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Immunosuppressive therapy in allograft transplantation: from novel insights and strategies to tolerance and challenges

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Abstract

Immunosuppression therapy is the key to successful post-transplantation outcomes. The need for ideal immunosuppression became durable maintenance of long-term graft survival. In spite of current immunosuppressive therapy regimens advances, surgical procedures, and preservation methods, organ transplantation is associated with a long-term poor survival and significant mortality. This has led to an increased interest to optimize outcomes while minimizing associated toxicity by using alternative methods for maintenance immunosuppression, organ rejection treatment, and monitoring of immunosuppression. T regulatory (Treg) cells, which have immunosuppressive functions and cytokine profiles, have been studied during the last decades. Treg cells are able to inhibit the development of allergen-specific cell responses and consequently play a key role in a healthy immune response to allergens. Mature dendritic cells (DCs) play a crucial role in the differentiation of Tregs, which are known to regulate allergic inflammatory responses. Advance in long-standing allograft outcomes may depend on new drugs with novel mechanisms of action with minimal toxicity. Newer treatment techniques have been developed, including using novel stem cell-based therapies such as mesenchymal stem cells, phagosomes and exosomes. Immunoisolation techniques and salvage therapies, including photopheresis and total lymphoid irradiation have emerged as alternative therapeutic choices. The present review evaluates the recent clinical advances in immunosuppressive therapies for organ transplantation.

Key words: *immunosuppression, transplantation, immunoisolation, immune rejection, mesenchymal stem cells, exosomes.*

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Introduction

Immunosuppression therapy is the key to successful post-transplantation outcomes. The need for ideal immunosuppression became durable maintenance of long-term graft survival. Targeting multiple immune pathways with the hope of decreasing both acute and chronic allograft rejection in the field of solid organ transplantation has evolved during the past two decades. Though the medication used at various transplant centers has some differences, current approaches to immunosuppressive therapy in general are similar. Basic maintenance immunosuppressive regimens usually involve three diverse classes, including calcineurin inhibitors, antiproliferative agents, and corticosteroids. Although long-term outcomes after transplantation have improved with the medication and surgical advances, the rate of allograft rejection remains high. This has led to an increased interest in innovative strategies maintaining adequate immunosuppression and declining graft rejection. Novel treatment approaches have been developed including the use of stem cell-based therapies, phagosomes and exosomes. Besides, alternative therapeutic choices have been introduced by merging immunoisolation techniques with salvage therapies. In this review we try to give an insight into recent clinical advances of immunosuppressive therapies for allograft transplantation.

Dendritic cells

Dendritic cells (DCs) are probably the most professional antigen presenting cells (APC) of the mammalian immune system. Dendritic cells react through cell-cell contact or secretion of cytokines towards antigens, and play an important role in regulating the balance between the immune response and tolerance [1, 2]. The most recent studies using DCs showed these cells as a promise or po-

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tential therapies in the field of cancers and other diseases. Dendritic cells prevent autoimmunity through two ways, consisting of induction of apoptosis in autoreactive T cells and by induction of anergy, deletion, or tolerance through cooperation with regulatory T cells (Treg) in the periphery. These cells are divided into the cDC and pDC types according to the function and surface markers [1, 3]. The pDC type has the ability to detect the self and microbial DNA, and is recognized with overexpression of CD123 and interferon α (IFN-α), which plays an important role in innate immunity [4], while under inflammatory conditions, and following Toll-like receptor (TLR) ligation and Ag uptake, cDC migrate to T cell areas of secondary lymphoid tissue to initiate adaptive immunity. Dendritic cells play a crucial role in the central and peripheral tolerance [5]. Central tolerance occurs in the thymus, in which thymoid DCs present self-antigens to the developing T cells, subsequently those T cells that show auto-reactivity above a certain threshold are eliminated. In the peripheral tolerance DCs fail to stimulate T cells sufficiently because of costimulatory factors and low expression of MHC molecules [6]. Furthermore, the absence of an appropriate antigen with expressing indoleamine 2,3-dioxygenase (IDO) by DC, prevent the proliferation of T cells and will eventually eliminate them [5]. Besides, DCs induce Treg ability to maintain the tolerance mode [7].

