Cell-Based Therapies in the Prevention of Solid Organ Transplant Rejection

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Abstract: Problem statement: Organ transplantation is a life-saving and increasingly common procedure, as it often serves as the only treatment available for end-stage organ disease. Although the constant development of new and more effective immunosuppressive drugs has revolutionized the prevention and treatment of acute graft rejection, these drugs have significant toxicity, greatly increase patient susceptibility to neoplasms and infection and exert little impact on chronic rejection. **Approach:** The literature was reviewed to illuminate the mechanisms by which the anti-donor immune response is initiated and how cellular therapies impact this response. **Results:** Data show that Donor Specific Transfusion, Apoptotic Cell therapies and Dendritic Cell therapies all function as a source of alloantigen to suppress the anti-donor T cell response. **Conclusion:** Cellular therapies hold promise in the prevention of solid organ allograft rejection, but require optimization and study in large animal models before clinical implementation.

Key words: Ag-Presenting Cells (APC), Donor Specific Transfusion (DST), Dendritic Cells (DC), Apoptotic Cell Therapy, allograft, transplant rejection

INTRODUCTION

Organ transplantation is becoming an increasingly important and common surgical procedure, as transplantation surgery often serves as the only life-saving treatment available for end-stage organ disease. Although the constant development of new and more effective immunosuppressive drugs along with better knowledge of their therapeutic application have revolutionized the prevention and treatment of acute graft rejection, these drugs have significant toxicity and greatly increase patient susceptibility to malignant neoplasias and infections. Further, the implementation of immunosuppressive agents has exerted little impact on the incidence of chronic rejection and therefore overall long-term graft survival has only improved modestly. Novel cell-based therapies that are able to down-regulate the immune response against donor Antigen (Ag), without inducing generalized immune suppression and its harmful side-effects, represent a promising avenue of research in transplantation. Cell-based therapies include Donor Specific Transfusion (DST) (the transfusion of donor peripheral blood mononuclear cells), donor-derived apoptotic cell therapy and negative immunization with donor- (or recipient-) derived Dendritic Cells (DC). DC-based therapies in particular have received significant attention over the past decade as possibly ideal

therapeutics in transplantation. Recent data though have shown that DC-based therapy, like DST and apoptotic cell-based therapies all function via a similar mechanism, that is as a source of alloantigen for presentation by recipient Ag-Presenting Cells (APC) to T cells.

Basics of solid organ transplantation: Allografts are grafted organs/tissues/cells transplanted between genetically disparate, MHC-mismatched individuals of the same species. The targeted Ag are called alloantigens (alloAg), are derived from Major Histocompatibility Complex (MHC) or minor histocompatibility Ag and are recognized by the adaptive immune response as non-self, or tissue incompatible (Afzali *et al*., 2007). Allo-recognition describes recognition of the allogeneic Ag by the recipient immune system and allo-response refers to the effector mechanisms recruited in the reaction to the transplanted cell/tissue/organ (Afzali *et al*., 2007).

Allografts are threatened by three types of rejection that are defined by both tempo of onset and histopathology. Hyperacute rejection occurs within minutes to h (usually within 48 h) after transplantation surgery and is mediated by deposition of pre-formed circulating antibodies against Ag on graft vascular endothelial cells and the consequent activation of

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complement and coagulation cascades, resulting in intravascular thrombosis, ischemia and necrosis. This results from pre-sensitization of the recipient, by previous blood transfusion, organ transplant, or pregnancy and in 1% of the general population for no known reason. Hyperacute rejection is largely preventable due to screening for antibodies against non-self HLA phenotypes and cross-matching, with subsequent pre-transplantation plasmaphoresis if necessary (Gallon *et al.*, 2002; Magee, 2006).

Acute rejection begins within weeks or months (5 days to 3 months is typical), or in rare cases even years, following transplantation and constitutes the main immediate threat to allograft survival. It is mediated by both innate and adaptive immune responses, however the advent of immunosuppressive drugs renders acute rejection largely preventable. Histopathology reveals diffuse interstitial infiltrate of $CD4^+$ and $CD8^+$ T cells with activated or memory phenotype (Ibrahim *et al.*, 1995).

Chronic rejection develops in months or typically years post-transplantation and is the most common cause of graft loss one year after transplantation (Pascual *et al.*, 2002; Sayegh and Carpenter, 2004). It results from both immune and non-immune factors. Typical features of chronic rejection include steady decline of organ function, interstitial fibrosis, chronic inflammatory infiltrate (i.e., lymphocytes, plasma cells), atrophy and gradual loss of parenchymal cells and chronic vascular arteriopathy, the latter a condition manifested by endothelitis, intimal proliferation, elastic fiber disruption, fibrosis and leukocyte infiltration of medium- and small-size arteries of the graft (Pascual *et al.*, 2002). Unfortunately, current immunosuppression protocols are ineffective at preventing or treating chronic rejection.

