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Immune Tolerance and Transplantation

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Abstract

Successful allogeneic hematopoietic stem cell transplantation (HSCT) and solid organ transplantation require development of a degree of immune tolerance against allogeneic antigens. T lymphocytes play a critical role in allograft rejection, graft failure, and graft versus host disease (GVHD). T cell tolerance occurs by two different mechanisms; i) depletion of self-reactive T cells during their maturation in the thymus (central tolerance) ii) suppression/elimination of self-reactive mature T cells in the periphery (peripheral tolerance). Induction of transplant tolerance improves transplantation outcomes. Adoptive immunotherapy with immune suppressor cells including regulatory T cells, NK-T cells, veto cells and facilitating cells are promising therapies for modulation of immune tolerance. Achieving mixed chimerism with the combination of thymic irradiation and T cell depleting antibodies, costimulatory molecule blockade with/without inhibitory signal activation and elimination of alloreactive T cells with varying methods including pre or post-transplant cyclophosphamide administration appear to be effective methods to induce transplant tolerance.

Immune Tolerance and Transplantation

Successful allogeneic hematopoietic stem cell transplantation (HSCT) and solid organ transplantation requires a certain degree of immune tolerance development against allogeneic antigens. Achievement of immune tolerance may prevent a host versus graft reaction, which leads to graft rejection and failure, as well as preventing a graft versus host reaction, which results in graft versus host disease (GVHD) in recipients of HSCT. Induction of immune tolerance decreases the risk of acute and chronic graft rejection after solid organ transplantation and can improve transplanted organ survival. Lymphocytes, specifically T lymphocytes, play a critical role in allograft rejection, graft failure, and GVHD. Therefore, in this review we will focus on *T cell tolerance*.

T Cell Tolerance and Thymopoiesis

Our immune system is very adaptive, able to mount an immune response to varying immunological targets. In some conditions the immune system becomes unresponsive to certain antigens ¹. T cell tolerance occurs by two different mechanisms. The first is the depletion of self-reactive T cells during their maturation in the thymus; only 1-2 % of thymocytes are able to reach a mature T cell status before they are released from the thymus.

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T cell clonal deletion is defined as the death of T cells with TCRs recognizing host antigens, which is the main tolerance mechanism during T cell development in the thymus ^{1,2}.

The second mechanism is the suppression/elimination of self-reactive mature T cells in the periphery; which involves either immunologically active suppressive cells such as regulatory T cells or the inactivation of autoreactive T cell clones by inhibitory molecules³. If T cells are unable to proliferate and produce cytokines such as IL-2 or IL-4 in response to antigens, they may become anergic (non-responsive) to those antigens. Sometimes suppressor/regulatory cells cause anergy or clonal deletion of T cells by secreting inhibitory cytokines or inducing T cell apoptosis in the periphery.

The central tolerance mechanism of thymic maturation will be discussed first, followed by the mechanisms of peripheral tolerance.

T cell development -Thymic Education/Maturation

T cell development begins after the entry of T cell precursors into the thymus. In mice, cell surface markers define multiple types of lymphoid precursors with T cell lineage potential; these include common lymphoid progenitors (CLP1 and CLP2), circulating T cell precursors (CTPs), early thymic precursors (ETPs) and early lymphoid precursors (ELPs) ⁴⁻⁹. The ability of circulating progenitors to migrate within the thymus is tightly regulated and requires the CC chemokine receptors CCR7 and CCR9, CD44, $\alpha 4$ and $\beta 2$ integrins, and P-selectin glycoprotein ligand-1 (PSGL1) (reviewed in ⁸). Lymphoid precursors from the BM migrate to the thymus and then will undergo subsequent differentiation under the control of factors including Interleukin-7 (IL-7) and Notch-1 ¹⁰. A small group of ETPs demonstrate both T and B cell developmental potential in the thymus. However, most of the ETPs lose their B potential and require Notch signaling for T cell development ^{11,12}. Progression beyond the DN3 stage depends on Notch-1 activation, which controls survival and proliferation through the DN3 transition to double positive (DP) cells in cooperation with pre-TCR signaling ¹³⁻¹⁵. DN thymocytes develop T-cell receptor (TCR) β - and α - locus rearrangements in the DN3 and DN4 stages. They then, start to express CD4 and CD8 as characterized by CD4+/8+ double-positive (DP) expression along with modest $\alpha \beta$ TCR expression. Petrie et al. demonstrated that cultured immature thymocytes originally expressing specific TCR alpha and beta chains may lose surface expression of the original TCR alpha, but not beta chains ¹⁶. These data provide evidence that TCR alpha gene rearrangement continues even after surface expression of a TCR alpha/beta heterodimer, up until positive selection ¹⁶⁻¹⁸. This process is controlled by recombinant activator gene (RAG) expression ¹⁹, that starts in CD25+ double negative thymocytes through DP thymocytes. Interestingly, RAG reinduction occurs in the DP stage which is required for mature T cell development ¹⁹ (Figure 1).

