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Hematopoietic Stem Cells and Solid Organ Transplantation

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Abstract

Solid organ transplantation provides life saving therapy for patients with end stage organ disease. In order that the transplanted organ survive, the recipient must take a lifelong cocktail of immunosuppressive medications that increase the risk for infections, malignancies and drug toxicities. Data from many animal studies have shown that recipients can be made tolerant of their transplanted organ by infusing stem cells, particularly hematopoietic stem cells, prior to the transplant. The animal data have been translated into humans and now several clinical trials have demonstrated that infusion of hematopoietic stem cells, along with specialized conditioning regimens, can permit solid organ allograft survival without immunosuppressive medications. This important therapeutic advance has been made possible by understanding the immunologic mechanisms by which stem cells modify the host immune system, although it must be cautioned that the conditioning regimens are often severe and associated with significant morbidity. This review discusses the role of hematopoietic stem cells in solid organ transplantation, provides an understanding of how these stem cells modify the host immune system and describes how newer information about adaptive and innate immunity might lead to improvements in the use of hematopoietic stem cells to induce tolerance to transplanted organs.

Introduction

Long-term outcomes in solid organ transplantation are limited by ongoing alloreactivity to the transplanted organ and non-specificity and toxicity of immunosuppressive medications. Current immunosuppressive medications are highly effective in suppressing host anti-donor responses, but they do so at the cost of increased risk for infectious and malignant complications. Therefore, it has been a long-time goal of physicians caring for patients with solid organ transplants to develop new therapies that essentially trick the recipient's immune system into accepting the transplanted organ as it's own (also called tolerance) and thereby avoid the need for immunosuppressive medications. Many investigators have shown that animals can be made tolerant to transplanted organs by the infusion of hematopoietic stem cells (HSCs) at the time of transplantation. It has in fact been known for many years that

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infusion of HSCs leads to engraftment of donor cells within the thymus and bone marrow of the recipient, which then modifies the recipient's immune response to donor antigens. While HSC transplantation has been shown to induce transplantation tolerance in rodents, monkeys and swine models, recent studies now show that HSCs might also induce tolerance in humans that receive a solid organ transplant (1). The mechanisms by which HSC infusions induce tolerance have been the subject of multiple published studies. As newer information is acquired about the way in which HSCs modify the host immune system it is likely that novel therapies will emerge that improve the ability of HSCs to induce tolerance in human transplant recipients. This review focuses on the role HSCs in immunologic tolerance and describes new concepts applicable to HSC transplantation in human solid organ transplantation.

What are stem cells?

Stem cells are undifferentiated, pluripotent, precursors that are able to transform into mature cells with specialized functions. A common factor among all stem cells remains their ability to exhibit extensive self-renewal and differentiation. Four different types of stem cells have been described including: embryonic stem cells (ESCs); adult stem cells (ASCs); induced pluripotent stem cells (iPS); and cancer stem cells (CSCs). ESCs are derived from pre-implantation epiblasts and are distinguished by their ability to maintain pluripotency. ASCs are postnatal derivatives of ESCs located throughout the body, and classified by their tissue of origin (e.g., hematopoietic, mesenchymal, neural, etc.). It is these types of stem cells, particularly HSCs that are used to induce tolerance to transplanted organs and are the focus of this review. iPS cells are differentiated adult cells that have been reprogrammed to assume a stem cell-like state of pluripotency. Each of the different types of stem cells can be distinguished by their cell surface phenotype (Table 1) (2–5).