The development and introduction of immunosuppressive drugs in the 1980s has greatly reduced the risk of acute rejection. Steroids, calcineurin inhibitors such as tacrolimus and cyclosporine that block TCR-dependent T cell activation, the MTOR inhibitor sirolimus, mycophenylate mofetil which inhibits purine biosynthesis and lymphocyte-depleting antibodies are currently employed in the clinic to prevent or mitigate acute rejection with great success. However, these agents non-specifically suppress the immune system, thus greatly increasing patient susceptibility to opportunistic infections and various cancers. Further, currently employed immunosuppressive regimens offer little protection against chronic rejection and have significant toxicity. Clearly, generation of therapeutics capable of donor Ag-specific suppression is ideal, if not necessary to reduce dependence on chronic pharmacologic agents.

Immune mechanisms of allograft rejection: The diversity and robustness of the alloresponse constitute major challenges to preventing graft rejection. Both the innate and adaptive immune responses are contributory. Mechanisms of graft damage include contact-dependent T cell cytotoxicity, granulocyte activation by Th1 or Th2 cytokines, NK cell-mediated cytotoxicity, delayed-type hypersensitivity like reaction and allo-Ab and complement activation (Moine *et al.*, 2002).

Ischemia-reperfusion injury: Ischemia-reperfusion injury refers to tissue damage resulting from the return of blood supply to tissue after a period of ischemia. This injury is Ag-independent and is responsible for initiating the events associated with rejection. Land *et al.* (1994) developed the "injury hypothesis" by showing that intra-operative treatment of cadaver-derived renal allografts with a free-radical scavenger reduced the incidence of acute rejection and improved long-term graft outcome (Land *et al.*, 1994). Tissue injury up-regulates pro-inflammatory mediators, inducing a robust innate immune response that in turn further promotes inflammation (LaRosa *et al.*, 2007). The innate immune response occurs prior to and independently of the adaptive immune response (Christopher *et al.*, 2002; He *et al.*, 2002; 2003), as RAG-deficient cardiac transplant recipients experience comparable cellular infiltration, chemokine receptor expression and pro-inflammatory cytokine expression with wild-type recipients 1 day post-transplantation (He *et al.*, 2002).

Innate immune cells express non-rearranged pattern recognition receptors that recognize not only conserved pathogen-derived molecules, as originally appreciated (Medzhitov and Janeway, 2002) but also self-derived molecules released from damaged or stressed tissue (Mollen *et al.*, 2006). Optimal inflammatory responses to liver damage by ischemia-reperfusion injury requires signaling through toll-like receptor 4 (TLR4) expressed on hematopoietic-derived phagocytes and activated by products of necrotic cells or extracellular matrix disruption (Shen *et al.*, 2005a; 2005b; Tsung *et al.*, 2005a; Zhai *et al.*, 2004). Interestingly, in humans, studies of lung transplant patients and kidney transplant recipients that are heterozygous for either of two TLR4 functional polymorphisms associated with LPS hyporesponsiveness both showed a reduced incidence of acute allograft rejection (Ducloux *et al.*, 2005; Palmer *et al.*, 2003). This is likely due to abundance of various redundant danger signals. Levels of the danger signal High-Mobility Group Box 1 (HMGB1) are increased following liver ischemia-reperfusion injury as early as 1 h following transplantation and neutralization of HMGB1 decreases markers of liver inflammation (Tsung *et al.*, 2005b). Likewise, inhibiting signals of receptor for Advanced Glycation End products (RAGE), the receptor for HMGB1, prolongs survival of fully allogeneic cardiac allografts (Moser *et al.*, 2007).

Notably, danger signals seem to persist within allografts long after transplantation, as T cell-deficient mice transplanted with mismatched skin or cardiac allografts that are allowed to heal for 50 days, rapidly reject their grafts upon T cell reconstitution (Anderson *et al.*, 2001; Bingaman *et al.*, 2000). If homeostatic proliferation is taken into account using a model devoid of secondary lymphoid organs but containing a normal T cell compartment, allografts display histological evidence of chronic rejection, but are not acutely rejected (Chalasani *et al.*, 2004).

Innate immune response: Polymorphonuclear cells (PMN), or neutrophils, rapidly infiltrate allografts following surgery and ischemia/reperfusion injury. Neutrophils have numerous cytotoxic and pro-inflammatory mechanisms, including release of pro-inflammatory cytokines and chemoattractants and production of reactive oxygen and nitrogen species. In a rat liver model of ischemia-reperfusion injury, depletion of neutrophils abrogates tissue damage (Jaeschke *et al.*, 1990) and neutralization of KC/CXCL1, a potent neutrophil chemoattractant, decreases PMN infiltration and prolongs graft survival (Morita *et al.*, 2001).