To maintain the graft and reduce the use of immunosuppressive drugs, tolerogenic DCs of donor or host have been replicated and used *in vitro*. These methods used to prevent T cell effects of immune reactions of host-versus-graft or graft-versus-host. Dendritic cells derived from the recipient organs pulse with the donor antigen cell such as allopeptides and used to reduce response against the donor T cell and to create tolerance.

Intrathymic inoculation of BM-derived mDC pulsed with a donor allopeptide one week prior to transplantation induces tolerance in heart transplantation and pancreatic islet in rat model [8, 9]. This method is suitable for children's heart surgery, because the young thymus is available during surgery. In one study it was shown that repetitive (2×) intravenous administration of BM-derived mDC, pulsed with cell-free lysate from donor splenocytes caused a significant survival among 40% of fully MHC-mismatched cardiac allografts in mice [10]. In a study, an IV injection of donor's iDC seven days before transplantation triggered a significant survival of the heart graft (vascularized) and pancreatic islet (non-vascularized) in the rat [11, 12]. In another study, an injection of pDC with CD154 antibodies significantly prolonged the survival of the heart graft in the mouse [13].

Given the above fact, DCs are needed to replicate a few days before transplantation, which is applicable to live-donor renal and liver transplantation, but not to organ transplantation from deceased donors. It is also shown that when the donor-DCs are injected to transplant mice, there is a possibility of their elimination by NK cells as shown in mice [14].

The regulatory T *cells*

The regulatory T cell (Treg) is a component of the immune system that plays a role in maintaining immune homeostasis and immunosuppressive response. There are generally two types of Tregs, including natural Tregs (nTregs) that are derived from the thymus. Another type is iTreg, which develops outside the thymus under the presence of antigen and tolerogenic condition. The iTreg cells are divided into two categories, containing secreting transforming growth factor β (TGF-β) (Th3 cells) and interleukin (IL)-10 (Tr1 cells) [15]. The nTreg constitutively express Foxp3 and CD25, thus a high and low expression of CD25 and CD127 is used to differentiate them from effector T cell used, respectively. The regulatory T cells undermine the operation of present antigens through direct action on DCs. These cells inhibit maturation of DCs via consuming the extracellular ATP synthesis and IL-2, and secretion of IL-10 and TGF-β. Treg cells also can kill conventional T cells (Tcons) using perforin and granzyme. Studies showed that nTreg plays an important role in protecting self-tolerance and preventing autoimmunity, while in vivo and in vitro activated iTreg plays a more important role in maintaining the graft and inducing tolerance during organ transplantation. Thus it seems that the main role of nTreg is tolerance to self-antigens, whereas iTreg is more responsible for tolerance to foreign antigens. Recent studies considering the application of regulatory T cells in transplantation are summarized in Table 1.

