5): 538-545.
132. Wang Q, Zhang M, Ding G, Liu Y, Sun Y, Wang J et al. Anti-ICAM-1 antibody and CTLA-4Ig synergistically enhance immature dendritic cells to induce donor-specific immune tolerance in vivo. Immunology letters 2003; 90(1): 33-42.
133. Moreau A, Varey E, Beriou G, Hill M, Bouchet-Delbos L, Segovia M et al. Tolerogenic dendritic cells and negative vaccination in transplantation: from rodents to clinical trials. Frontiers in immunology 2012; 3: 218.
134. Ferrer IR, Wagener ME, Song M, Ford ML. CD154 blockade alters innate immune cell recruitment and programs alloreactive CD8+ T cells into KLRG-1(high) short-lived effector T cells. PloS one 2012; 7(7): e40559.
135. Larsen CP, Elwood ET, Alexander DZ, Ritchie SC, Hendrix R, Tucker-Burden C et al. Pillars article: long-term acceptance of skin and cardiac allografts after blocking CD40 and CD28 pathways. Nature. 1996. 381: 434-438. 1996. Journal of immunology (Baltimore, Md : 1950) 2011; 186(5): 2693-2697.
136. Ha J, Bingaman AW, Durham MM, Pearson TC, Larsen CP. Aggressive skin allograft rejection in CD28-/- mice independent of the CD40/CD40L costimulatory pathway. Transplant immunology 2001; 9(1): 13-17.
137. Graca L, Honey K, Adams E, Cobbold SP, Waldmann H. Cutting edge: anti-CD154 therapeutic antibodies induce infectious transplantation tolerance. Journal of immunology (Baltimore, Md : 1950) 2000; 165(9): 4783-4786.
138. Honey K, Cobbold SP, Waldmann H. CD40 ligand blockade induces CD4+ T cell tolerance and linked suppression. Journal of immunology (Baltimore, Md : 1950) 1999; 163(9): 4805-4810.
139. Kim EY, Lee EN, Lee J, Park HJ, Chang CY, Jung DY et al. Two-signal blockade with anti-CD45RB and anti-CD154 monoclonal antibodies inhibits graft rejection via CD4-dependent mechanisms in allogeneic skin transplantation. Experimental & molecular medicine 2006; 38(3): 284-294.
140. Rothstein DM, Livak MF, Kishimoto K, Ariyan C, Qian HY, Fecteau S et al. Targeting signal 1 through CD45RB synergizes with CD40 ligand blockade and promotes long term engraftment and tolerance in stringent transplant models. Journal of immunology (Baltimore, Md : 1950) 2001; 166(1): 322-329.