NK cells are also important contributors to allograft rejection. Based on the 'missing self' hypothesis, NK cells recognize cells lacking expression of self-MHC class I molecules. NK cells are not sufficient to reject solid organ allografts, as Rag-/- or SCID mice, that lack T and B cells, fail to reject skin or heart allografts (Bingaman *et al.*, 2000; Kitchens *et al.*, 2006). NK cells do however contribute to tissue damage and amplify graft inflammation through release of the pro-inflammatory cytokines IFN-γ and TNF-α and through contact-mediated cytotoxicity (Obara *et al.*, 2005). Further, NK cell depletion in CD28-/- mice, whose T cells are unable to receive co-stimulation, prolongs fully MHC-mismatched cardiac allograft survival (Maier *et al.*, 2001), suggesting that NK cells influence the adaptive immune response (McNerney *et al.*, 2006).

Macrophages (MΦ) are also believed to be important for rejection, although their importance may be organ or model dependent. MΦ contribute to an inflammatory response in multiple ways. They phagocytose necrotic debris, secrete pro-inflammatory cytokines, produce reactive nitrogen and oxygen species and present Ag to effector T cells (Wyburn *et al.*, 2005). In rat renal allografts, MΦ begin infiltrating allografts within 24 h following surgery and proliferate in situ (Grau *et al.*, 1998) and in human acute renal rejection, MΦ accumulate in significant numbers (Hancock *et al.*, 1983). Also in a rat renal transplant model, liposomal clodronate administration 1 d post-transplantation, which depletes the majority of MΦ, reduces allograft damage (Jose *et al.*, 2003), although liposomal clodronate also depletes other subsets of monocytes and DC thus complicating interpretation of the data. The production of iNOS in particular seems important for allograft rejection, as its neutralization prolongs cardiac allograft survival in mice (Roza *et al.*, 2000; Worrall *et al.*, 1995).

Adaptive immune response: There are two mechanisms by which donor-reactive T cells recognize alloAg: the direct and the indirect pathways of allorecognition (Fig. 1) (Game and Lechler, 2002). By the direct pathway, recipient T cells recognize intact donor MHC molecules expressed on the surface of donor APC transplanted along with the allograft (i.e. donor DC, macrophages, endothelial cells) (Lombardi *et al.*, 1989). Ischemia-reperfusion injury and surgical trauma activate donor DC inducing their migration as "passenger leukocytes" to recipient secondary lymphoid organs, where they prime donor-reactive T cells (Anderson *et al.*, 2001). The precursor frequency of direct pathway T cells is extremely high, roughly 1-10% (Baker *et al.*, 2001) of the T cell pool. This direct T cell alloreactivity likely results from cross-reactivity between intact allogeneic MHC molecules and self-MHC-foreign peptide complexes (Lombardi *et al.*, 1989).

By the indirect pathway, recipient T cells recognize self-MHC molecules presenting donor-derived allopeptides on recipient APC (Benichou *et al.*, 1992; Liu *et al.*, 1996). The precursor frequency of indirect pathway T cells is extremely low (1:100,000-200,000) and similar to that for any other conventional/nominal Ag. It is unknown whether recipient APC mobilized into the graft acquire alloAg, then traffic to secondary lymphoid organs to prime indirect pathway T cells, or whether alloAg derived from the graft, either in the form of passenger leukocytes or soluble MHC molecules, enters secondary lymphoid organs where it is taken up by lymphoid-resident DC for presentation. Either way, recipient APC internalize donor Ag and process it into peptide for presentation by self-MHC to indirect pathway T cells.

Recently, a third "semi-direct" pathway of allorecognition has been identified in mouse models. By the semi-direct pathway, intact donor MHC molecules are acquired by recipient APC and are presented intact to direct pathway T cells (Herrera *et al.*, 2004).

The semi-direct pathway is one proposed model challenging the existing paradigm that direct pathway T cells are primed independently of recipient APC and the indirect pathway (Fig. 2a, d). Alternatively, the 4-cell hypothesis suggests that indirect pathway CD4^{+} T helper cells stimulated by recipient APC provide unlinked bystander help to direct pathway CD8^{+} T cells stimulated by donor APC (Fig. 2b, c). Indirect CD4^{+} T cells could also provide CD40-mediated stimulation of

recipient APC that in turn might stimulate the direct pathway response through an unknown mechanism (Fig. 2b), or via interaction between a B cell receptor, if the recipient APC were a B cell, with donor MHC:alloAg on the surface of donor APC (Fig. 2c).

