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Harnessing Apoptotic Cells for Transplantation Tolerance: Current Status and Future Perspectives

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Abstract

Purpose of review—The use of donor apoptotic cells is an emerging therapy for inducing transplantation tolerance. In this review, we will discuss current understanding of mechanisms of this approach, as well as crucial aspects necessary for successful translation of this approach to clinical transplantation.

Recent findings—Transplantation tolerance by donor apoptotic cells is mediated by their homeostatic interaction with recipient phagocytes, and subsequent expansion of suppressor cell populations as well as inhibition of effector T cells via deletion and anergy. To ensure their tolerogenicity, it is critical to procure non-stressed donor cells, and to induce and arrest their apoptosis at the appropriate stage prior to their administration. Equally important is the monitoring of dynamics of recipient immunological status, and its influences on tolerance efficacy and longevity. Emerging concepts and technologies may significantly streamline tolerogen manufacture and delivery of this approach, and smooth its transition to clinical application.

Summary—Hijacking homeostatic clearance of donor apoptotic cells is a promising strategy for transplantation tolerance. Timing is now mature for concerted efforts for transitioning this strategy to clinical transplantation.

Keywords

Apoptotic cells; transplantation; tolerance; suppressor cells; sensitization; nanoparticles

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Author Contributions

A.D. and X.L. conceptualized and wrote the manuscript. X.L. edited and finalized the manuscript.

Compliance with Ethical Guidelines

Conflict of Interest

Xunrong Luo and Anil Dangi declare no conflict of interest.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

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Introduction

To achieve immunosuppression-free graft survival by inducing transplantation immune tolerance has been a long-sought goal of the transplant field. Clinical tolerance for human allogeneic kidney transplantation has now been achieved, using protocols incorporating donor stem cell transplantation that results in transient or permanent donor chimerism [1–5]. This approach, however, often requires highly toxic conditioning regimens to prepare the recipients for donor bone marrow transplant. In addition, long-term risks for graft versus host disease (GVHD) remain formidable.

A conceptually different approach is to induce peripheral tolerance by providing donor antigens in an immunologically quiescent manner. One such approach is by using donor apoptotic cells. Billions of apoptotic cells are generated and cleared every day in the body in order to maintain its health and function [6**]. Significant progress has been made in understanding the highly complex cellular signaling network orchestrating such rapid, highly efficient and immunoquiescent clearance of apoptotic cells [7]. It is now generally agreed that apoptotic cell clearance suppresses inflammation in its local milieu [8]. Therefore, harnessing such immunosuppressive potential of apoptotic cell clearance for the therapeutic purpose of inducing transplantation tolerance is a rational approach and has been attempted by numerous groups [9].

As transplant antigens are of donor origin, i.e. donor major, or minor histocompatibility complexes (MHC or MiHC), or non-MHC linked non-self-antigens [10], providing apoptotic cells of donor origin would be necessary to deliver the entire spectrum of relevant donor antigens for inducing donor-specific transplant tolerance. Indeed, several approaches of infusion donor apoptotic cells in this context have been experimented. Most notably, UVB and γ-irradiation have been used as apoptotic stimuli to generate donor apoptotic splenocytes [11–15] followed by their infusion to the recipients. In rodent models of allogeneic cardiac, aortic and islet transplantation, such an intervention results in preventing acute allograft rejection in the complete absence of immunosuppression, and in some cases also in preventing chronic rejection [16, 17]. Our lab has serendipitously discovered that donor splencoytes simply treated with a chemical cross-linker called ethylene carbodiimide (ECDI) undergo rapid and efficient early apoptosis [18*]. When infused intravenously, they are readily phagocytized by recipients' splenic antigen-presenting cells (APCs) [19], and induce robust donor-specific tolerance in murine models of allogeneic and xenogeneic transplantation [18*, 19–24]. This approach is currently being tested in non-human primate models of allogeneic and xenogeneic pancreatic islet transplantations with promising results (Hering, Miller and Luo, unpublished data). Independently, a recent phase I/IIa clinical trial has been published using a single infusion of donor early apoptotic mononuclear cells for prophylaxis of GVHD in 13 patients receiving allogeneic bone marrow transplantation [25**]. The study demonstrated the remarkable safety and potential efficacy of this approach in reducing acute GVHD [25**]. Collectively, these data highlight the potential use of donor apoptotic cells for inducing donor-specific tolerance for clinical transplantation. Table 1 summarizes published preclinical and clinical studies employing donor apoptotic cells for transplantation tolerance induction.