Stem cells grow and differentiate in environments called "niches"; and duplicating these niches has been a challenge for clinical application. Niches are physiologically defined microenvironments with properties that regulate and support the balance of quiescence, self-renewal and differentiation. In the bone marrow, adhesion molecules and components of the extracellular matrix are important for anchoring adult HSCs to the stroma, allowing for regulation of survival, proliferation and differentiation (6–8). Both cellular and extracellular matrix elements of the stem cell niche are essential for normal stem cell function. For instance, the extracellular matrix molecules osteopontin and hyaluronic acid, as well as membrane-bound stem cell factor (mSCF), regulate HSC quiescence, homing, trans-marrow migration and lodgment in the niche (9–11). Inflammatory signaling molecules including interferons, tumor necrosis factor-alpha and toll-like receptors (TLRs) are also recognized to play an important role in regulating stem cell responses involving growth, proliferation and differentiation (12). Niche-associated elements mediate the many growth and development pathways of residing cells, and it is often the relationship between stem cells and their respective niches that direct phenotypic variations between stem cell types.

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Why are stem cells of interest in solid organ transplantation?

Since the mid twentieth century, scientists have used HSC infusions (administered through the injection of donor bone marrow) to modify host immune responses in experimental models of autoimmunity and solid organ transplantation (13). Infusion of allogeneic donor bone marrow can lead to a state of mixed hematopoietic chimerism, where the genetically distinct donor HSCs engraft in the host and differentiate into lymphocytes of donor origin that coexist with those of the host.

Mixed hematopoietic chimerism was first associated with alloantigen tolerance by Owen in 1945 when freemartin cattle (fraternal twins sharing a placental circulation) were shown to be chimeric, and tolerant of one another (14, 15). Kashiwagi and Starzl introduced the concept of mixed chimerism in human transplantation in 1969 when they identified donor immunoglobulins circulating in the blood of recipients of liver allografts (16). Inducing tolerance through mixed chimerism became of great interest in experimental models of transplantation during the 1970s and 80s. However, it became clear that the induction of tolerance required host conditioning with harsh toxic agents, such as total lymphoid irradiation (TLI) and/or whole body irradiation (WBI) (17–25). Improving on the early experimental methods, Cobbold and Waldmann developed a preconditioning regimen using monoclonal antibodies to deplete the host of T cells; however, high doses of irradiation were still needed to achieve anything more than transient chimerism (26). Sharabi and Sachs took these early observations further and hypothesized that perhaps the monoclonal antibody regimen used by Cobbold failed to deplete mature T-cells residing in thymus. While a thymectomy could potentially overcome this problem, it would also leave the host without thymic stromal elements essential for educating naïve host T cells. They eventually reported successful long lasting chimerism by using a conditioning regimen of selective thymic irradiation (TI) and sub-lethal WBI along with T cell depletion (26, 27). Since these early days, a number of other preconditioning regimens have been tried along with infusion of donor HSCs to improve tolerance induction to transplanted organs. Other experimental models have been developed to eliminate irradiation preconditioning by either administering higher number of HCSs, selective T cell subset depletion and/or co-stimulatory blockade (28, 29).

Larger animal models, such as miniature swine, with greater similarities than rodents to human biology, were used in preparatory experiments for transitioning into clinical studies. In 1988, Pennington and Sachs pioneered the use of bone marrow transplantation in partially inbred, major histocompatibility complex (MHC)-defined miniature swine conditioned with total body irradiation (TBI). Initial attempts were associated with high rates of graft versus host disease (GVHD), but long lasting hematopoietic chimerism was achieved in the pig model when swine anti-CD3 antibody became available. In fact, the addition of host T cell depletion to the regimen achieved host tolerance to genetically disparate pig skin and kidney transplants (30–33). Non-human primates models have also been developed as a bridge to clinical practice. Cynomolgus recipient monkeys conditioned before transplant with donor bone marrow, sub-lethal TBI, TI and thymoglobulin, and then with cyclosporine A for four weeks after receiving an orthotopic histocompatibility antigen-mismatched kidney transplant

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developed clear evidence of hematopoietic chimerism. Eleven of 13 animals developed chimerism while 10 of 13 survived long-term without rejection (34).

