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REVIEW

Tolerogenic dendritic cell therapy in organ transplantation

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SUMMARY

Although the occurrence of acute rejection was significantly reduced and the allograft survival at 1 year was massively improved by the development of pharmacological immunosuppressive drugs, little progress has been made regarding long-term graft survival. Cell therapy appears to be an innovative and promising strategy to minimize the use of immunosuppression in transplantation and consequently increases long-term graft survival. The strength of cell therapy is that it will induce graft-specific tolerance and not a general immunosuppression of the patients. Several candidates, such as tolerogenic dendritic cells, have been gaining interest as an efficient means of promoting antigen-specific tolerance over recent years. Studies performed in rodent models have demonstrated the feasibility and efficacy of tolerogenic dendritic cells for the induction of tolerance in transplantation. In parallel, protocols to generate human tolerogenic dendritic cells *in vitro* have been defined, and some phase I clinical trials in autoimmune diseases have been recently performed to evaluate the safety of tolerogenic dendritic cell therapy. In this review, we will focus on the potential therapeutic interest of these cells in transplantation as well as their generation and characterization in humans. Finally, we will describe our current clinical trial using autologous tolerogenic dendritic cells in transplantation.

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Acute and chronic graft rejection

Today, transplantation appears to be the most efficient treatment to replace the loss of kidney function in patients suffering from end-stage renal disease [1]. However, transplants can be rejected by the recipients. Two major immunological mechanisms occur during allograft rejection: the innate nonspecific reaction, with proinflammatory signals that play an important role, and the donor-specific adaptive response. In the context of allogeneic transplantation, donor antigen-presenting cells (APCs) migrate to the secondary lymphoid organs and induce alloreactive naive T cells to differentiate into effector T cells that in return migrate into the graft. In the meantime, the inflammation boosts and maintains the adaptive immune T-cell response.

The adaptive response results from the presentation of alloantigens by APCs, mainly DCs, and their allorecognition by recipient T cells. Three different pathways of allorecognition exist (direct, indirect, and semidirect) [2,3]. Following transplantation, donor DCs are induced to mature by proinflammatory signals such

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as IL-1β, TNF-α and CD40 and induced to migrate out of the graft to the secondary lymphoid organs, where they prime the host T cells via the direct pathway. In the indirect pathway, donor DCs disappear rapidly from the graft and migrate to the draining lymph nodes (LNs), where they die. Dying DCs may be a source of alloantigens for recipient APCs present in the draining secondary lymphoid organs that stimulate T cells. This pathway was recently highlighted by Celli and collaborators in a murine model of skin transplantation [4]. The third pathway is the semidirect allorecognition. In this pathway, recipient APCs acquire intact allogeneic MHC–peptide complex from donor APCs by direct cell-to-cell contact or via release and uptake of small vesicles called exosomes, allowing $CD8^+$ T-cell stimulation. The same recipient APCs are also able to stimulate $CD4^+$ T cells by presenting peptides of allogeneic MHC by self-MHC class II [5–8]. The direct pathway is considered the main mechanism that leads to early acute graft rejection, whereas the indirect pathway is more commonly implicated in chronic rejection. Because of their central position linking innate and acquired immune responses and controlling immunity and tolerance, DCs appear to be a key component of the modulation of graft rejection.

Immunosuppressive drugs and cell therapy

Up until now, the success of transplantation is because of the emergence of immunosuppression, allowing the control of the recipient immune response. With the generation of new immunosuppressive drug combinations, many clinical units now achieve 1-year graft survival rates of 90% [9]. Moreover, the percentage of 1-year kidney graft (from living donors) survival reaches 94.3% for patients transplanted during 2000–2001 in the USA [10] with lifelong standard immunosuppression using one calcineurin inhibitor (CNI; cyclosporine A or tacrolimus) and mycophenolate mofetil (MMF) with or without initial transient induction with antithymoglobulin (ATG) or anti-IL2R mAb (basiliximab). Although significant improvement has been made in graft survival at 1 year, little progress has been obtained in long-term graft survival. In fact, long-term use of immunosuppressive (IS) drugs increases the risk of developing infections and cancer [11,12] and is also directly associated with serious nonimmune toxicities, such as nephrotoxicity [13], dyslipidemia [14], and cardiovascular diseases [15]. At 10 years post-transplantation, all graft biopsies exhibit CNI-induced nephrotoxicity, and approximately 60% of grafts present signs of chronic allograft nephropathy, the predominant cause of late graft loss after renal transplantation [16,17].