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In the rest of this review, we will discuss critical variables pertaining to the efficacy of donor apoptotic cell-based tolerance therapies. We will further discuss emerging technologies, while exploiting the same concept of apoptotic cell clearance, may significantly simplify tolerogen manufacture and/or delivery.

Brief overview of mechanisms

APCs are the first point of encounter between the host and the infused apoptotic donor cells. APCs are critical regulators in maintaining homeostasis as well as in initiating innate and adaptive immune responses. Phagocytosis of apoptotic cells by APCs creates a local immunosuppressive milieu by promoting the expression of anti-inflammatory cytokines such as IL-10, TGF-β, indoleamine 2,3-dioxygenase, and nitric oxide while suppressing the production of inflammatory cytokines such as IL-1β, IL-6, IL-12, IL-23, TNF-α, IFN-γ [13, 26]. Besides regulating the production of cytokines, phagocytosis of donor apoptotic cells also substantially influence the expression of cell-surface molecules on the phagocytes. Following uptake of apoptotic cells, dendritic cells express low levels of antigen presenting and co-stimulatory molecules such as MHC II, CD80 and CD86, and are refractory to further stimulation by activating signals such as LPS or TNF-α [13]. In addition, we have shown that splenic $CD11c^{+}$ dendritic cells up-regulate expression of co-inhibitory molecules PD-L1 and PD-L2 upon uptaking donor apoptotic cells [19], and are subsequently involved in the deletion or anergy of allo-reactive T cells [19, 20]. The role of $CD4^{+}CD25^{+}Foxp3^{+}$ immunosuppressive regulatory T cells (Tregs) in mediating transplantation tolerance by apoptotic cells is also well documented. We and others have shown that Tregs expand in response to infusions of donor apoptotic cells, accumulate in allografts and are obligatory for graft protection [14, 15, 19, 20, 27]. In addition to Tregs, another immunosuppressive population expanded by donor apoptotic cell infusions is myeloid-derived suppressor cells (MDSCs), including $Gr1^{HI}$ and $Ly6C^{HI}$ MDSCs [21, 23]. In models of cardiac transplantation [21, 23] and islet transplantation (Dangi, unpublished data), MDSC populations are observed to be obligatory for transplant tolerance induced by this approach.

Based on the above understanding, a critical “checkpoint” of this strategy for ensuring tolerance efficacy is the initial encounter between host APCs and the infused apoptotic donor cells. We will now discuss important considerations for strict “quality control” of this encounter for the goal of achieving tolerance.

Critical aspects for preparing tolerogenic donor apoptotic cells

Successful manufacturing of tolerogenic donor apoptotic cells will likely require strict controls of the following parameters:

Control of stage of apoptosis

Early- and mid-stage apoptosis differ in the degree of translocation of the membrane phospholipid, phosphatidyserine (PS), although are both characterized by a yet intact plasma membrane. Late-stage apoptosis, on the other hand, is characterized by the loss of integrity of the plasma membrane, and releasing of the intracellular content to its surrounding. Early- and mid-stage apoptosis send specific signals to phagocytes tasked with the cleanup. Series

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of synapses bridging such apoptotic cells and their interacting phagocytes result in suppression of inflammatory cytokines with simultaneous induction of anti-inflammatory cytokines [9, 28]. The ultimate effect is to allow removal of apoptotic cells by the phagocytes without rendering inflammation. In contrast, releasing of intracellular contents, such as genomic/mitochondrial DNA, heat shock proteins, etc., during late-stage of apoptosis or further secondary necrosis engages receptors for damage-associated molecular patterns (DAMPs) and delivers a rather different set of signals. These signals are often inflammatory, leading to immune activation.