Human T cell development in the thymus

In humans, the phenotype of lymphoid precursors is different from that detected in mice. Human stem cells express CD34, whereas in mice, lineage negative, SCA-1 and c-Kit positive cells contain true stem cells. These CD34+ precursors seed the thymus and differentiate into multiple lineages, including T, B and NK cells ^{20,21}. Lin- CD34+ CD10+ CD24- progenitors with a low myeloid potential have been reported to generate B, T, and natural killer lymphocytes and co-express recombination activating gene 1 (RAG-1), terminal deoxynucleotidyl transferase (tdt), PAX5, interleukin 7 receptor alpha, and CD3e. These progenitors are present in both cord blood and BM, but can also be found in the peripheral blood and thymus throughout life ²².

Positive Selection

All thymocytes undergo positive and negative selection during their maturation in the thymus². The average DP cell life span is 3-4 days. The fate of each DP cell depends upon the ability of its newly rearranged TCR to appropriately interact with MHC molecules. Most MHC molecules display self-peptides and positive selection involves the recognition of the MHC-self peptide complex²³. More than 95% of DP cells do not have any specificity to an MHC ligand, and these thymocytes die by neglect instead of positive selection^{24,25}. A small proportion of DP cells are able to bind an MHC ligand with mild avidity; this induces DP maturation to the CD4+ or CD8+ single-positive (SP) stage²⁴.

T cell clonal deletion in the thymus; Negative Selection

Intra-thymic T cell tolerance occurs via deletion of T cell clones autoreactive to self-antigens presented by hematopoietic cells and thymic epithelial cells^{26,27}. If the T cell receptor has high affinity to self-antigens (self MHC and peptide complex), this leads to the deletion of thymocytes by apoptosis^{28,29}. Thymocytes that express a low affinity TCR survive and continue to the next maturation step. The difference between low affinity and high affinity TCRs is critical for thymocyte fate but the mechanisms of this discrimination are not clearly documented^{2,24}. Fas is a member of the TNF receptor family and plays a role in the development of apoptosis in semi-mature medullary thymocytes³⁰, which is dependent upon specific antigen concentration in in vivo murine models³¹. Strong TCR ligation stimulates rapid onset of Fas-dependent apoptosis of naïve T cells³². The second required signal of negative selection is costimulation, especially interaction between antigen presenting cells (APC) and CD28^{33,34}. In the absence of costimulation, anti-TCR antibody stimulation results in significant apoptosis of thymocytes³³. Further studies in mice lacking B7-1 (CD80) and/or B7-2 (CD86) demonstrated a role for both B7-1 and B7-2 in negative selection. CD28 mediates these B7-dependent signals that promote negative selection³⁵. CTLA-4 delivers signals that inhibit selection, suggesting that CTLA-4 and CD28 have opposing functions in negative selection³⁵. Thymic medullary tissue is an APC-rich area in which thymocytes interact with varying costimulatory molecules (such as CD80 and CD86) that are expressed on thymic epithelial cells (TEC) and thymic dendritic cells, suggesting their involvement in negative selection. On the other hand, high-level TCR signaling results in apoptosis through the Fas/FasL pathway^{32,36}, which occurs in the thymic cortex (Figure 1). In the last decade a new transcriptional regulator was discovered in the thymus, named Autoimmune Regulator (AIRE)³⁷. *Aire* is primarily expressed in medullary thymic epithelial cells. It promotes self-tolerance by inducing transcription of a wide array of tissue-specific antigens (TSAs) in the thymus and plays a critical role in negative selection (reviewed in³⁸). In the absence of functional AIRE, medullary TECs express a severely restricted array of self-antigens which results in severe autoimmune disease^{37,38}. The role of Aire has not been defined in post-transplant tolerance.