The early animal studies led the way for HSC transplantation as a way to prolong human kidney allograft survival. In 1999 the Spitzer and Sachs team at the Massachusetts General Hospital treated a 55 year old female with end stage renal disease secondary to multiple myeloma with combined histocompatibility leukocyte antigen (HLA)-matched bone marrow and kidney transplant after conditioning with cyclophosphamide, antithymocyte globulin (ATG), and thymic irradiation. Cyclosporine, as the only post-transplant immunosuppressive therapy, was tapered and discontinued on day 73 after transplantation. No rejection episodes occurred and renal function remained normal 5 years after discontinuation of all immunosuppressive therapy (35, 36). Ciancio et al showed that infusion of donor bone marrow cells significantly improved long-term allograft survival and that the degree of hematopoietic chimerism correlated with the improvement in allograft function in both deceased and live kidney donor recipients, although in none of their subjects was immunosuppression discontinued (36, 40). Later, Millan and colleagues at Stanford reported four patients who were given combined kidney and HSC transplants following nonmyeloablative post-transplant conditioning with TLI and ATG. One patient had humoral rejection, one was able to wean off all immunosuppressive maintenance and the fourth patient did not reach the point of drug withdrawal (37). In 2006 the Massachusetts General group treated six patients with renal failure due to multiple myeloma with simultaneous kidney and bone marrow transplantation from HLA-identical sibling donors following nonmyeloablative conditioning with cyclophosphamide, peri-transplant ATG and thymic irradiation (38). Cyclosporine was given for approximately 2–3 months post-transplant in the majority of patients, followed by donor leukocyte infusions. Three patients lost detectable chimerism but accepted their kidney grafts off immunosuppression for 2 to >7 years. Two patients achieved full donor chimerism, but resumed immunosuppression to treat graft-versus-host disease. Only one patient experienced rejection, following cyclosporine withdrawal, but responded to acute immunosuppressant treatment, which was later successfully withdrawn. Following this experiment, Kawai and Sachs treated five end-stage renal disease patients with combined bone marrow and kidney transplants from HLA single-haplotype mismatched living related donors through use of a nonmyeloablative preparative regimen (39). Irreversible humoral rejection occurred in one patient. In the other four recipients, all immunosuppressive therapy was discontinued within 9 to 14 months after the transplant and renal function remained stable for 2 to 5.3 years. Ciancio, in another trial of haplotype mismatched donor stem cell infusions, used almetzumab as an induction agent and found the benefit of the donor lymphocyte infusions to be eliminated by the induction agent, possibly because chimerism was prevented by almetzumab (43). In a more recent trial, Leventhal & Ildstad reported fifteen HLA-mismatched living donor renal transplant recipients who underwent low intensity conditioning (fludarabine, cyclophosphamide, TBI), followed by a living donor kidney transplant (40). Maintenance of immunosuppression, tacrolimus and mycophenolate was weaned over one year. All but one patient demonstrated peripheral blood macrochimerism after transplantation. Engraftment failure occurred in one highly sensitized recipient, but complete immunosuppression withdrawal was successful by one year post-transplantation in all patients with durable chimerism. There was no evidence

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of graft versus host disease and renal transplantation loss occurred in only one patient who developed sepsis following an atypical viral infection. Table 2 provides a listing of these studies in which bone marrow infusions containing HSCs have been used to induce tolerance to solid organ transplants in humans (36–44). Other studies have certainly also been done support the importance of donor specific transfusions for transplant tolerance, including the Trivedi group in Hyderabad (45) and further studies on genomic markers is providing new information from groups that have been working in this area for some time (46, 47).

How do HSCs induce tolerance?