T cells are both necessary and sufficient for allograft rejection of almost all tissues. Acute graft rejection is considered T cell dependent, as several studies demonstrate that mice lacking T cells accept fully MHC-mismatched allografts and that T cell reconstitution results in rejection. Due to the high precursor frequency of direct pathway T cells (approximately 1000 fold greater than indirect pathway T cells), it is assumed that the direct pathway is the more significant contributor of acute rejection (Larsen *et al*., 1990; Lechler and Batchelor, 1982; Talmage *et al*., 1976). However, as the supply of donor APC within the graft wanes over time, the contribution of the direct pathway decreases. Human studies confirm that the direct pathway response is strongest in the period immediately following transplantation. Comparatively, alloAg is shed from the graft continuously and due to epitope spreading (Ciubotariu *et al*., 1998), the significance of the indirect pathway increases (Valujskikh *et al*., 1998). As such, it is considered the main mediator of chronic rejection, which is confirmed in human studies of chronically rejected heart, kidney and lung (Ciubotariu *et al*., 1998; Frasca *et al*., 1998; Hornick *et al*., 2000; Lee *et al*., 2001; Liu *et al*., 1996; Reznik *et al*., 2001; Valujskikh *et al*., 1998; Vella *et al*., 1997).

Notably, there is evidence supporting the ability of the indirect pathway to mediate acute rejection. In human recipients of heart, kidney and liver allografts, in vitro detection of the indirect response shows strong correlation with episodes of rejection (Dalchau *et al*., 1992; Vella *et al*., 1997) and immunization of animals with peptide derived from allogeneic MHC (thus presented through the indirect pathway) causes allograft rejection (Fangmann *et al*., 1992a; 1992b). Finally, using a cardiac allograft transplant model in mice, Auchincloss *et al*. (1993) showed that the indirect pathway is sufficient to elicit graft rejection in the absence of direct allorecognition (Auchincloss *et al*., 1993). The relative contributions of the indirect and direct pathway were evaluated in skin, cornea and retina and results reveal that the importance of each pathway appears to be organ dependent (Illigens *et al*., 2002). As expected, skin allografts have a pronounced direct pathway response, likely attributable to their high passenger APC load. Comparatively, cornea had a more potent indirect pathway response, again not surprising given its low level of MHC molecule expression. Such studies are yet to be performed in heart transplantation, although given the low number of passenger leukocytes, one might expect increased importance of the indirect response.

T cells contribute to allograft rejection by various mechanisms. Contact mediated cytotoxicity and release of pro-inflammatory cytokines are both potent mechanisms of allograft damage. Typically, the allograft response is Th1, IFN-γ mediated, however both Th1 and Th2 effector responses can cause allograft rejection (Moine *et al*., 2002). Further, T cells stimulate other immune cells to cause damage. B cell function and the alloAb response depends on indirect pathway CD4^{+} T cell help because B cells recognizing Ag via B cell receptors internalize, process and present antigenic peptides loaded in self-MHC to T cells, that in turn, provide the necessary help for B cell effector function and Ab class switching (Steele *et al*., 1996; Terasaki, 2003). Indirect pathway T cells could also stimulate recipient MΦ or DC within the graft to release pro-inflammatory molecules in a DTH-like response. This previously has been associated with chronic rejection, however it is possible that the cytotoxic molecules released by MΦ/DC could contribute to acute rejection, particularly since one stimulated MΦ/DC could damage numerous surrounding donor cells simultaneously, while one CD8^{+} cytotoxic T cell targets only one donor cell at a time.