Mesenchymal stem cells

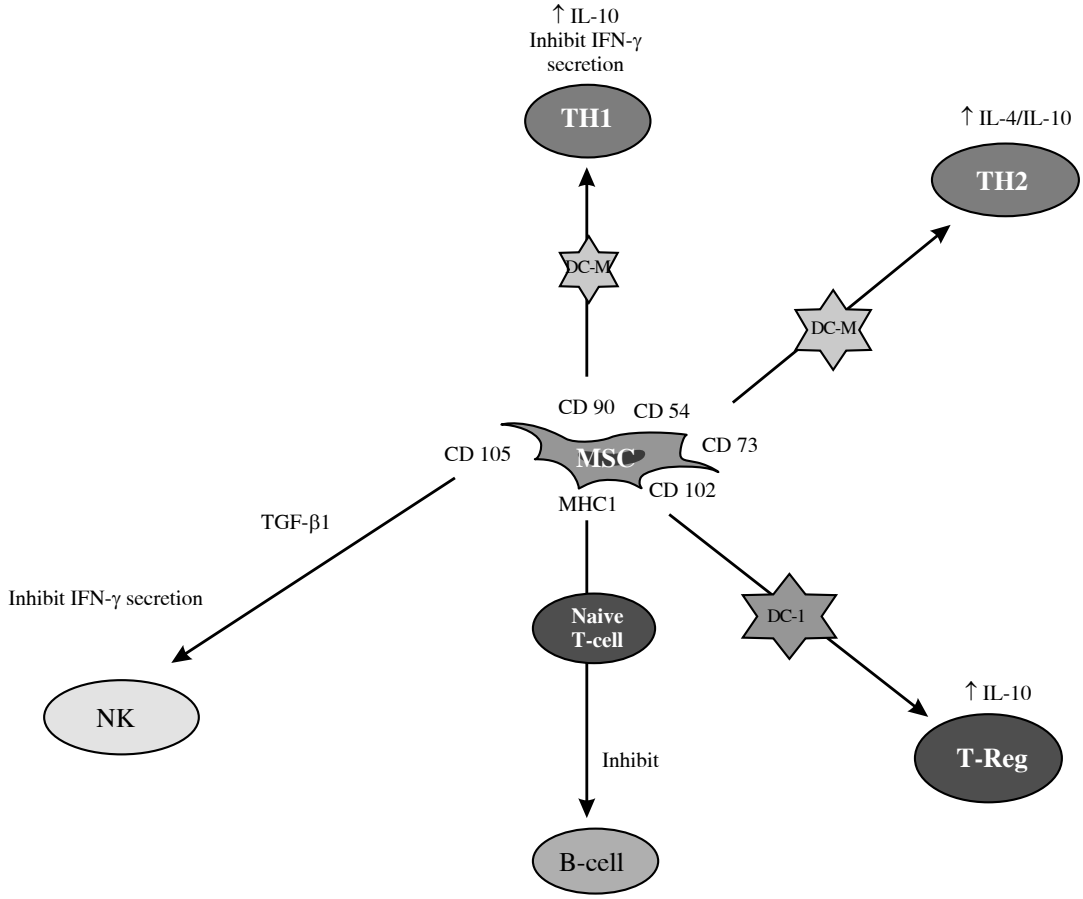
Mesenchymal stem cells (MSCs) are of stromal origin and have been isolated from hair follicles, teeth, bone marrow, lungs, and adipose tissue [29]. Several studies have shown that MSCs have the potential to differentiate into endoderm and bone cells [30]. However, apart from this, MSCs also have great capacity to modulate the immune system through influencing T and B cells. Besides, MSCs influence T cell proliferation via cell-to-cell contact (programmed cell death-1 pathway) or factors such as TGF-α1 (transforming growth factor α1), IDO (indoleamine 2,3-dioxygenase), HO-1 (heme oxygenase-1), HGF (hepatocyte growth factor) and PGE2 (prostaglandin E2) [31-34]. Mesenchymal stem cells also decrease the proliferation of B cells via PD-1 and PD-L1 pathways and partly by using soluble factors in the blood [35]. Furthermore, MSCs reduce expression of MHCs and CD86, CD83 and CD40 molecules (Fig. 1).

Mesenchymal stem cells inhibit the maturation of myeloid-DCs derived from monocytes, and ultimately help to confront with antigen presenting cells (APCs) [36-39]. In