Therefore, reduction of the dependence on immunosuppressive drug therapy and the induction of donor-specific tolerance are the major objectives for transplantation research. Cell therapy appears to be an innovative strategy to induce the long-term acceptance of transplants [18]. For this reason, cell therapy using regulatory immune cells has been recently considered in transplantation. Many significant studies have focused on understanding the prospective value of targeting specific immune regulatory cells from either the myeloid or lymphoid lineages. For instance, the contribution of various types of regulatory T cells (Tregs) to transplantation tolerance has been demonstrated, and several clinical trials are currently evaluating the safety of $CD4^+CD25^+Foxp3^+$ Tregs in kidney and liver transplantation [19]. Furthermore, several groups recently suggested that B cells might also be involved in tolerance. Indeed, the analysis of peripheral blood mononuclear cells has shown a B-cell signature from recipients that spontaneously accept their kidney transplants in the absence of immunosuppression (operational tolerance) [20–22]. Consequently, recent studies also highlighted the potential of regulatory B-cell therapy in transplantation in rodents [23–25]. Regarding myeloid cells, the safe administration of human regulatory macrophages in kidney transplantation was reported a few years ago by the team of Geissler [26]. On the other hand, the efficacy of myeloid-derived suppressor cell (MDSC) therapy in transplantation was also demonstrated in different models of transplantation in mice [27–30]. In this review, we will focus on the protective potential of tolerogenic dendritic cells (TolDCs) in organ transplantation.

Dendritic cells and immune tolerance

Dendritic cells are a heterogeneous population classified, in humans, into different subsets, including conventional myeloid DCs (mDCs), plasmacytoid DCs (pDCs), and Langerhans cells, based on their ontogeny, phenotypes, and functionality [31]. Many studies have shown that DCs are dual APCs capable of linking innate to adaptive immunity or inducing tolerance of specific antigens. Myeloid DCs and pDCs are activated following pathogen ligation to Toll-like receptor (TLR) or antigen (Ag) uptake. pDCs are the professional type 1 interferon (IFN)-producing cells during viral infection. Furthermore, these cells express TLR7 and TLR9, which

recognize viral nucleic acids, leading to their production of a large amount of IFN [32]. On the other hand, mDCs can be resident or migratory. Lymphoid organs, such as the spleen, LNs, and thymus, contain resident mDCs that capture and present Ag to T cells. In peripheral tissues, mDCs are migratory cells. These APCs are specialized in Ag capture, processing, and presentation. Upon activation, they migrate to the LNs, where they prime T cells. It has been suggested that migratory DCs bring Ag to the LNs, where they could transfer the Ag to resident $CD8^+$ DCs. These resident DCs would then efficiently present this Ag to T cells to induce T-cell priming [33]. In general, immune responses are mediated by DCs in a mature state under inflammatory conditions, whereas immune tolerance is induced by TolDCs with an immature phenotype.

Tolerogenic dendritic cells are essential for the maintenance of both central and peripheral tolerance. In mice, depletion of all subsets of DCs results in fatal autoimmunity [34]. Central tolerance is achieved through the negative selection of self-foreign Ag-reactive thymocytes and the induction of natural T regulatory cells (nTregs). nTregs are thymic-derived $CD4^+CD25^+$ $Foxp3^+$ T cells that constitute 1–4% of peripheral T cells in young patients and approximately 1% in healthy elderly patients [35]. TolDCs play a significant role in the maintenance of peripheral tolerance against self-Ag in the steady state. Moreover, immune peripheral tolerance is defined by the ability to induce the reduction or complete inhibition of immunogenic responses against Ag in order to prevent over-reactivity of immunity to microbes, allergens, etc.