Cellular therapies in solid organ transplantation: The concept of utilizing cellular therapies to induce allograft tolerance has its roots in the earliest studies of transplantation. Billingham *et al*. (1953) showed that infusion of donor allogeneic cells into newborn mice resulted in acceptance of skin allografts in the absence of immunosuppression (Billingham *et al*., 1953). More recently, Sayegh *et al*. (1993) demonstrated that intra-thymic injection of donor allopeptides prolongs subsequent allografts of the same MHC, further indicating that exposure of recipients to donor Ag prior to transplantation has a tolerizing effect. Currently, there are three types of cellular therapies proposed for use in transplantation. Donor-Specific Transfusion (DST) refers to the transfer of donor splenocytes in mice, or peripheral blood mononuclear cells in humans, directly from donor to recipient with little manipulation. DST has been employed in the clinic for decades and in some cases successfully decreased the anti-donor immune response and prolonged allograft survival. However, prevention of acute rejection was not universally achieved and DST was associated with risk of recipient sensitization, thus the advent of pharmacologic immunosuppressive agents replaced DST as the main prophylactic for transplant recipients. Ironically, the negative side effects of pharmacologic immunosuppression coupled with the new goal of achieving operational tolerance, defined as long-term freedom from all immunosuppression with normal graft function, has resulted in a renewed interest in cellular therapies.
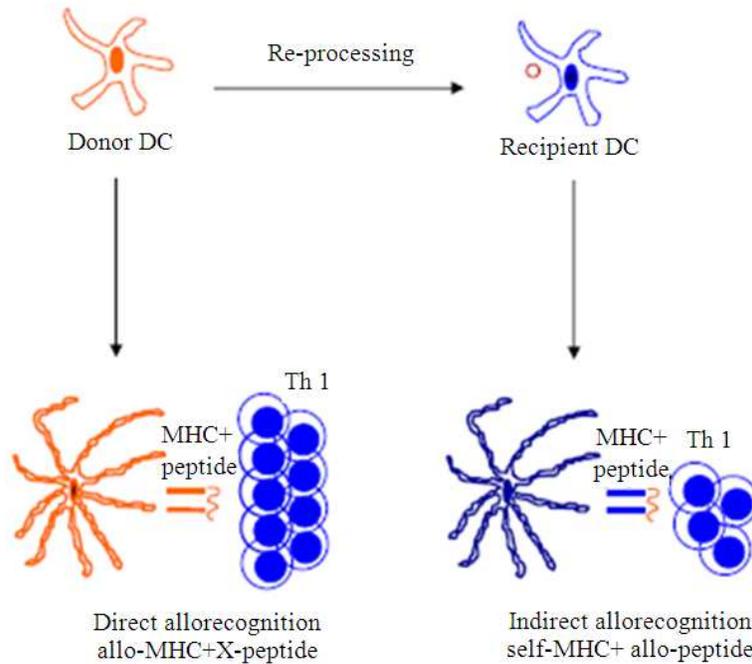
Fig. 1: Pathways of allorecognition. In the direct pathway, donor DC directly interact with anti-donor T cells. In this case, T cells recognize alloMHC: peptide complexes on the surface of donor DC. In the indirect pathway, recipient DC re-process donor alloAg derived from donor APC into allopeptide for presentation by self-MHC to anti-donor T cells.
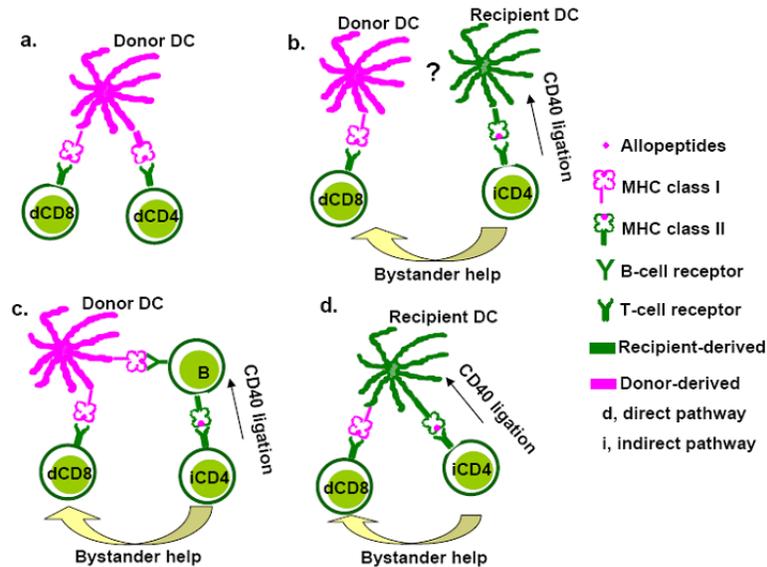

Fig. 2: Models of direct pathway CD8^{+} T cell priming. (a) Direct pathway T cells are primed by donor APC independently of recipient APC or indirect T cells. (b) Indirect pathway CD4^{+} helper T cells stimulated by recipient APC provide unlinked bystander help to direct pathway CD8^{+} T cells stimulated by donor APC. (c, d) Indirect CD4^{+} T cells provide CD40-mediated stimulation of recipient APC that in turn might stimulate the direct pathway response via (c) interaction between a B cell receptor, if the recipient APC were a B cell, or (d) a recipient DC with donor MHC:alloAg on its surface.

Advancements in understanding peripheral tolerance mechanisms has led to development of newer cellular therapies including donor apoptotic cell therapy and tolerogenic DC therapies.