Several immunologic mechanisms have been observed in experimental models that help to explain how donor HSCs might induce host tolerance to alloantigens. When donor bone marrow is infused into the host, HSCs engraft within the recipient bone marrow and thymus and repopulate the host immune system with lymphocytes of donor origin. As shown in the schematic in Figure 1, the presence of donor progenitors within the thymus leads to the development of T cells that recognize donor antigens expressed by the transplanted organ as self, and thus the host becomes tolerant of the allograft. The coexistence of host and donor hematopoietic cells is called chimerism and it is this chimerism within the host that drives central tolerance mechanisms. Central tolerance is defined as tolerance that occurs while developing immune cells are still present in the thymus (48). Increasing evidence highlights central tolerance as the key mechanism of long-lasting HSC-induced allograft tolerance (49). This type of tolerance in many experimental models is dependent on engraftment of the allogeneic HSCs in the recipient thymus, and many experimental models have shown that newly developed donor-reactive T cells are deleted, resulting in systemic donor-specific tolerance (22, 29, 50, 51). Central tolerance lasts as long as the HSCs of donor origin persist in the host (52, 53).

Due to the dynamic environment of the hematopoietic system following HSC transplantation, levels of chimerism exist and are categorized as either complete (full) or mixed chimerism. As implied, full chimerism exists when all hematopoietic elements are of donor origin, while mixed chimerism is the coexistence of both donor and recipient hematopoietic elements in varying proportions (28). The recipient preconditioning regimen determines the amount of chimerism that can be achieved. In general, complete chimerism requires a more extensive preconditioning regimen, which is associated with greater risk of GVHD and a lower retention of immunocompetence than mixed chimerism (52). Therefore, induction of mixed rather than complete chimerism is favored in clinical protocols that test HSC-induced immunologic tolerance to allografts.

Although central tolerance is a dominant mechanism for HSC-induced tolerance to alloantigens, it may not be complete, in part because not all donor antigens are expressed by donor HSCs in the host thymus, and because T lymphocytes with low affinity for self-antigens may escape the selection process and enter the peripheral lymphoid circulation. When self-reactive T cell populations evade the intrathymic selection processes, peripheral mechanisms are needed to maintain immunologic tolerance. In a transplant setting, with a mild preconditioning regimen aimed at inducing mixed chimerism, the survival of mature alloreactive T cells in the periphery may also be controlled through peripheral mechanisms,

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such as extra-thymic deletion of alloreactive lymphocytes and T cell anergy (54–56). Deletion of alloreactive lymphocytes occurs by activation of death-domain-containing receptors that ultimately cause apoptotic cell death (53). T cell anergy occurs when the T cell receives incomplete activation signals. T cell activation requires the presentation of antigen (provided by antigen presenting cells) through the T cell surface receptor, in addition to a costimulatory signal provided through CD28 costimulatory surface molecules. In the absence of CD28 signaling the T cell becomes hyporesponsive to the antigen presented through the TCR, also termed anergy. (53). Studies from several laboratories demonstrated that co-stimulation blockade is an essential component of allograft tolerance protocols (29, 57–59). In some experimental tolerance studies, co-stimulatory blockade was associated with peripheral deletion of donor-reactive mature host CD4+ T cells (57). However, co-stimulation blockade in combination with mixed chimerism has been shown to also anergize alloreactive host T cells that survived preconditioning (60). In yet other studies, the absence of a co-stimulation signal has been shown to lead to both T cell anergy and apoptosis (54, 55, 60).