Peripheral Tolerance

After positive and negative selection, mature T cells are released from the thymus into the peripheral circulation and secondary lymphoid organs. Negative selection in the thymus effectively deletes thymocytes that have high affinity TCR to self-peptide-MHC complexes. Peripheral immune tolerance mechanisms are critical for controlling mature T cells with low/moderate affinity TCRs to self MHC/peptide complexes (reviewed in³). Small amounts of T lymphocytes escape selection in the thymus; these can be eliminated in the periphery by deletion. Induced anergy and/or suppression by other immunologically active cells (regulatory cells/suppressor cells) also play roles in lessening the effects of these self-reactive T cell clones that escaped thymic selection.

Peripheral Deletion

Varying levels of antigenic stimulation of mature T cells in the periphery can result in T cell clonal deletion. A small amount of antigenic stimulation can induce T cell tolerance by partial down-regulation of T cell receptors (TCR) on self-reactive CD8⁺ cells³⁹. However, T cell apoptosis is generally required for the induction of peripheral transplant tolerance⁴⁰. Clonal deletion of autoreactive T cells occurs through apoptosis via activation of the Fas/FasL pathway and the Bim dependent mitochondrial pathway³. Tissue-associated self-antigens can be cross-presented by APCs to naïve CD8⁺ T cells⁴¹, which creates the potential for the development of autoimmunity. However, cross-presentation of self-antigen also leads to deletion of those autoreactive CD8⁺ T cells⁴². Davey et al. showed that self-tolerance can be maintained by the deletion of activated CD8⁺ T cells via Bim activation, which leads to BCL-2 inhibition and apoptosis of T cells⁴³. Bim deficient and Fas deficient (lpr/lpr) mice show defective peripheral tolerance induction, leading to massively increased size of their lymph nodes and spleen and development of autoimmunity, suggesting that both molecules play a role in peripheral deletion of T cells^{44,45}.

T cell Anergy and Costimulatory Signals

T cell activation requires two signals: i) TCR signal ii) costimulatory signal. T cells are not able to mount an immune response without a second costimulatory signal. CD28 is the main co-stimulatory receptor and has two ligands, B7.1 (CD80) and B7.2 (CD86), that are expressed on APCs (Figure 2). CD28 signals are critical for T cell activation, proliferation and survival after T cell interaction with APCs⁴⁶. CD28 activation results in increased expression of cyclins and cyclin dependent kinases (cdk), downregulation of cdk inhibitor cdk27kip1 and upregulation of glucose metabolism through phosphoinositol 3-kinase (PI3K) and Akt activation⁴⁷⁻⁵⁰. CD28 controls T cell survival by enhancing BCL_{XL} expression in T cells, which prevents T cell death from apoptotic signals such as Fas activation or IL-2 withdrawal⁵¹. Activation of T cells in the absence of CD28 results in an anergic state (reviewed in⁵²) with defective phospholipase activation and intracellular calcium mobilization⁵³, which is also associated with increased levels of the cdk inhibitor p27kip1^{50,54}. Importantly, blockage of CD28/B7 interaction promotes transplant tolerance in animal models⁵⁵.

Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4) and Inhibition of CD28 Costimulatory Signal

CTLA-4, a member of the immunoglobulin superfamily, was the first inhibitory molecule discovered, and has a similar structure to CD28. Both molecules bind to CD80 and CD86 on antigen-presenting cells. Lack of CTLA-4 results in severe lymphoproliferative disease and lethality in rodents⁵⁶. CTLA-4 inhibits CD28 dependent T cell activation, cell cycle progression and IL-2 production of T cells^{57,58}. Interestingly, CTLA-4 inhibition is more pronounced after initiation of T cell activation and anti-CTLA-4 antibody activity is optimized with continuous CD28 signaling, suggesting that upregulation of CTLA-4 is CD28 dependent⁵⁸.