In vitro strategies for rendering donor cells apoptotic for transplantation tolerance include γ-irradiation [12], UV-B irradiation [13], and chemical treatments such as with ethylene carbodiimide (ECDI) [20] or paraformaldehyde [29]. In using these approaches, efforts have been made to ensure that the treated donor cells enter an "early" apoptotic stage [12, 13, 18*], marked by annexin V^+ but propidium $iodide^-$, without progressing to "late" apoptotic or even secondary necrotic stage, marked by propidium $iodide^+$. The need for this quality control will likely limit the "shelf-life" of apoptotic cell products. After being induced to undergo apoptosis, the cells will likely follow a kinetic process through the different stages of apoptosis, therefore exhibiting only a finite shelf-life during which they maintain their tolerogenic property. Another pragmatic consideration is the storability of donor cells prior to apoptosis induction, especially in settings of deceased donor transplantation. In this regard, we have reported that frozen and thawed donor cells may contain a large proportion of necrotic cells which compromise their suitability for manufacturing apoptotic cells and their ability for inducing transplantation tolerance [18*].

A long-standing concern of using donor apoptotic cells is the potential hazard of sensitization. This concern has been substantiated by studies showing that *in vitro* generated, drug-conditioned donor-derived dendritic cells that demonstrate tolerogenic features *in vitro* could in fact be sensitizing *in vivo* [30, 31, 32*]. A common feature of these studies is that the infused donor-derived dendritic cells, while alive when injected (i.e. not induced to become apoptotic prior to injection), experienced a rather short life-span once injected before being quickly phagocytosed by recipient dendritic cells [32*]. However, the modality of their death prior to their ingestion has not been examined. In fact, depending on whether such donor-derived dendritic cells were generated by Flt-3 ligand or by GM-CSF, a dichotomous response of tolerance vs. sensitization results upon their infusion to the recipients [30]. Evidence suggests that when the *in vivo* death of donor APCs is triggered by NK cell-mediated apoptosis involving caspases [33], tolerance ensues [34]. These findings underscore that strictly ensuring the pathways of ultimate demise may be necessary for the desired host immune responses. Therefore, for clinical translation, a quality control program for ensuring early apoptotic cells to encounter host APCs will likely be critical to the success of transplantation tolerance induction.

Control of cell stress

In addition to deliberate apoptosis-inducing treatments mentioned above, certain stress signals within the donor, including hypoxic, oxidative, and endopasmic reticulum (ER) stresses, may also destine the retrieved donor cells to undergo apoptosis. One such example

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is cell death induced by stress from active microbial infections [35–37]. Such cell stress is closely linked to unfolded protein response signals downstream of toll-like receptors (TLRs) [38], therefore participates in inflammasome activation [39, 40], supports production of pro-inflammatory cytokines [41] and consequent inflammatory rather than homeostatic clearance of the dying cells [42]. Alternatively, cell stress may also trigger autophagy which can act to inhibit apoptosis via inhibition of apoptosis-associated caspase [43]. Therefore, employing "stressed" donor cells may not be able to induce tolerance, and may even result in sensitization. In this regard, we have observed that cells retrieved from donors infected with murine cytomegalovirus (MCMV) were unable to induce transplant tolerance following their treatment with ECDI in contrast to cells from un-infected donors (Dangi, unpublished data). In addition to causing aberrant apoptosis, microbial infections of donor cells may further compromise tolerance efficacy [44] by directly transmitting pathogens to the recipient. Quality control for excluding "stressed" cells awaits identification of precise biomarkers of cell stress, and will likely also be crucial to the success of this tolerance approach, particularly in deceased donor transplantation.