DST in transplantation of solid organ allografts: Quezada *et al*. (2003) showed that DST significantly prolongs skin allograft survival through peripheral deletion of indirect pathway CD4$^+$ T cells and increased numbers of regulatory T cells (Treg). Brouard and Soulillou and colleagues demonstrated that infusion of splenocytes without additional immunosuppression leads to long-term survival of cardiac allografts through expansion of Treg and that transfer of long-term survivor splenic T cells to new allograft recipients transfers long-term allograft survival in an Ag-specific manner (Lair *et al*., 2007). Importantly, in the Quezada study, the injected living donor splenocytes did not directly interact with CD4$^+$ T cells (Quezada *et al*., 2003). This finding suggests that living splenocytes upon i.v. injection in some way transfer alloAg to recipient APC for indirect presentation rather directly interacting with direct pathway T cells.

Apoptotic cell therapy in transplantation of solid organ allografts: Our group has investigated apoptotic cell therapy in allograft survival and demonstrated in mice that i.v. administration of donor-derived UV-B-irradiated apoptotic splenocytes 7 d prior to transplantation significantly prolongs survival of heart allografts in the absence of immunosuppression (Wang *et al*., 2006). Moreover, combination of donor apoptotic splenocytes with suboptimal blockade of the CD40-CD154 pathway with a single dose of anti-CD154 Ab results in long-term survival of cardiac transplants for more than 100 d (Wang *et al*., 2006). The therapeutic effect of donor apoptotic cells was donor-specific and required interaction of the apoptotic cells with recipient DC in secondary lymphoid organs. It also depended on the physical properties of the donor leukocytes, since administration of donor necrotic cells did not affect graft survival (Wang *et al*., 2006). We further demonstrated that i.v. administered donor-apoptotic cells are rapidly phagocytosed by recipient splenic DC, which present apoptotic cell-derived allopeptides in self-MHC to indirect pathway T cells (Wang *et al*., 2006). Interestingly, splenic transgenic CD4$^+$ T cells specific for indirect pathway Ag proliferated in response to injection of BALB/c apoptotic splenocytes, but did not upregulate expression of the T cell activation markers CD25, CD44, CD69 and CD152 and secreted lower amounts of IL-2 and IFN-γ upon ex vivo re-stimulation when compared to controls. Importantly, the defective activation of anti-donor indirect pathway CD4$^+$ T cells resulted in their peripheral deletion, as

their numbers decreased significantly in spleen, LN, blood and peripheral tissues, 14 d after administration of apoptotic cells.

Besides inducing peripheral deletion of donor-reactive T cells, administration of donor apoptotic splenocytes in combination with suboptimal CD40-CD154 blockade promotes differentiation/expansion of donor-specific CD4$^+$ Treg (Wang *et al*., 2006), reduces significantly the level of circulating alloAb in cardiac allograft recipients (Wang *et al*., 2006) and in aortic allograft transplantation, a model of chronic rejection, results in significant inhibition of the histopathological features of chronic vascular arteriopathy, the classic feature of chronic rejection (Wang *et al*., 2009).

Dendritic cell-based therapies in transplantation of solid organ allografts: DC are a heterogeneous population of hematopoietic-derived APC that orchestrate the adaptive immune response to self- and foreign-Ag. DC are defined by surface expression of MHC class-II molecules, expression of the integrin and complement receptor CD11c (in mice) and their unique ability to stimulate naïve T cells (Banchereau *et al*., 2000). DC respond to both endogenous and exogenous danger signals such as pathogen-associated molecular patterns on microorganisms, products secreted by activated MΦ and parenchymal cells and stimulatory signals from activated T cells (Banchereau *et al*., 2000). Since they are the only APC capable of priming naïve T cells, they serve as a crucial link between innate and adaptive immunity (Banchereau *et al*., 2000; Banchereau and Steinman, 1998).