CTLA-4 has a significantly higher binding affinity for the B7 molecules than that of CD28 and acts as a competitive antagonist of CD28⁵². The presence of inhibitory effects of CTLA-4 in CD28 deficient mice demonstrates that CTLA-4 can function independently from CD28⁵⁹. This inhibitory function of CTLA-4 is mediated by its cytoplasmic tail which interacts with other T cell signaling molecules including tyrosine kinases, MAP kinases and the PKB/AKT pathway (reviewed in⁵²). CTLA-4 Ig is a soluble form of the extracellular domain of CTLA-4, which binds B7 with high avidity, and treatment with CTLA-4 Ig results in immunosuppression with decreased T cell dependent antibody secretion⁶⁰. Treatment with CTLA-4 Ig in conjunction with BM transplantation induces long-term allograft survival and donor unresponsiveness in murine models⁶¹.

Programmed Death-1 (PD-1)/PD-1 Ligands

PD-1 (CD279) is a member of the CD28 family that is expressed on activated T, B, and myeloid cells. PD-1 ligands (PD-L1 and PD-L2) are expressed on T cells, B cells, antigen-presenting cells, endothelial cells, and tumor tissues. PD-L1 (CD274, B7-H1), a transmembrane glycoprotein belonging to the immunoglobulin (Ig) superfamily plays an integral role in the regulation of immune tolerance and homeostasis. PD-1/PD-L1 interactions lead to inhibitory signals, and ligation of PD-1 occurs during autoimmunity, allergy, allograft rejection, antitumor immunity, and chronic virus infection⁶²⁻⁶⁴. PD-1 KO mice develop autoimmunity suggesting that PD-1 has a role in immune tolerance^{65,66}. PD-1 is expressed on T cells after activation and delivers co-inhibitory signals via an immunoreceptor tyrosine-based switch motif in the cytoplasmic domain^{67,68}. The ability of PD-1 to block T cell activation requires receptor ligation, suggesting that co-localization of PD-1 with CD3 and/or CD28 may be necessary for inhibition of T cell activation⁶⁸. PD-1 signals interfere with CD28 mediated activation of phosphatidylinositol-3-kinase (PI3K) and subsequently inhibit interleukin-2 production resulting in an anergic state in T lymphocytes⁶⁹. PD-1 has been recently shown to play a role in control of homeostatic proliferation of CD8+ T cells in lymphopenic hosts by inducing apoptosis of T cells⁷⁰. Interestingly, peripheral deletion of alloreactive CD8+ T cells is related to the PD-1/PD-L1 pathway, but tolerization of CD4+ T cells occurs independently from this pathway in murine transplant models⁷¹. PD-L1 also interacts with CD80 to inhibit T cell activation⁷² and this interaction is required for maintenance of peripheral tolerance⁷³. Mice deficient for the PD-L1 gene or wild-type mice treated with anti-B7-H1 blocking monoclonal antibody (mAb) exhibit exacerbated autoimmunity associated with activation of self-reactive CD4+ and CD8+ T cells^{74,75}. PD-L2 is a second ligand for PD-1 which inhibits activation of and cytokine production by T cells⁷⁶. The role of PD-L2 in the development of post transplant tolerance is not clearly documented. PD-L2 may limit CD8+ T cell proliferation in recipients of HSCT⁷⁷.