Control of workload

Based on the above rationale, care should be taken to ensure that the work load of clearing apoptotic cells does not exceed the host's capacity of clearance, because residual apoptotic cells may then be allowed to progress to late stage apoptosis or even secondary necrosis, and consequently induce inflammation instead of tolerance. We have previously demonstrated that 4×10^8 cells/kg is the ideal dose of donor apoptotic cells for successful induction of tolerance in rodent models [18*]. However, the optimal dose for clinical application in human transplantation has not been defined. Ongoing experiments in non-human primates will hopefully address this question (Hering, Miller and Luo). Alternatively, we have observed that repetitive small doses of donor apoptotic cells are feasible and have additive efficacy for inducing graft protection [21]. This approach may be safer as the host's capacity of homeostatic clearance is much less likely to be exceeded. Another consideration in this regard is the potential need for individual dose adjustment when the intended recipient carries certain diseases known to be associated with defects in apoptotic cell clearance, such as certain autoimmune diseases [45] or wide-spread atherosclerosis [46]. For such recipients, the use of donor apoptotic cells may need to be further scaled down or avoided altogether. A standardized assay using recipient-derived phagocytes for assessing clearance capacity of donor apoptotic cells will be highly desirable for determining the ideal dose of donor apoptotic cells to use in a given individual.

Recipient conditioning and monitoring

In applying pre-emptive donor apoptotic cells to transplant recipients for tolerance induction, several important recipient factors should be considered.

Prior sensitization

It has been observed that the same apoptotic donor cell product, while tolerogenic in naïve hosts, is ineffective or even sensitizing in inflammatory hosts. This scenario was initially dissected in models of tolerance by donor specific transfusion (DST). Once transfused, the

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donor cells quickly become the target of recipient NK cells, are rendered apoptotic and ingested by recipient APCs. In humans, the sensitization state of the recipients determines whether DST is tolerizing or sensitizating [47]. In a carefully designed sensitized murine transplant model, Burns *et al* show that pre-existing donor specific antibodies (DSAs) act as opsonins to the infused DST. Uptake of opsonized donor cells by APCs leads to their maturation, enhances their priming of alloreactive T cells, and ultimately prevents induction of transplantation tolerance [48*]. In this process, classical complement activation triggered by the preformed antibodies to donor antigens on the transfused donor cells and complement-dependent cytokine and chemokines secretion [49] likely play a role in further augmenting the adaptive immune response, consequently making the DST sensitizing rather than tolerizing.

Thus, a highly clinically relevant question is: how to effectively induce transplantation tolerance in sensitized recipients using apoptotic donor cell based strategies? A useful framework to conceptualize a solution is to address two separate compartments in a sensitized host: i.e.: (1) pre-formed anti-donor antibodies, and (2) donor-specific memory cells.

Pre-existing anti-donor antibodies may be detrimental to tolerance induction by apoptotic donor cells, either by themselves as described above [48*] or in conjunction with allo-specific memory B cells [50, 51]. Fortunately, therapeutic modalities to remove allo-antibodies, at least transiently, are clinically readily available. A combination of plasmapheresis and IVIG is frequently employed in settings of antibody-mediated rejection to remove allo-antibodies. For tolerance induction in sensitized recipients, it will be crucial to define the extent and duration for which pre-existing anti-donor antibodies should be removed. Our own data from studies of donor ECDI-SP in sensitized recipients suggests that DSAs, if present at a low level, may not interfere with tolerance efficacy. In fact, their production may be further suppressed by donor ECDI-SP treatment (Dangi, unpublished data). Further studies are needed to fully understand how antibody strength, subtypes, rebound, complement-fixing ability, and the nature of their interaction with donor apoptotic cells may differentially influence the outcome of tolerance by this approach.

The second barrier to tolerance induction in a sensitized recipient is the presence of donor-specific memory T cells. Allo-specific memory T cells can be generated by prior rejection [52] or by infection through heterologous immunity [53]. Such memory T cells respond rapidly to repeat antigen stimulation, are less dependent on conventional costimulation, and are consequently more resistant to tolerance therapies such as by apoptotic donor cells [52]. Based on understanding of the biology of memory T cells, therapeutic strategies for controlling these cells include: (1) targeting alternative costimulation pathways, such as OX40/OX40L [54] and CD27/CD70 [55], thought to be more commonly used by memory T cells for their activation and effector functions; (2) newer small molecules and biologics, such as 15-deoxyspergaulin analogue [56], sphingosine-1 phosphate receptor agonist [57], and anti-LFA-1 [58]; or (3) combinatorial therapies. These therapeutic interventions aiming to directly inhibit memory cells should now be tested in restoring tolerance efficacy by apoptotic donor cells in sensitized recipients. In our own studies of donor ECDI-SP, by utilizing T cell receptor transgenic T cells, we have found that a combinatorial therapy