Additional proposed mechanisms of immune modulation that allow HSC-induced allograft tolerance have been proposed and involve regulatory and suppressor cells. The ability of transplanted HSCs to create populations of regulatory and suppressor T cells has in fact now become of interest for cellular therapies in clinical transplantation. Of particular interest are “veto cells”, and non thymic-derived “adaptive” T regulatory cells (Tregs) induced in the periphery in response to antigen. While multiple different lineages may make up the larger category of “veto cells”, activated CD8+ T cells with ‘veto’ activity have been implicated in the induction of peripheral tolerance in HSC transplant models (61). By definition, veto cells engage and remove T lymphocytes that are reactive to veto cell antigens through MHC class I ligation as well as through the Fas/FasL signaling pathway (62). In mixed chimerism transplant models, veto cells have gained particular interest due to the ability of peripheral blood HSCs of donor origin to create CD8+ veto cells that can remove anti-donor CD8+ T cells of host origin in the bone marrow and peripheral lymphoid tissues (63). Furthermore, recipient CD8+ T cells with veto power can eliminate donor CD8+ T cells that recognize recipient MHC class I molecules in order to attenuate GVHD (56). While recent animal studies have suggested promise for the use of veto cells to induce tolerance to solid organ transplants and GVHD, currently we lack sufficient in vivo data to support translation to a clinical setting. Regulatory T cells on the other hand have recently been recognized to be integral for maintaining donor-specific tolerance in transplant models and are the focus of several clinical trials in transplantation (64). Recent studies have highlighted the possibility of creating peripherally derived CD4+CD25+ Tregs through in vivo manipulations, and have illustrated the ability of extrathymic-derived Treg populations to induce donor-specific tolerance (65–70). Several studies have provided evidence that Foxp3 expression is induced in CD4+CD25+ T cells in the periphery upon encounter with antigen by way of non-professional APCs, while in the presence of transforming growth factor- β (TGF-β) (71). These data suggest that CD4 Tregs are promising targets for tolerance strategies aimed at alloreactive T cells during the induction of tolerance to alloantigens. While regulatory T cells are important early after tolerance induction, it is thought that long-term tolerance requires additional mechanisms, such as deletion or anergy (72). Recently T cell exhaustion,

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which has been well-known to arise during an immune response to chronic infections and cancers, has been added to the list of possible mechanisms of transplant tolerance (73). Whether through peripheral or central mechanisms, growing evidence continues to highlight transplanted HSCs as crucial elements for the induction of initial and lasting allograft tolerance in multiple different experimental transplant models.

How does the innate immune system influence hematopoietic stem cell transplantation?

While much of the interest in HSC-induced transplant tolerance has focused on the adaptive immune system, recent data suggest that innate immunological processes might also play an important role. The ability of transplanted HSCs to home and engraft in a myeloablated hematopoietic microenvironment appears to be modified by signals derived through the innate immune system. Following myeloblative preconditioning, irradiative damage to the bone marrow microenvironment leads to release of chemo-attractant cytokines and chemokines, which facilitate HSC homing (74). Recent data from Pitchford and Kim have highlighted the importance of complement activation during homing of HSCs to their niches following myeloablative preconditioning (75, 76). Using mice deficient in C3 and C5 complement fragment proteins, these investigators each showed impaired engraftment of HSCs from complement component deficient mice relative to their wild-type counterparts (77). The process of HSC homing to the niche microenvironment is highly dependent on membrane bound receptors, as well as chemo-attractant gradients produced by host cells in the niche microenvironment. The highly studied relationship between the bone marrow niche-associated α-chemokine CXCL12 (stromal-derived factor-1; SDF-1) and its respective HSC transmembrane receptor CXCR4 has consistently yielded evidence to suggest a vital role in homing, retention, and mobilization of HSCs to and from the niche (78–80). Kim and colleagues have documented how degradation of CXCL12 occurs due to induction of a highly proteolytic BM microenvironment following myeloablative preconditioning. Subsequently, activation of the innate immune response leads to upregulation of BM niche complement component cleavage fragments and cationic peptides (e.g. cathelicidin and β_2-defensin), which act to preserve HSC responsiveness toward the lowered SDF-1 chemotactic gradient (75, 81). Additionally, prostaglandin E2 fibrinogen fragments, hyaluronic acid and bioactive lipids have all been recently placed on an emergening list of innate immune response-associated positive regulators of the CXCL12-CXCR4 axis during HSC homing to the BM niche (82–86). It is evident that the processes of homing/lodgment, and subsequently the engraftment of transplanted HSCs formulate a prerequisite of events needed to establish graft-derived hematopoiesis, mixed chimerism and, in turn, immunocompetence.