CD40/CD40L Pathway

CD40 and its ligand CD154 (CD40L) are members of the tumor necrosis factor (TNF)/TNF-receptor family. CD40 is constitutively expressed on APCs such as B cells, macrophages, dendritic cells, and thymic epithelium, but can also be found on endothelial cells and fibroblasts. CD154 (CD40L) is expressed on activated T cells and NK cells^{78,79}. Signaling through CD40/CD154 is critical for activation of B and dendritic cells. CD40 ligation leads to secretion of IL-1, TNF- α , and IL-12, and to endothelial cell secretion of monocyte chemotactic factors^{80,81}. Signaling downstream of the CD40 molecules is mediated by TRAFs and leads to the activation of NF κ B, which results in the upregulation of MHC molecules and co-stimulatory molecules in B cells (reviewed in⁸²). CD154 is rapidly induced on CD4+ and some CD8+ T cells following T cell activation after stimulation with cognate antigen. CD40/CD40L activation primarily provides a costimulatory signal to the APC rather than to the T cell, but eventually T cells are indirectly stimulated after APC activation⁸³. The blockage of the CD40/CD40L pathway alone or in combination with CTLA-Ig results in tolerance to MHC-mismatched skin and cardiac grafts in murine models^{84,85}. Moreover, treatment with anti-CD154 antibody with or without CTLA-Ig prevents acute allograft rejection in non-human primates^{86,87}.

Suppression of immune responses; regulatory cells

The suppressive functions of T cells on the immune system has been known since the 1970s⁸⁸, but a clear definition of T regulatory cells was not achieved until the mid 90s. Regulatory T cells (T_{reg}) play a major role in the development of tolerance by suppression of immune responses (reviewed in⁸⁹). Other immunologically active cells can also suppress

immune responses including NK-T cells, double negative (CD4-CD8-), CD8+CD28- cells, and veto cells.

Regulatory T Cells

Regulatory T cells were first defined as CD4+CD25+ double positive cells with suppressive functions on immunological response in rodents⁹⁰. Approximately 5-10 % of peripheral CD4+ cells express IL-2 receptors on their surface. The depletion of CD4+CD25+ cells results in development of autoimmune disease^{91,92}. Normally, the thymus produces immunoregulatory CD4+CD25+ T cells that are anergic to TCR stimulation and are able to suppress proliferation of other T cells⁹³. They might have intermediate affinity to MHC/peptide resulting in an escape from negative selection in the thymus⁹⁴.

Naturally occurring T_{reg} specifically express a transcription factor (Foxp3), which is the main inducer, regulator, and survival factor in T_{reg} development and function^{95,96}. Only CD4+CD25+ single positive thymocytes exhibit expression of Foxp3 in the thymus. Foxp3 transduced CD4+CD25- cells are capable of suppressing other T cells and autoimmune colitis in murine models⁹⁵. Retroviral transduction of Foxp3 into CD25- T cells upregulates CD25 and CTLA-4 expression on their surface. CD4+CD25+ cells are increased in Foxp3 transgenic mice and other T cell subsets also gain suppressive properties in the presence of over-expression of Foxp3⁹⁷. Naïve T cells in the periphery can also acquire Foxp3 expression and suppressive function in conditions such as chronic antigenic stimulation in the presence of transforming growth factor (TGF)-β1⁹⁸⁻¹⁰⁰. TGF-β1 signaling is required for the maintenance of immune suppressive activity and expansion of regulatory T cells^{101,102}. Interestingly TGF-β1 deficient mice have a reduced number of peripheral T_{reg} while preserving normal thymic T_{reg} development. A defect in TGF-beta-mediated signaling in T_{reg} is associated with a decrease in Foxp3 expression and suppressor activity¹⁰². Other types of T regulatory cells can be induced in the periphery in the presence of interleukin-10 (IL-10)¹⁰³, called Tr1 cells. Tr1 cells secrete IL-10 and TGF-β1 and their suppressive activities are independent of Foxp3 expression¹⁰⁴. Both naturally occurring T_{reg} cells and Tr1 cells are hyporesponsive to stimulation of their TCR but can slowly proliferate in presence of IL-2. Recently, CD8+ Foxp3+ T_{reg} have been shown to have a suppressive function in autoimmune disorders and after allergen immunotherapy as well as in GVHD¹⁰⁵⁻¹⁰⁷.