In the periphery, DC exist in 3 different stages of activation/maturation: immature, semi-mature or quiescent and mature or activated (Fig. 3). In the steady-state, quiescent DC are highly phagocytic and express low surface levels of MHC : peptide complexes and the co-stimulatory molecules CD80 and CD86 (Banchereau *et al*., 2000; Banchereau and Steinman, 1998; Cella *et al*., 1997). DC mature upon exposure to pro-inflammatory stimuli. During maturation, DC decrease phagocytic ability and increase their surface expression of MHC class-I and -II: peptide complexes, CD40, CD80 and CD86 (Cella *et al*., 1997). The MHC: peptide complexes presented by DC bind the T cell receptor (TCR) (signal 1), while CD80 and CD86 bind CD28 (signal 2) on the surface of the T cell. This induces secretion of IL-2, which is a potent agonist for T cell activation/proliferation (Lenschow *et al*., 1996). Additionally, the interaction between CD40 on the DC and CD40Ligand (CD40L or CD154) on the T cell surface further enhances DC and T cell stimulation (Grewal and Flavell, 1998).
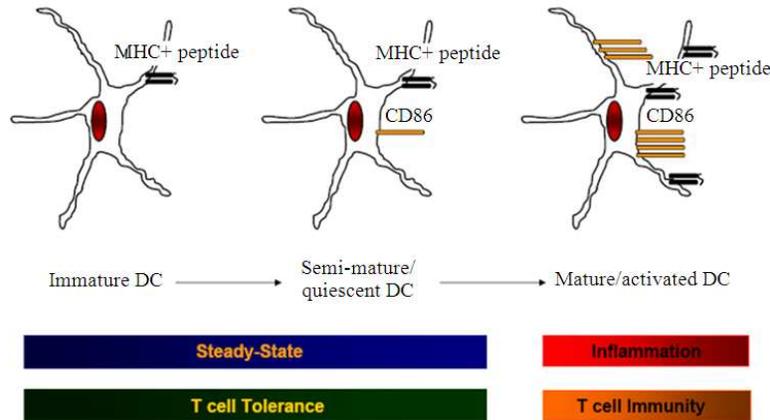
Fig 3: Stages of DC maturation. DC can exist as either immature (express low levels of MHC: peptide complex without co-stimulatory molecules CD80 or CD86), semi-mature or quiescent (express low levels of MHC:peptide complex along with low levels of co-stimulatory molecules), or mature or activated (express high levels of MHC:peptide complex and high levels of co-stimulatory molecules).

Mature DC secrete soluble mediators (signal 3) which help direct the immune response, such as IL-12p70, which polarizes T cells toward a Th1 response (Banchereau *et al*., 2000; Banchereau and Steinman, 1998; Cella *et al*., 1997).

DC as inducers of T cell tolerance: Although most T cells recognizing self-peptides with high affinity are eliminated centrally in the thymus through negative selection (Brocker *et al*., 1997), a percentage of self-reactive T cells escape thymic deletion and access the periphery. An efficient mechanism in the periphery is therefore necessary to prevent activation of self-reactive T cells and avoid autoimmunity.

Quiescent DC expressing MHC: Peptide complexes (signal 1) with low levels of co-stimulatory signals (signal 2) provide sub-threshold stimulation to auto-reactive T cells, resulting in defective T cell activation (Schwartz, 1990; Steinman *et al*., 2003; Steinman and Nussenzweig, 2002). Incomplete T cell activation results in poor cellular proliferation followed by deletion, anergy and likely differentiation/expansion of Treg cells, all mechanisms leading to T cell hypo-responsiveness or tolerance (Steinman *et al*., 2003; Steinman and Nussenzweig, 2002).

DC therapies in transplantation of solid organ allografts: The ability of DC to tolerize T cells in an Ag-specific manner, coupled with the ability to propagate large numbers of DC in vitro, has heralded the use of tolerogenic/immunosuppressive DC as therapeutics for transplantation and autoimmunity. Tolerogenic DC are in an immature or quiescent state, in that they express low levels of MHC: peptide complexes with or without low amounts of co-stimulatory molecules (Fig. 3) and are impaired in their ability to produce the Th1-driving cytokine IL-12p70. A number of methods, including culture-conditioning with different cytokines or growth factors, treatment with various pharmacologic agents and genetic engineering (Table 1) (Morelli and Thomson, 2007) have been developed to increase DC tolerizing potential and/or render tolerogenic DC resistant to maturation, to combat the risk of *in vivo* maturation of the administered DC and thus patient sensitization.

These pharmacologic or genetic manipulations affect DC differentiation and function by various mechanisms (Morelli and Thomson, 2007). Some tolerogenic DC express high levels of co-inhibitory molecules such as PD-L1 on their surface, or have a lower net ratio of co-stimulatory to co-inhibitory molecule expression (i.e., CD86 : PD-L1). Secretion of inhibitory cytokines/mediators also is variable, as some tolerogenic DC release IL-10, which has been shown to inhibit T cell expansion (Li *et al*., 2005). Further, tolerogenic DC can induce activation-induced cell death through FasL expression or induce Treg through IDO expression (Bohana-Kashtan and Civin, 2004; Mellor *et al*., 2004). A number of different types of tolerogenic DC, many with different phenotypic and functional characteristics have been studied in mouse models of heart transplantation using a heterotopic cardiac allograft model. These tolerogenic DC therapies prolong allograft survival with a Mean Survival Time (MST) between 20 and 50 days (Emmer *et al*., 2006; Lan *et al*., 2006; O'Connell *et al*., 2002; Tang *et al*., 2006; Turnquist *et al*., 2007). Typically, an increased percentage of Treg is observed along with decreased T cell effector responses.