The influences of the innate immune response on the process of HSC transplantation extend past those seen during the process of homing and engraftment. Several pattern recognition receptors (PRRs) have been found to significantly alter HSC proliferation, differentiation and survival. PRRs are made up of four families of receptors known as toll-like receptors (TLRs), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), C-type Lectin receptors (CLRs) and RIG-1 like receptors (RLRs). Recent data support that both

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TLRs and NLRs are expressed by HSCs and they play key roles as regulators of HSC activation in response to inflammation, danger-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) in vivo (87–89). TLRs are a family of innate immune receptors characterized as transmembrane proteins with the ability to recognize a variety of PAMPs and DAMPs (90). Experiments by Nagai et al found that murine HSCs expressed functional TLRs with the ability to activate HSC proliferation and differentiation upon receptor-specific stimulation (91). Furthermore, Sioud et al provided evidence for the presence of functional TLRs on human CD34+ bone marrow progenitors with capabilities of forcing differentiation and production of pro-inflammatory cytokines upon stimulation (92). In vivo murine models of chronic infection utilizing minimal-dose LPS treatments caused phenotypic changes to HSC populations through TLR dependent pathways and permanently reduced their capacities for repopulation and self-renewal following transplantation (93). Additionally, exposure of HSCs to *Candida albicans* leading to activation of TLR dependent signaling pathways directed HSCs toward proliferation, differentiation and diminished reconstitution ability (94). Similar to TLRs in their ability to recognize PAMPs and DMAPs, NLRs make up a body of intracytoplasmic innate receptors with the ability to mediate apoptosis and secretion of the pro-inflammatory cytokines. Developing evidence from recent clinical studies have found increased mortality and acute GVHD in recipients of allogeneic HSC transplants (HSCT) due to single nucleotide polymorphisms (SNPs) in the NOD2 receptor-coding gene (95–97). Strikingly, clinical findings showed that both donors and recipients with these SNP mutations in the NOD2 lead to increase recipient prevalence of transplant-related mortality and GVHD following HSCT (98, 99). The finding that donor and/or recipient NOD2 SNPs impact outcomes following HSC transplantation suggests that targeting NLRs might improve HSC engraftment, homing, lodgment and subsequent development of tolerance to transplanted allografts.

Conclusions

Given the potential for improving outcomes in solid organ transplantation, substantial efforts have been aimed at finding ways to improve the use of HSCs to tolerize recipients to transplanted allografts. Over the past two decades it has become clear from animal and human studies that transplant tolerance can be achieved through infusion of donor HSCs. The mechanisms by which tolerance is achieved have focused on alterations of central and peripheral adaptive immunity, and modifying these mechanisms has led to remarkable translation of the findings from animal studies into the clinic. Alteration of local stem cell niches, by decreasing the amount of irradiation and preserving thymic stromal cell content and structure, has been an important advance. Additionally, innovations in preconditioning protocols aimed at maximum elimination of pre-existing host T cells, by using targeted monoclonal antibodies, has been another advance that has allowed HSCs to become a viable therapeutic strategy for induction of tolerance. New data suggest that the innate immune system plays an important role in HSC survival in the host and therapies targeted at innate immunity are likely to emerge in the future. HSC transplantation is a promising therapy that allows specific downregulation of immune responses to transplanted organs. As our understanding of the mechanisms by which tolerance is achieved expands, HSC

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transplantation at the time of solid organ transplant will have broader applications and provide lifesaving therapy for patients with end-stage organ disease.