Function of regulatory T cells

CD4+CD25+ T cells suppress the proliferation of CD4+ as well as CD8+ T cells, which requires direct cell contact with T_{reg}¹⁰⁸. Survival and function of T_{reg} is dependent on presence of IL-2; IL-2 deficient mice develop lymphoproliferation and severe autoimmune disease^{109,110}. Expression of IL-2R (CD25) on their surface and signaling through IL-2R is required for optimum T regulatory function¹¹¹. T_{reg} are anergic after stimulation and therefore, IL-2 secretion from conventional T cells is critical for development of suppressive activity of T_{reg}.^{112,113} The crosstalk between T effector cells and T regulatory cells is shown in Figure 3. As mentioned above, IL-10 is required for Tr1 development in the periphery. Interestingly, CD4+CD25+T_{reg} suppressive function *in vitro* does not require IL-10 and TGF-β1 secretion, as has been shown with suppressor T cells from IL-10-deficient and TGF-β1 deficient mice, which seem to suppress effectively^{108,114,115}. On the other hand CD4+CD25+ T_{reg} can secrete IL-10 in vivo and autoimmunity can be suppressed by regulatory cells through IL-10 secretion¹¹⁶. T_{reg} interaction with CD80/CD86 through the CTLA-4 molecule may lead to suppression of effector T cells¹¹⁷. Interaction of CTLA-4 expressing T_{reg} with APCs induces the activation of indoleamine 2,3-dioxygenase (IDO), which results in both a local deprivation of tryptophan and the production of inhibitory molecules known as kynurenines^{118,119} (Figure 3). CTLA-4 expressing CD4+CD25+

regulatory cells show a stronger suppressive activity than their CTLA-4- counterparts¹²⁰. Moreover, blocking CTLA-4 on T_{reg} can abrogate the suppression of T effectors¹²¹. CD28-deficient CD25⁺ CD4⁺ T cells can also suppress activation of normal T cells, indicating that CD28 is not required as a costimulatory molecule for activation of the regulatory T cells. T_{reg} may also act as cytotoxic T cells that expresses granzyme A after activation and are able to kill activated CD4⁺ and CD8⁺ T cells by a perforin dependent mechanism¹²².

Transplant tolerance and T regulatory cells

CD4⁺CD25⁺ T cells play a role in regulating the allo response and tolerance induction. Depletion of CD25⁺ T cells results in faster rejection of allogeneic skin grafts and increases graft versus host disease (GVHD) related mortality in murine models^{90,123}. The presence of CD4⁺CD25⁺ T cells in *in vitro* mixed lymphocyte reaction cultures can induce tolerance to alloantigen¹²⁴. Infusion of CD4⁺CD25⁺ regulatory T cells after stem cell transplantation decreased GVHD related mortality in recipients of allogeneic HSCT^{123,125}. T_{reg} induce tolerance to minor histocompatibility antigen-mismatched skin grafts^{126,127} and are involved in tolerance and prevention of graft rejection after organ transplant¹²⁸⁻¹³⁰. T_{reg} may be a long-awaited T cell population for adoptive therapy to induce tolerance in clinical settings specifically after transplantation (reviewed in¹³¹). Hippen and coworkers from University of Minnesota reported large scale expansion of human natural regulatory cells from peripheral blood with well-preserved suppressive function¹³². The same group also showed that the infusion of ex vivo expanded T_{reg} into adults transplanted with umbilical cord blood is safe and might be able to prevent GVHD in clinical settings¹³³. Finally Di Lanni and colleagues evaluated the effects of pre-transplant adoptive transfer of T_{reg} lymphocytes in 28 patients with haploidentical HSCT. They found that T_{reg} infusion prevented GVHD, promoted immune reconstitution and improved immunity to opportunistic infections¹³⁴. These data suggest that adoptive therapy with donor derived T_{reg} can prevent GVHD after allogeneic HSCT.