Table 1. Recent studies using DCs in the various fields of transplantation

Author, year	Host	Graft	Method	Findings
Sagoo *et al*., 2013 [16]	NOD/scid/IL-2Rγ–/– mice	skin graft	alloantigen-specific Tregs	significantly improved protection of human skin allografts
Wu *et al*., 2013 [17]	immunodeficient BALB/c.rag2–/–.cγ–/– mice	islet allograft	*ex vivo* expanded $CD25^{high}CD4^{+}$ human Treg	co-transfer of Treg prolonged islet allograft survival and suppression of proliferation and interferon-γ production by T cells
Issa *et al*., 2013 [18]	humanized mouse model	skin allografts	*ex vivo* expanded human Treg	stable long-term transplant survival along with a reduction in the CD8+ human cellular graft infiltrate
Takasato *et al*., 2013 [19]	C57BL/6 mice	cardiac allograft	*ex vivo* expanded antigen-specific iTreg	iTregs induced via the indirect pathway had the greatest ability to prolong graft survival and suppress angiitis. iTregs generated *ex vivo* also induced long-term engraftment without using MHC peptides
Wolf *et al*., 2012 [20]	C57BL/6 (H2b) mice	umbilical cord blood transplant	Tregs expanded *in vivo* by TNFRSF25	significant prolongation of median graft survival from 8 days to 17 days. Treated animals showed increased accumulation of Foxp3+Tregs within the graft and decreased infiltration of inflammatory cells
Guo *et al*., 2012 [21]	BALB/c (H-2d) mice	corneal allograft	*in vitro* expanded $CD4^{+}$ $CD25^{high}$ $Foxp3^{+}$ Treg	prevented fully MHC-mismatched corneal allograft rejection
Yi *et al.,* 2012 [22]	NOD-SCID IL2rγ–/– mice	islet xenograft	*in vitro* expanded autologous Treg in the absence or presence of (IL-10)	Treg prevented islet xenograft by inhibiting graft infiltration of effector cells and their function
Nadig *et al*., 2011 [23]	BALB/c. *rag2–/–.Il2rγ–/–* ($H2^{d}$) mice	Aortic xenograft	$CD25^{hig}hCD4^{+}$ and $CD127^{lo}CD4^{+}$ expanded Treg	Treg cells with a low expression of CD127 provide a more potent therapy to conventional Treg cells
Cao *et al*., 2009 [24]	NOD/SCID mice	human PBL	*ex vivo* expanded CD4+CD25+ Tregs	co-transfer of Tregs with human PBL significantly enhanced survival, reduced GVHD symptoms, and inhibited human IgG/IgM production
Tsang *et al*., 2009 [25]	C57BL/6 mice	heart allograft	$CD4^{+}$ $CD25^{+}$ regulatory T cells	Tregs can induce indefinite survival of BALB/c hearts transplanted into BL/6 recipients when combined with short-term immunosuppression
Kitazawa *et al*., 2008 [26]	Lewis ($RT\text{-}1^{l}$) and DA ($RT\text{-}1^{a}$) rat	cardiac transplan-tation	*in vitro* expanded nTreg in the presence of supCD28 MAb	significant prolongation of full MHC-mismatch cardiac graft survival
Feng *et al*., 2008 [27]	NOD/scid	skin and islet allografts	CD25+CD4+ Treg	prevent rejection of both skin and islet allografts mediated by effector T cells
Veronese *et al*., 2007 [28]	NOD-scid IL2rγ*null* mice	islet allograft	*ex vivo* expanded human CD25+CD4+ Treg	human islet's survival was significantly prolonged following adoptive transfer of Tregs

2002, a research revealed that the injection of allogeneic MSCs prolonged skin graft survival in an animal model [40]. Many studies have been conducted on the immunomodulatory properties of MSC since then. Some studies have focused on the tolerance induced by MSCs against immune rejection and graft-versus-host disease (GVHD) [41]. Another study discusses the functions of MSCs on the T cells infiltrating the central nervous system (CNS) [42]. Thus, it was reported that the injection of MSCs in the experimental autoimmune encephalomyelitis (EAE) mice, may reduce peptide-specific antibodies [43]. In another report, systematic injections of MSCs in the mouse rheumatoid arthritis (RA) model lead to a reduction in inflammatory factors such as IFN-γ and activation of T-regulatory cells to reduce inflammation [44]. One more important feature of MSC is engraftment, which makes the migration of these cells into damaged tissues for regenerating or repairing those tissues. This characteristic is a very clinically important feature. In a preclinical model study, wound sites and pro-inflammatory environments



DC-M – dendritic cells-mature, DC-I – dendritic cells-immature, IL – interleukin, T-Reg – T-regulatory cells, NK – natural killer cells, PGE2 – prostaglandin E2, TGF – transforming growth factor

Fig. 1. Immunomodulatory role of mesenchymal stem cells (MSC)

were found capable of enhancing the engraftment of MSCs such as in lung fibrosis in mice induced by bleomycin in mice. However, the majority of MSCs were found in lung following systemic administration in normal recipients, which, disappeared gradually [45]. Recently, it was reported that the MSCs allogenicity does not disturb the stem cells engraftment during the wound healing process, which is an important therapeutic application of these cells. In general, there are two ways of the MSCs delivery: first is the intravenous infusion that leads to MSCs migration to the inflammatory organs [46], and the second one is local injection that causes the accumulation of MSCs in the damaged tissue [47].