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consisting of donor ECDI-SP, anti-CD40L and rapamycin, but not individual therapies alone, is highly effective in eliminating alloantigen-specific memory T cells and promoting long-term allograft survival in sensitized recipients (Dangi, unpublished data). Our finding suggests that donor apoptotic cells, when combined with additional targeting strategies, may in fact be an effective modality for controlling memory T cells. Studies uncovering mechanisms of combinatorial therapies will likely be highly informative for designing effective tolerance strategies for sensitized recipients.

Tolerance longevity

While most studies have focused on tolerance *induction*, it is in fact the understanding of tolerance *maintenance* that will have a direct impact on our ability to ensure lasting tolerance once it is induced. While induction of tolerance by apoptotic cells has been shown to involve a multitude of mechanisms including regulation, deletion and anergy, it appears that maintenance of tolerance relies on anergy more than any other mechanism [59, 60]. Our own studies using donor ECDI-SP support this notion and reveal that once tolerance is established, thorough depletion of $CD25^{+}$ cells does not result in breaking of tolerance or precipitation of graft rejection. In contrast, blocking PD-1/PD-L1 interaction does lead to tolerance reversal in previously tolerized recipients [19] likely by reverting T cell anergic [59, 61, 62]. These findings suggest that tolerance by apoptotic donor cells could potentially be de-stabilized by signals capable of breaking T cell anergy. One such signal is infection. Using *Listeria monocytogenes*, Chong and Alegre *et al* have shown that microbial infection results in TLR signaling and proinflammatory cytokine production, leading to loss of T cell anergy and acute rejection of previously accepted allografts in tolerant hosts [63]. They went on to show that transplantation tolerance abrogated in this manner can spontaneously restore phenotypically [64*]; however, the re-established tolerance exhibits an altered gene signature from that of the original tolerant state, alluding to a molecular compromise of the robustness of the restored tolerance [65]. Another highly clinically relevant infection in transplantation is CMV. Using donor ECDI-SP for tolerance induction and maintenance, we have observed that CMV infection abrogates tolerance induction as well as tolerance maintenance. At least one mechanism implicated in CMV-mediated tolerance impairment is their ability to modulate the differentiation and function of myeloid derived suppressor cells via induction of type I interferon (Dangi, unpublished data). This leads to subsequent enhancement of host antigen presentation and T helper cell responses [66], as well as impairment of Treg numbers and function [66, 67]. As compelling data from independent groups have now converged on the detrimental effects of pathogens on tolerance longevity, there is now an urgent need to examine the individual mechanism of tolerance impairment by each clinically relevant pathogen, and to design individualized therapeutic strategies aimed to preserve tolerance in settings of such infections.

Emerging concepts and technologies

In vivo apoptosis

An emerging concept for antigen-specific tolerance alternative to infusing *ex vivo* generated apoptotic cells is to induce apoptosis *in vivo*. The feasibility of this approach was uncovered from studies delineating the tolerogenic mechanism of anti-CD3 monoclonal antibody. This