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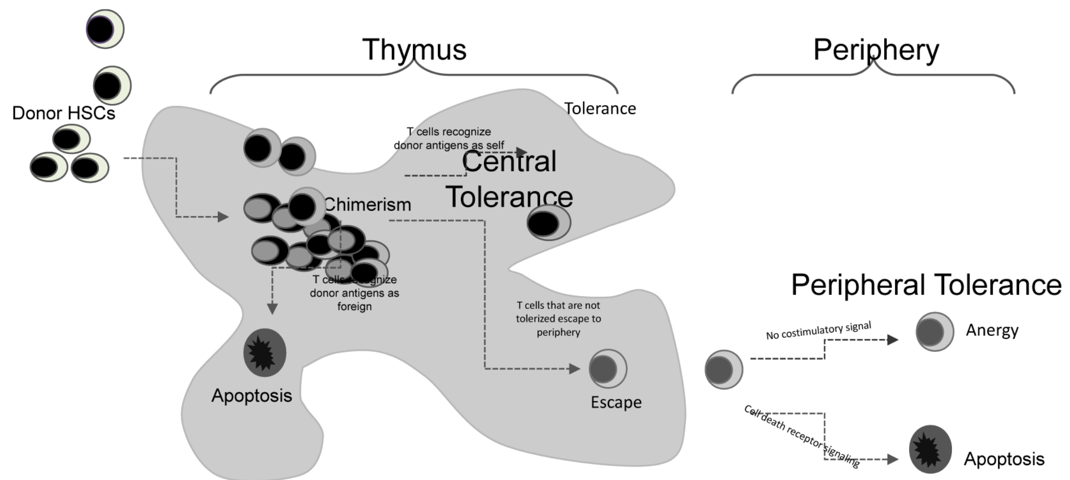

Figure 1. Schematic of mechanisms of HSC-induced tolerance

Donor HSCs home to the recipient thymus, where they integrate with recipient thymic cells and develop donor/recipient chimerism. The result of chimerism is that central tolerance mechanisms ensue allowing mature T cells to develop that can recognize donor antigens as self (tolerized). T cells that recognize donor antigens as foreign undergo apoptosis. T cells that escape to the periphery undergo peripheral tolerance mechanisms that result in either anergy (in the absence of costimulation) or apoptosis (in the presence of costimulation).

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Table 1

Phenotypic markers distinguishing stem cells

Stem cell types*	**Surface marker**
ESC	SSEA-3, SSEA-4, CD9, CD56, Class-I HLA, Thy1 [3,5]
HSC	CD34, CD59, Thy, CD38^{low}, CD135, CD48, CD159 [2,3]
MSC	STRO-1, VCAM-1, Sca-1, BMPR-IA/ALK3, BMPR-IB/ALK6, BMPR-II, CD73, c-kit, Class-I HLA, Thy-1, CD105/ endoglin [3]
CSC	CD44, CD24, CD133, CD166, SSE-1, SSE-4 [4]

*ESC-embryonic stem cell; HSC – hematopoietic stem cell; MSC – mesenchymal stem cell; CSC – cancer stem cell

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Table 2

Tolerance induction studies utilizing donor bone marrow infusions.