Table 1: Methods of generating tolerogenic DC *in vitro*

Cytokines, growth factors	Pharmacologic mediators	Genetic engineering
↓GM-CSF	Immunosuppresstive or anti-inflammatory drugs	Recombinant viral vectors or naked DNA:
↑IL-10	Cyclosporine	CD95L (FasL)
↑TGFβ1	Rapamycin	CTLA4-Ig
↑VEGF	Tacrolimus	IL-10
	Deoxyspergualin	TGFβ1
	Mycophenolate mofetil	IDO
	Sanglifehrin A	Soluble TNFR
	Corticosteroids	CCR7
	A spirin	Dominant-negative I_KB
	1 α, 25-dihydroxyvitamin D_3	Kinase
	N-acety-L-cysteine	ODNs;
	Cyclic AMP inducers	NF-$_K$b-specific decoy
	Glucosamine	RAN interference:
	Cobalt protoporphyrin	RELB
	ILT receptor ligands	IL-10

Although these different tolerogenic DC vary phenotypically in vitro, the similar effect on allograft survival and anti-donor T cell responses suggests similar mechanism of action in vivo.

It has been assumed that therapeutic tolerogenic DC, once administered i.v. to prospective graft recipients, interact directly with anti-donor T cells. Given the preponderance of the direct pathway in acute allograft rejection, it has further been assumed that the ability to down-modulate the direct pathway response makes DC therapies superior to alternative cellular therapies (DST and apoptotic cell therapy) in transplantation.

Our group has investigated the mechanisms by which DC therapy functions in vivo to prolong cardiac allograft survival in a mouse model. We demonstrated that donor-derived DC rapidly die once transfused into the prospective graft recipient and that apoptotic cell fragments derived from the injected therapeutic DC are taken up by the recipient's DC and processed into donor alloAg for presentation via recipient MHC molecules to indirect pathway CD4^{+} T cells (Divito *et al*., 2010). If the recipient DC are quiescent, then this process induces defective activation of indirect pathway CD4^{+} T cells with preferential survival of Treg (Divito *et al*., 2010). We have further shown that recipient DC are necessary for DC therapy prolongation of allograft survival using CD11c-DTR bone marrow chimeric mice to selectively deplete recipient but not donor DC (Wang *et al*., 2012). Finally, we showed that apoptotic cell therapy, DST and DC therapy all act via the same mechanism of action, that is, they serve as a source of donor alloAg for recipient DC, rather than through direct interaction with anti-donor T cells (Divito *et al*., 2010).

A brief statement on T cell-based therapies in transplantation: In addition to the above named cellular therapies, the possibility of employing regulatory and/or anergic T cells generated and expanded in vitro, ex vivo, or even in vivo, to modulate the anti-graft response has received great attention over the past decade. Various methodologies of generating regulatory and/or anergic T cells have been studied in multiple animal models of organ transplantation with initial promising results (reviewed by (McMurchy *et al*., 2011)) and Tregs generated in vitro have been utilized in clinical trials for graft-versus-host disease in stem cell transplantation, demonstrating its clinical feasibility (Brunstein *et al*., 2011; Ianni *et al*., 2011; Trzonkowski *et al*., 2009). Given the expanse of literature on T cell based-therapies in transplantation, as well as in autoimmunity and graft-versus-host-disease, they will not be discussed further here, except to say that T cell-based therapies are a major area of research in the transplantation community and warrant further research.

CONCLUSION

Despite the promising results of cell-based therapies in animal models, it is important to acknowledge caveats to current research and roadblocks to clinical translation. First, most research on cellular therapies in transplantation has been conducted using young inbred mice maintained in clean or nearly pathogen-free conditions, which therefore may possess low numbers of memory T cells compared to outbred animals. Comparatively, transplant rejection in humans is mediated by both naïve and memory T cells and as such, the ability of cellular therapies to tolerize not only recipient DC-naive T cell interaction, but also other non-professional recipient APC capable of activating anti-donor memory T cells, will likely be critical for successful therapy.

Second, safety is a major area of concern. What if a preparation of cellular therapeutic contained traces of effector cells? Or if a batch of the tolerizing agent was ineffective? And if administration of a cellular therapy

had a deleterious effect, could the adoptively transferred cell be eliminated from the patient? In reality, it is likely that cellular therapeutics would never be instituted in the clinic as a single therapy, but rather in combination with additional pharmacologic immunosuppression. This may help alleviate some of the risks associated with cellular products. Further, clinical experience with cellular therapeutics is being gained from the fields of stem cell transplantation and cancer.

Solid organ transplantation remains a clinical challenge despite its increasing prevalence. Many questions remain in regards to the immunopathogenesis of allograft rejection and whether cellular therapies will ultimately have a role in preventing rejection. Regardless, optimization and application of cellular therapies represents an active area of research in transplant immunology and still holds promise for clinical use.

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