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antibody has long been used to treat autoimmune disorders and transplant rejection, and has largely been thought to exert its effect through rapid depletion of T cells and generation of Tregs. However, it has been recently revealed that in fact this antibody induces immune tolerance by engaging phagocytes such as macrophages and immature dendritic cells to produce TGF-β in the process of ingesting and digesting apoptotic T cells [68]. This concept of harnessing the potential of *in vivo* apoptosis for the purpose of immune tolerance induction was further supported by a recent study demonstrating the ability of other *in vivo* apoptosis-inducing regimens to similarly achieve immune tolerance [69*]. In this work, the authors used a number of strategies to induce apoptosis of cells of hematopoietic origin, including systemic sub-lethal irradiation, depletion of B cells or CD8 T cells with specific monoclonal antibodies. In doing so, the resulting apoptotic cells trigger professional phagocytes to produce TGF-β, which in turn directs naïve CD4 T cells to differentiate into $Foxp3^+$ Tregs. The antigen specificity of this approach is determined by the provision of antigenic peptides during the burst of TGF-β that confer antigen specificity to the *in vivo* differentiated $Foxp3^+$ Tregs. In murine models of autoimmunity, this approach has been shown to effectively establish antigen-specific immune tolerance to EAE and colitis. Effort should now be made to test the efficacy of this approach in alloimmune tolerance. Conceivably, donor specificity can be restricted by the transplanted organ itself which carries the full spectrum of relevant donor antigens, directing donor-specific Tregs to be induced from naïve CD4 T cells under the apoptosis-induced TGF-β milieu. Certainly, several questions will first need to be addressed in setting of alloimmune tolerance. These include: (1) what cell populations will be the best to induce apoptosis; (2) what source of alloantigens will be the best for driving donor-specific Treg induction; (3) what strategies specific to alloantigens will be necessary to maximize the robustness of this approach in transplant, particularly in light of the large allo-specific T cell clone size in comparison to that present in autoimmunity. However, if successful strategies are identified, this approach could potentially eliminate many concerns of the *ex vivo* approach discussed above.

Nanoparticles for tolerogenic delivery of donor antigens

Instead of donor apoptotic cells, solubilized donor antigens in the form of donor cell lysate coupled to apoptotic recipient cells are able to induce transplant tolerance with equal efficacy [18*, 70]. This finding is of pragmatic importance, because it obviates the need for procuring large numbers of fresh donor cells for manufacturing apoptotic donor cell products, which can be logistically cumbersome at the time of deceased donor organ donation. Solubilized donor lysate can also be frozen for storage and later thawed for coupling when needed, providing additional flexibility to this approach. These findings prompted us to further test synthetic particles as an acellular carrier for delivering solubilized donor antigens for tolerance induction. Compared with cells, synthetic particles can be manufactured with more consistency and reproducibility. Solubilized donor antigens coupled to poly(lactide-co-glycolide) (PLG) particles significantly inhibit anti-donor responses, improve transplant graft survival [71*] and prevent GVHD [72]; and when combined with transient low dose rapamycin induce permanent donor-specific tolerance [71*]. Synthetic carriers can further serve as a versatile platform for additional functionalization to enhance tolerogenic efficacy of the particles. For example, PLG particles modified with phosphatidylserine have been reported to be particularly efficient in

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promoting expansion of Tregs while suppressing activation of allo-reactive T cells [73]. Nanoparticles can also be engineered for targeted delivery of anti-inflammatory biologics [74, 75]. An interesting recent study demonstrates that nanoparticles containing encapsulated antigens and rapamycin can be directly injected into local lymph nodes (LNs) [76*], resulting in local LN reorganization, systemic Treg expansion and inhibition of autoimmunity in a mouse model of multiple sclerosis [76*]. These data collectively highlights the enormous potential of synthetic particles for delivering alloantigens for transplantation tolerance induction.

Conclusions

The recent decade saw a major breakthrough in our ability to induce clinical transplantation tolerance via establishing bone marrow chimerism. However, the use of apoptotic donor cells may present an alternative and less toxic approach for tolerance induction. A great deal has been learned of the mechanisms and limitations of this tolerance approach. Aiming for clinical translation, it is of paramount importance to establish the following: (1) a standard for quality control of apoptosis and cell stress to ensure immunological quiescence when the cells are infused; (2) a standard for assessing recipient phagocytic competency; (3) a standard for recipient immune monitoring that can accurately predict recipient sensitization, tolerance robustness and tolerance stability, and allow for tolerance personalization. Conceivably, the first clinical trial using donor apoptotic cells in organ transplant recipients will need to assess the risk of recipient sensitization by cells manufactured strictly according to the above standards. At the same time, emerging concepts (e.g. *in vivo* apoptosis) and technologies (e.g. nanocarriers) will immensely streamline the current process of donor apoptotic cell manufacturing and delivery, and ultimately make its clinical translation readily achievable.