Other suppressive cells

Natural Killer (NK)-T Cells

These are T cells that express NK cell associated markers (CD16 and CD56 in humans, NK1.1, DX5 in mouse) together with T cell markers and TCR $\alpha\beta$ on their surface. NK-T cells secrete high levels of IL-4 and IFN- γ . In mice, NK1 T cells express a restricted TCR repertoire with an invariant TCR alpha chain and recognize the products of the conserved family of MHC class I-like CD1 genes¹³⁵. The presence of allogeneic donor NK1.1+ TCR α + T cells in the BM can protect mice against lethal GVHD in recipients of allogeneic HSCT¹³⁶. Total lymphoid irradiation (TLI) can induce tolerance after organ and HSC transplantation by selectively increasing NK-T cells in the host¹³⁷. Host conditioning with TLI and ATG prevents GVHD by an NK-T cell dependent mechanism¹³⁸. Recent reports suggest that NK-T cells promote immune tolerance through an interaction with T_{reg} by increasing expression of negative costimulatory molecules (like PD-1) on T_{reg}, which is mostly dependent on enhanced IL-4 secretion by NK-T cells¹³⁹. Taken together, NK-T cells play a specific role in immune tolerance and strategies to enrich NK-T cells can induce transplant tolerance. Cytokine stimulation with IFN- γ , IL-2 and anti-CD3 expands human CD3⁺ CD56⁺ NK-T cells, and these cells show MHC-independent cytotoxicity (cytokine induced killer cells-CIK). The safety and feasibility of CIK cell infusion have been shown after autologous HSCT¹⁴⁰. Adoptive immunotherapy with CIK cells induces anti-tumor responses with minimal GVHD in patients with relapsed hematological malignancies after allogeneic HSCT¹⁴¹.

Veto cells

Veto activity was defined in 1980 by Miller as the capacity to suppress cytotoxic T lymphocyte (CTL) precursors directed against antigens expressed on veto cells, but not against third party antigens^{142,143}. Interestingly, the most potent veto cell activity was found in CD8+ CTL lines or clones^{144,145}. The specificity of the veto effect mediated by CTL clones is antigen specific and MHC restricted. Veto activity occurs by apoptosis through the Fas/FasL pathway^{145,146}. Other cells, including varying BM subsets, have been reported to have veto activity and augment immune tolerization¹⁴⁷⁻¹⁴⁹. Murine and human hematopoietic stem cells also have veto cell activity^{150,151}, and tolerance can be induced by using high doses of stem cells especially in MHC-mismatched donor/host combinations (reviewed in¹⁵²). Megadose human CD34+ stem cells have been successfully used in haploidentical transplantation, which results in high-level engraftment of MHC disparate stem cells¹⁵³.

Inducing tolerance; clinically applicable strategies

Multiple strategies have been explored to optimize immune tolerance after transplantation. T cell depletion is one of the well-known strategies to enhance immune tolerance and decrease graft versus host disease. Increased susceptibility to infections and increased relapse have been observed after T cell depletion in recipients of allogeneic HSCT.

Pre or post-transplant chemotherapy can be used to induce immune tolerance to transplanted organs or to the host. Cyclophosphamide (CTX) is a well-known chemotherapeutic agent; preclinical and clinical studies demonstrated that pre or post-transplant CTX promotes immune tolerance in recipients of allogeneic HSCT¹⁵⁴⁻¹⁵⁶. Recent studies have demonstrated that in particular post-transplant cyclophosphamide can improve engraftment and reduce GVHD¹⁵⁷. This topic is discussed in detail in another section in this issue.

Generation of mixed chimerism is another strategy that has been explored in clinical settings. As previously discussed, intrathymic clonal deletion is one of the main mechanisms leading to the development of transplant tolerance. Combining thymic irradiation with T cell depleting antibodies results in a multi-lineage mixed chimerism and skin graft tolerance without GVHD in recipients of MHC-mismatched BMT in murine models^{158,159}. Addition of CTX to conditioning protocols with TI provides successful engraftment and long-term chimerism in fully-MHC mismatched murine BMT recipients. Durable chimerism after transplantation prevents chronic rejection and induces graft survival as shown in experimental animal models¹⁶⁰. This strategy was successfully translated to the clinic to induce organ tolerance in recipients of both BM and kidney transplantation¹⁶¹⁻¹⁶³. A similar strategy combining kidney transplantation and Hematopoietic stem cell transplantation has been reported with durable chimerism and donor specific tolerance¹⁶⁴. Interestingly, immunosuppression was discontinued one year after transplant in patients who achieved durable chimerism after HSC+ facilitating cell (FC) infusion. BM derived FC were initially identified as CD8+ cells that did not express TCR on their surface and enhanced engraftment of allogeneic HSC¹⁴⁸. Subsequently, FCs were shown to be predominantly composed of a plasmacytoid precursor dendritic cell subpopulation that produces IFN-alpha and TNF-alpha, and can be activated by toll-like receptor (TLR)-9 ligand stimulation, and expanded by Flt-3 ligand¹⁶⁵.