In 2007, a phase I clinical trial revealed that peripheral injection of bone marrow-derived MSCs could improve the post-liver transplantation survival [48]. One year later, FDA approved clinical trials for application of MSCs in patients with multiple sclerosis and cartilage defects, which could become potential clinical strategies in the future.

Aerosolized immunosuppression, macrolides and statins

One of the new approaches is the aerosolized immunosuppressive regimen used specifically for lung transplantation. Many studies reported the use of various aerosolized immunosuppressive regimens and showed their effect in terms of safety, clinical improvement, increasing the graft survival, and prevention of post-transplantation infections (Table 2) [49]. Meanwhile, r*egimens* that include aerosolized *corticosteroids* have fueled a *controversy* regarding the use of such therapies in the transplant recipients (Table 2) [50].

Recently, researches have shown that macrolides not only decline the production of pro-inflammatory cytokines such as IL-6 and IL-8 and chemotaxis or apoptosis in activated neutrophils, but also increase anti-inflammatory cytokines, such as IL-10 [62].

It was found that using antibacterial agents against infection and inflammation, such as azithromycin in patients with lung

Table 2. Available literature considering the use of various aerosolized immunosuppressive and corticosteroid regimens in the transplantation procedure

Author, year	Country, number of patients	Patient	Aerosolized method	Findings
Immunosuppressive regimens				
Lemarie *et al*., 2011 [51]	France, 11	lung carcinoma	aerosolized gemcitabine	safe, with minimal toxicity
Hayes *et al*., 2010 [52]	USA, 1	bronchiolitis obliterans syndrome (BOS)	aerosolized tacrolimus	clinical improvement in functional capacity and oxygenation
Iacono *et al*., 2006 [53]	USA, 58	lung-transplant recipients	aerosolized cyclosporine	improved survival and extended periods of chronic rejection-free survival
Calvo *et al*., 1999 [54]	Spain, 52	lung-transplant recipients	aerosolized amphotericin B	prevented fungal infection in the postoperative period
Iacono *et al*., 1997 [55]	USA, 9	lung-transplant recipients	aerosolized cyclosporine	reversal of acute lung rejection
Keenan *et al*., 1997 [56]	USA, 18	lung-transplant recipients	aerosolized cyclosporine	treatment of refractory acute allograft rejection
Nathan *et al*., 1994 [57]	USA, 9	lung-transplant recipients	aerosolized pentamidine	safe and effective form of pneumocystis carinii pneumonia (PCP) prophylaxis
Corticosteroid regimens				
Bashoura *et al*., 2008 [58]	USA, 17	constrictive bronchiolitis in hematopoietic stem cell transplantation (HSCT)	aerosolized fluticasone propionate	high-dose inhaled corticosteroids may be effective
Naef *et al*., 2007 [59]	Switzerland, 20	lung-transplant recipients	aerosolized fluticasone propionate	itraconazole co-medication substantially increases systemic levels of inhaled fluticasone
Whitford *et al.*, 2002 50]	Australia, 30	lung-transplant recipients	aerosolized fluticasone propionate	ineffective for the prevention of BOS
De Soyza *et al.*, 2001 [60]	UK, 120	lymphocytic bronchiolitis following lung transplantation	aerosolized budesonide	a useful addition to systemic immunosuppressants in controlling airway inflammation posttransplant
Whitford *et al*., 2000 [61]	Australia, 30	lung-transplant recipients	aerosolized fluticasone propionate	lung function was not altered over the 3 months of treatment

transplants significantly improved respiratory function and their survival [63]. Another study showed that azithromycin reduced the mortality rate in liver-transplant recipients [64]. These findings *motivate* some transplant centers to use these therapies *in the early post-transplant* period. But the long-term use of macrolides can cause antimicrobial resistance [65].