Tolerogenic dendritic cells are thought to exert their actions using different mechanisms. TolDCs can induce T-cell anergy and clonal deletion. T-cell anergy occurs because TolDCs lack co-stimulation molecules. In the presence of Ag but without CD80 and CD86, T cells become anergic and lose their ability to proliferate [36]. TolDCs can also induce naïve and memory T-cell apoptosis via the Fas/FasL pathway [37] and the expression of indoleamine 2,3-dioxygenase (IDO) by TolDCs [38]. In addition, TolDCs have the capacity to induce tolerance in the peripheral tissue via the expansion or induction of several subtypes of regulatory lymphocytes, which are mainly classical induced $CD4^+CD25^{hi}Foxp3^+$ Tregs [39] and $LAG\text{-}3^+CD49b^+CD25^+Foxp3^{+/-}$ regulatory T-cell type 1 (Tr-1) [40] but are also $CD8^+$ Tregs [41], regulatory B cells [42], and double-negative T cells (DNT cells; $TCR\alpha\beta^+$, $CD3^+$, $CD4^-$ $CD8^-$ $NKRP1^-$) [43]. This last type of Tregs has been shown to produce IFN-γ, which was essential for tolerance induction in a rat cardiac allotransplantation model using syngeneic TolDCs. Anti-IFN-γ treatment of recipient mice caused the abrogation of tolerance induction [43]. Tregs have been shown to be involved in the tolerance process and to prevent allogeneic skin graft rejection in mice [44]. In a pilot study analyzing human renal allograft infiltrates, Bestard *et al.* [45] have shown that more Tregs were present in donor-specific hyporesponder patients both in the peripheral blood and in renal infiltrates compared to normoresponders, suggesting that Tregs play an important role in kidney graft acceptance. While TolDCs drive the differentiation of Tregs, Tregs in return also modulate the phenotype and function of DCs. [46,47]. This tolerogenic feedback between TolDCs and Tregs is illustrated by the fact that (i) no or weak CD80/CD86 co-stimulation is required to induce $Foxp3^+$ iTregs, whereas PD-L1 expression on the TolDC surface promotes the expansion of Tregs and (ii) IL-10 and TGF-β secreted by Tregs inhibit DC maturation and facilitate the maintenance of DCs in a tolerogenic state.

Finally, TolDCs promote central and peripheral tolerance via the expression of immunomodulatory molecules [e.g., PD-L1, PD-L2, heme-oxygenase-1 (HO-1), human leukocyte antigen-G (HLA-G), TNF-related apoptosis-inducing ligand, galectin-1, and DC-SIGN] and the production of IS factors such as IL-10, transforming growth factor-beta (TGF-β), IDO, IL27, and nitric oxide (NO) [35,48–51]. These environmental factors determine the induction of tolerance. Expression of HO-1 was correlated with an immature state of TolDCs [52] and with the inhibition of allogenic T-cell proliferation [53]. In our own model of rat cardiac allotransplantation, the prolongation of allograft survival because of TolDC treatment was abrogated by the administration of a specific HO-1 inhibitor, demonstrating that HO-1 is involved in the prolongation of allograft survival mediated by TolDCs [54]. Moreover, TolDCs express Epstein–Barr virus-induced gene 3 (EBI3). This member of the IL-12 family also has a crucial role. In fact, in our *in vivo* model of tolerance induced by TolDCs, anti-EBI3 treatment of the recipient rats leads to the rejection of the heart transplant [43].

Ex vivo TolDC generation

Tolerogenic dendritic cells are classically generated *ex vivo* from blood monocytes ($CD14^+$) in humans, from bone marrow precursors in rodents, or from $CD34^+$ cells in nonhuman primates. Precursors are isolated by microbeads, cell sorter, or elutriation. TolDCs