Selective removal of allo-reactive T cells is another approach to decrease GVH reaction and induce tolerance after stem cell transplantation. The T cell proliferation that occurs in haplotype-mismatched mixed lymphocyte reactions provides a platform for elimination of allo-reactive T cells. An immunotoxin coupled to a T cell activation marker (CD25) successfully depletes allo-reactive T cells without having any harmful effects on stem

cells¹⁶⁶. The same group reported successful ex vivo depletion of host-reactive donor T cells from peripheral blood stem cell (PBSC) transplant allografts using an anti-CD25 immunotoxin, while the remaining T cell population had an intact third-party response in most cases¹⁶⁷. However, possible depletion of CD4+CD25+ regulatory cells by this method raised a concern regarding the induction of long-term immune tolerance and efficacy in the mitigation of GVHD risk. A non-FCR binding anti-CD3 antibody has been used to induce apoptosis in selectively antigen-activated and cycling T cell populations¹⁶⁸. This antibody might have a role in the therapy of GVHD^{169,170} but it has not yet been reported as a tolerance-inducing agent in clinical trials. Campath is an antibody against CD52 that was evaluated in recipients of renal allografts and found to be ineffective for inducing tolerance despite successful T cell depletion^{171,172}, suggesting that the degree of T cell depletion may not always be correlated with better tolerance to alloantigens.

Many challenges to optimize transplant tolerance remain¹⁷³. Many successful preclinical studies in mice and rats have not resulted in positive results when translated to humans¹⁷⁴. Large animal models including nonhuman primate models are necessary to confirm the studies done in rodents. A number of examples teach us to be careful in designing early stage clinical trials. One such example is a first in human clinical trial with a CD28 superagonist, which resulted in life-threatening cytokine storm following administration of this agent to normal healthy volunteers¹⁷⁵. In this clinical trial, the superagonist antibody was used at a small fraction of the dose that was used safely in nonhuman primates. The difference in CD28 superagonist binding affinity in humans and non-human primates might explain the disparity in severe side effects. Anti-CD154 antibody administration has also induced unexpected side effects including thrombosis, which precludes conducting further clinical trials of this agent^{176,177}. The lack of standardized clinical tests for the evaluation of immune tolerance is another problem in developing clinical trials.

Conclusion

Induction of transplant tolerance improves outcomes after HSCT and solid organ transplantation. Adoptive immunotherapy with immune suppressor cells including regulatory T cells, NK-T cells, veto cells and facilitating cells, achieving mixed chimerism with thymic irradiation, costimulatory molecule blockade with/without inhibitory signal activation and elimination of alloreactive T cells with varying methods including pre or post-transplant cyclophosphamide administration appear to be effective methods to induce transplant tolerance. Clinically applicable transplant tolerance induction methods will broaden options to treat GVHD, facilitate anti-tumor effects, and shorten the period of post-transplant immune deficiency, thus leading to the development of new treatment strategies.

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Figure 1.

Thymopoiesis; T cell development in the thymus includes negative and positive selection of thymocytes. DN; double negative thymocytes, TEC; thymic epithelial cells, cTEC; cortical TEC, mTEC; medullary TEC, DC; dendritic cells, DP; double positive thymocytes, RAG; recombination activating genes, TCR; T cell receptor.

Figure 2. T Cell-APC interaction with costimulatory molecules. CTLA-4; Cytotoxic T- Lymphocyte Antigen 4, TCR; T cell receptor, MHC; Major histocompatibility molecules, ICOS; Inducible T-cell costimulator, PD-1; Programmed death-1, PD1-L; Programmed death-1 ligand.

Figure 3. Main mechanisms of T regulatory cell function. T_{reg} suppress effector T cell function through cell contact (Figure 3A) and inhibitory cytokine and granzyme secretion (Figure 3B). T Eff; effector T cells, IDO; indoleamine 2,3-dioxygenase, Trp; Tryptophan, Kyn; kynurenines, IFNs; interferons