Studies have reported that statin, a *cholesterol synthesis* inhibitor in the body, may act as an anti-inflammatory agent through inhibiting 3-hydroxy-3-methylglutaryl-coaenzyme A. The first research on the use of statins in transplantation was done on heart-transplant recipients [66]. The reduction of MHCII expression in the endothelial cells and macrophages following the use of statins has also been reported. Thus, this group of drugs may play a crucial role in the onset of the inflammatory process [67]. Another study showed that statins enhance the proliferation of Tregs and inhibit the expression of pro-inflammatory cytokines [68, 69]. Besides, decreasing cholesterol levels in heart-transplant recipients reduce the organ rejection rate. However, other studies showed that statin is an ineffective therapy in preventing rejection in kidney transplantation, thus the effects of statins in transplantation may prove to be organ specific [70].

Extracorporeal photopheresis

One of the developed procedures to deal with two more important consequences of hematopoietic stem cells

transplantation, including GVHD and graft rejection, is the extracorporeal photopheresis (ECP) method. This method decreases the number of T-cells in lymphoma via three mechanisms containing, leukopheresis, incubation of mononuclear cells with 8MOP, and photo-activation of incubated cells with UVA radiation [71].

These cells are then returned to the body and undergo apoptosis [72], increase the T-regulatory levels and anti-inflammatory factors [73]. In a study conducted on lung-transplant recipients receiving either immunosuppressive or ECP treatments, the ECP group was significantly associated with a reduction in the rate of decline in lung function [74]. Although this approach seems to help in improving graft outcome, important questions regarding the use of ECP in the clinical setting, such as length of therapy, concomitant use of immunosuppressive therapy, and cost effectiveness continue to remain unanswered [75]. However, each 6-month ECP therapy requires 24 treatments performed over 4 hours costing $7,000 with no insurance coverage [74].

Exosomes and phagosomes

Formerly, it was reported that presentation of donor MHC antigen before transplantation, could induce immune tolerance in the transplant recipient [76]. Exosomes are antigen-presenting vesicles 50-100 nm in diameter, which can be easily isolated by ultra-centrifugation. Exosomes are generated by a variety of cells, including enterocytes, mast cells, DCs, T and B lymphocytes and tumor cells [77, 78].

Though DCs and tumor cell-derived exosomes such as leukemia cell-derived exosomes (LEXs) have been used to develop antitumor vaccines, their biological properties and antitumor effects are not well described [79]. Thymocyte-derived exosomes reported to have the ability to induce T-regulatory and immune suppression [80, 81]. In a study on the animal model of heart transplantation, exosomes induced a significant prolongation of allograft survival, and long-term graft survival in some recipients [76]. Other researchers showed that mature DCs-derived exosomes can activate the response of T cells and may cause skin graft rejection. Hence immature DC-derived exosomes significantly prolong allograft survival in the heart-transplant recipients [82, 83].

Recently, alloantigen using phagosomes, a vesicle formed around a particle absorbed by phagocytosis, has been developed. The PLGA (polylactic-co-glycolic acid)-containing phagosomes, a nanoparticle sequestering into the phagosome, showed a biochemical composition similar to the original phagocytic plasma membrane. When these phagosomes are exposed to immature DCs of another strain, DCs express low levels of MHC class II and CD86 maturation markers, secrete low levels of the activating cytokines IL-2 and IL-12, and increase IL-10 secretion [84]. In a study, it was reported that phagosome-based alloimmunization reduces cellular immune response and antibody levels significantly [85]. Such findings may encourage the researchers to use PLGA-phagocytosis as a convenient tool in the tolerogenic context of alloantigen administration.

Apoptotic cells

The potent anti-inflammatory and immunoregulatory effects of apoptotic cells on the antigen presenting cells have been recently reported [86, 87]. Broad spectrums of factors are likely help to determine the tolerogenic or immunogenic role of DCs next to uptake of apoptotic cells. Early stage apoptotic cells are more likely known to *induce* tolerance than late stage ones [88, 89]. A number of molecules on the surface of apoptotic cells interact with other cellular receptors and released cytokines [90]. Besides, DCs maturation status can play a role in the induction of tolerogenicity or immunogenicity.