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Table 1

A summary of studies employing donor apoptotic cells for inducing tolerance in various transplant models.

Transplantation	Treatment regimen	Key findings	Ref.
Murine bone marrow transplantation (allogeneic)	Apoptotic cells (apoptosis induced by either γ- or UVB-irradiation or anti-Fas Ab)	Improved engraftment of hematopoietic cells independent of the origin of the apoptotic cells: donor, recipient or third party	[11]
Murine bone marrow transplantation (allogeneic)	Donor apoptotic cells (apoptosis induced by γ-irradiation)	Improved engraftment and prevented humoral response	[16]
Murine heart transplantation (allogeneic)	Donor apoptotic cells (apoptosis induced by UVB-irradiation) plus CD40-CD154 blockade	1. Improved graft survival mediated by tolerogenic DCs. 2. Indefinite graft survival mediated by Tregs.	[13]
Murine bone marrow transplantation (allogeneic)	Donor apoptotic cells (apoptosis induced by γ-irradiation) and effects by conventional immunosuppression.	Improved engraftment and increased Tregs which were further enhanced by sirolimus	[27]
Murine pancreatic islet transplantation (allogeneic)	Donor apoptotic cells (splenic antigen-presenting cells fixed with ethylene carbodiimide)	Prolonged survival of islets; mediated by Tregs and intact PD1-PDL1 signaling.	[20]
Murine aortic transplantation (allogeneic)	Donor apoptotic cells (apoptosis induced by UVB-irradiation)	Prevented chronic allograft vasculopathy; mediated by preventing indirect allorecognition.	[17]
Murine skin transplantation (partial mismatch)	Mismatch peptide antigen coupled to splenocytes employing ethylene carbodiimide	Prolonged graft survival, decreased CD154 expression on CD4 Th cells prevented CD8 CTL response.	[70]
Murine pancreatic islet transplantation (allogeneic)	Donor apoptotic cells (splenic antigen-presenting cells fixed with ethylene carbodiimide)	Prolonged graft survival; mediated by depletion of T cells indirectly recognizing alloantigens while anergizing T cell clones directly recognizing alloantigens.	[19]
Xenogeneic pancreatic islet transplantation (rat to mouse)	Donor apoptotic cells (splenic antigen-presenting cells fixed with ethylene carbodiimide) plus B cell depletion using anti-CD20 mAb	Prolonged survival of xenografts; mediated by suppression of xeno-specific T cell-priming and memory cell generation.	[22]
Murine pancreatic islet transplantation (allogeneic)	Donor apoptotic splenocytes (apoptosis induced by UVB-irradiation)	Prolonged survival of islets; mediated by increased Tregs and toleraogenic DCs.	[15]
Murine heart transplantation (allogeneic)	Donor apoptotic cells (splenic antigen-presenting cells fixed with ethylene carbodiimide)	Prolonged survival of allografts; mediated by increased numbers of $Ly6C^{HI}$ and $Gr1^{HI}$ myeloid derived suppressor cells.	[23]
HLA-Matched Allogeneic Bone Marrow Transplantation (Phase I/IIa Clinical Trial)	Apoptotic cells (mononuclear enriched cell fraction from a sibling HLA matched donor; apoptosis induced by freezing/thawing and a treatment with methylprednisolone)	Apoptotic cell infusion is safe for clinical use and a potentially effective prophylaxis for acute GVHD occurring after myeloablative conditioning.	[25**]
Xenogeneic pancreatic islet transplantation (pig to mouse)	Donor apoptotic cells (splenic antigen-presenting cells fixed with ethylene carbodiimide) plus rapamycin and B cell depletion using anti-CD20 mAb	Prolonged survival of xenografts; mediated by inhibiting early anti-donor Th17 response.	[24]