Study reference	Patient #s/gps	BM donor/kidney donor	Conditioning regimen/Induction	Post-transplant immunosuppression	Immunosuppression discontinued?	Follow-up time/Outcome
Spitzer et al, 1999(36)	1 pt with multiple myeloma	BM - HLA identical sibling KD - HLA identical LKD sibling	Cyclophosphamide (60mg/kg on d −5 and −4) Anti-thymocyte globulin (15mg/kg (on d −1, +1, +3 and +5) Thymic irradiation (700cGy on d −1) Cyclosporine (5mg/kg on d −1)	Cyclosporine (6mg/kg Q12hr)	Yes	Follow-up – 170 days Creatinine - 0.7mg/dl
Ciancio et al, 2001(41)	Experimental group 63 pts – donor BM Control group 219 pts – no BM	BM – HLA-DR-matched BM KD - HLA-DR-matched DKD	OKT3 mAb	Tacrolimus Methylprednisolone Mycophenolate mofetil	No	Follow-up – mean 4.7 years Creatinines - Control group – 2.15mg/dl Experimental group – 1.7mg/dl Acute rejection Control group - 15% Experimental group – 13% Chronic rejection Control group – 19% Experimental group – 3%
Millan et al, 2002(37)	4 pts	BM – HLA mismatched HSCs KD – HLA-mismatched LKD	Anti-thymocyte globulin (1.5mg/kg on d0; ¾ pts given same dose of ATG on days +1,3,5,9,14) Total lymphoid irradiation (80 cGy on Day +1 and repeated x 10 to total dose of 800 cGy)	Prednisone, Cyclosporine	Yes - 1 pt No – 3pts	Follow-up- 116–374 days Creatinines – 1–1.7mg/dl Rejection - 1/4 humoral rejection
Ciancio et al, 2002(42)	Experimental group – 47 pts- donor BM Control group – 39 pts – no donor BM	BM – matched to kidney donor KD – HLA mismatched LKD	OKT3 mAb Daclizumab	Tacrolimus Prednisone Mycophenolate mofetil	No	Follow-up- 48 months Creatinines - no statistical differences Rejection – no statistical differences
Fudaba et al, 2006(38)	6 pts with multiple myeloma	BM - HLA identical siblings KD - HLA identical LKD siblings	Cyclophosphamide (60 mg/kd/d on d −5, −4) Thymic irradiation (700 cGy on day 1) Anti-thymocyte globulin (15–20mg/kg/d on day−1, +1,3,5)	Cyclosporine Donor leukocyte infusions	Yes – 4pts No – 2 pts	Follow-up- 2–7.3 years Creatinines – 0.9 – 5.6mg/dl Rejection – 1 pt

Study reference	Patient #s/gps	BM donor/kidney donor	Conditioning regimen/Induction	Post-transplant immunosuppression	Immunosuppression discontinued?	Follow-up time/Outcome
Kawai et al, 2008(39)	5 pts with multiple myeloma	BM - HLA haplotype mismatched KD – HLA haplotype mismatched	Cyclophosphamide (60m/kg/d on d −5, −4) Anti CD2 Ab (0.6mg/kg on day −1, 0, +1) Thymic irradiation (700 cGy on d −1) Rituximab (375 mg/SA^2 on d −7, −2)	Cyclosporine Prednisone	Yes – 4pts No – 1pt	Follow-up – 2months – 9.2 years Creatinines - 1.2 – 1.8 mg/dl Rejection - 1/5 humoral rejection
Kawai et al, 2013(100)	5 additional pts- extension of 2008 study	BM HLA haplotype mismatched KD HLA haplotype mismatched	Cyclophosphamide(60mg/kg on d −5, −4) Anti CD2 Ab (0.6mg/kg on d −1, 0 and +1) Thymic irradiation (700 cGy on d −1) Rituximab (375mg/kg/BSA^2 on d −7, −2)	Cyclosporine Prednisone	Yes – 3pts No – 2pts	Follow-up – 2mo – 9.2yrs Creatinines – 0.8 mg/dl - ESRD Rejection - NR
Ciancio et al, 2013(44)	Experimental group – 4 pts donor HSCs Control group – 5 pts no – donor BM	BM - 2–3 haplotype mismatch KD - 2–3 haplotype mismatch	Almetzumab	Tacrolimus Mycophenolate mofetil (Converted to sirolimus in 1 patient)	Yes- 4pts in HSC group	Follow-up – 31– 63 months Creatinines – Experimental group - 1.25 mg/dl Control group - 1.41 mg/dl Rejection – Experimental group--1/4 with rejection; Control group - 2/4 with rejection
Leventhal et al, 2013(40)	15 pts	BM- HSCs enriched for facilitating cells KD - HLA mismatched LDK	Fludarabine (30mg/kg on d −4, −3, −2) Cyclophosphamide(50mg/kg on d −3, +3) Total body irradiation(200 cGy)	Tacrolimus Mycophenolate mofetil Sirolimus	Yes – 6pts No – 4pts	Follow-up 30 months Creatinine <2mg/dl in all Rejection NR

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