Several studies have shown that an increase in the surface marker of the immature DCs, including MHCII, CD40, CD80, CD83, CD86, even if these cells are exposed to LPS and TNF-α, does not happen in case of the exposure of these cells to apoptotic cells [91, 92]. Also, if early stage DCs are exposed to apoptotic cells, the expression and secretion of inflammatory cytokines, including IL-1α, IL-1β, IL-6 and tumor necrosis factor α (TNF-α), decreases, while the expression of anti-inflammatory factors such as TGF and IL-10 increases [93, 94]. *Apoptotic cells* that carry *donor MHC* molecules has been *used* in many animal models to prevent adverse reaction following *transplantation*, showed favorable results [95-97].

Apoptotic cells usage has many advantages, include transferring a strong signal of immunosuppression to DCs [91], are a rich source of MHC molecules [92], are easy to prepare and with relatively safe intravenous injections [98]. Following the intravascular injection, apoptotic cells are efficiently captured by splenic DCs [99], which present the apoptotic cell derived antigens to T cells [100].

Post-cardiac transplantation intravenous injection of apoptotic cells in a mouse model led to reduction in B and T cell responses against the donor antigens, blocking of CD40-CD154 and prolonged allograft survival [101].

Immunoisolation

Immunoisolation is a new proposed method that aims to isolate and hide nonself (the graft cell) antigens from the *host's* immune system. In 1980, Lim and Sun showed that transplantation of the encapsulated-islets can recover in diabetic eugelycemia in rats [102]. Immunosuppression-free transplantation needs a protective cover that does not interfere with the cells viability and function, and keeps them out of reach of the immune system to avoid transplant rejection. Biomaterials used to encapsulate the transplant cells should be biocompatible and allow for penetrating

nutrients, hormones and oxygen into the cells. The most commonly applied biomaterials are alginate [102], chitosan [103], agarose [104] and polyethylene glycol [105]. However, these biomaterials allow T cells, macrophages and cytokines such as IL-1β, TNF-α and IFN-γ to easily penetrate into the capsules and damage and destroy the encapsulated islets. Thus, a polyamino acid layer, which is coated by alginate, can be used for this purpose.

The positive and negative charges of poly-amino acid and alginate react to form a complex.

Two major amino acid polymers are PLL (Poly L-Lysine) and PLO (Poly L-Ornithine), which PLL is the most *popular* one. Poly L-Ornithine has some advantages compared to the PLL, including better performance in preventing the infiltration of immune cells into the capsule. Hence, PLO has more resistance against mechanical stresses, such as changes in osmotic pressure.

Immuno-blocking is a cutting-edge technology, in which the inner surface of blood vessels is coated with a *nano*-barrier membrane (*NB-LVF4*) to hide the endothelial antigens from the immunological system. NB-LVF4 is injected through the arterial line of the graft immediately prior to transplantation to cover its inner surface. This nano-barrier membrane allows the passage of nutrients and oxygen [106, 107].

In a study on a canine model of renal allograft using a bioengineered interface consisting of a NB-LVF4 was associated with a reduction in the stimulation index up to 99.98%, and prolonged allograft survival has been achieved in the absence of systemic immunosuppression [106]. In another study using NB-LVF4 as a targeted drug delivery system, allograft rejection occurred in controls by 7 days, while the group treated with NB-LVF4 showed mean onset of rejection on day 30. Thus, they claimed that treatment with the NB-LVF4 membrane delays the onset of allograft rejection in the absence of systemic immunosuppression [108]. However, one of the major limitations of this approach is the need to use tolerogenic regimen besides the use of NB-LVF4; besides, this method does not have immunosuppression properties and only provides a 30-day window to use the immunosuppression regimen.

Summary

Immunosuppressive therapy has contributed significantly to improved survival after solid organ transplantation. Nevertheless, treatment-related adverse events and persistently a high risk of chronic graft rejection remain as major obstacles to long-term survival after transplantation. Improvements in procedures to monitor immunosuppression, the development of new agents, and better understanding of transplant immunobiology are essential for further improvements in outcome.

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