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are generated *ex vivo* by culturing precursors with culture medium containing granulocyte–macrophage colony-stimulating factor (GM-CSF) (mainly at a low dose) and eventually with IL-4, IL-10 (DC10), or TGF-β or drugs such as vitamin-D3, rapamycin, tacrolimus, or dexamethasone [55–58]. The active form of vitamin D (vitD3, $1,25(OH)_2D3$) appears as a potent immunomodulatory agent. Indeed, tolerogenic DCs generated in the presence of vitD3 display the features of tolerogenic DCs described below [55,59]. About their effects on T cells, VitD3-treated DCs prevent T-cell priming and induce the apoptosis of effector T cells [59]. Interestingly, VitD3-treated DCs also promote antigen-specific regulatory T cells, leading to infectious tolerance through the re-education of proinflammatory mature DCs into DCs with regulatory properties by Tregs [60]. Moreover, other protocols, such as DC treatment with CD40, CD80, and CD86 antisense oligonucleotides [61] or with an NF-κB inhibitor [62], have also been reported. At the end of the culture period, DCs should display the following features to be recognized as tolerogenic cells: (i) the lack or low expression of cell surface markers of MHC-II, costimulatory molecules (CD80 and CD86), or activation markers (CD40), (ii) resistance to maturation stimuli (tested by pathogen and/or inflammatory signals, that is, lipopolysaccharides, CD40 ligand, DC maturation cocktail, TNF-α), (iii) the low potential of induction of allogenic T-cell proliferation in a mixed lymphocyte reaction (MLR), (iv) the ability to produce IL-10 in response to stimulation, and (v) eventually, the ability to favor Treg proliferation [63]. In our laboratory, we generated mouse and human TolDCs with only a low dose of GM-CSF, as previously described by Lutz *et al.* [64]. For human injection, human TolDCs have to be produced using Good Manufacturing Practice (GMP) in a clinical grade facility. The feasibility of generating *ex vivo* DCs with tolerogenic properties has now been proven. However, two major concerns exist about the use of TolDCs in transplantation: the inflammatory mediators and the immunosuppressive drugs. Indeed, organ transplantation induces inflammation in the graft microenvironment that could gradually lead to TolDC maturation [65]. To avoid this possibility, one strategy consists of the injection of stable semi-mature TolDCs that exhibit a semi-mature phenotype, remain stable *in vivo* following exposure to an inflammatory environment (compared to classical immature DCs), and are able to prolong organ graft survival [66,67]. On the other hand, IS treatment could affect the ability of TolDCs to induce tolerance. In fact, several studies performed in mice and humans showed that IS-modified DCs display higher tolerogenic features and are highly potent at prolonging graft survival (Table 1) [56,57,68–93]. Rapamycin-conditioned DCs are particularly studied because of their ability to favor the expansion of alloantigen $Foxp3^+$ Tregs and increase DC migration [57,94,95]. Conversely, DC migration is impaired in cyclosporin-conditioned DCs [81]. Moreover, the administration of IS drugs to rodents, such as cyclosporin [85,96,97], MMF [98], tacrolimus [82,99], or rapamycin [57], in combination with TolDCs does not impair TolDC efficiency.

Animal models with TolDCs in transplantation

The first experimental report suggesting that TolDCs might be used in the field of transplantation was published by Fu *et al.* [100]. They observed that the cardiac graft survival time was increased in mice that received donor DC progenitors. *In vitro*, these DCs induced alloantigen-specific T-cell anergy. Since these studies, DCs with tolerogenic properties have been explored extensively in small animal models and a meta-analysis has demonstrated their potency to prolong allograft survival in multiple transplantation models [101]. In rodents, donor-generated TolDCs, alone or with IS drugs, have been shown to prolong cardiac allograft survival [57,64,82,102–107]. However, Peche *et al.* [108] have demonstrated the superiority of recipient-derived DCs (nonpulsed) to prolong cardiac allograft survival in comparison with donor-derived DCs (nonpulsed) (for review, see [35,49,50,63,65,109]). Nonhuman primates (NHPs) provide attractive preclinical models for testing TolDCs in transplantation. Generation of DCs from the bone marrow or from monocytes with tolerogenic properties was previously shown *in vitro* [53,54,110,111], and, in 2013, Ezzelarab *et al.* [112] demonstrated that the infusion of regulatory DCs prolongs kidney allograft survival in a clinically relevant rhesus macaque model. In this study, DCs were isolated from donor blood monocytes, generated with VitD3 and IL-10 and infused intravenously 7 days before renal transplantation. All recipients received CTLA4-Ig (B7-CD28-blocking fusion molecule) to minimize the risk of host sensitization. Recently, the same team aimed to decipher the effect of CTLA4-Ig on the transcription factor Eomes in memory T cells in their model of NHP renal allograft. They showed that prolonged renal allograft survival is associated with $Eomes^{low}$ $CTLA4^{high}$ donor-reactive $CD8^+$ suppressive memory T cells [113].

Table 1. Effects of immunosuppressive (IS) drugs on dendritic cells.

		Tacrolimus	Cyclosporin	Rapamycin	Mycophenolate mofetil	Prednisolone
Ability of DCs to perform antigen processing and presentation	Endocytic capacity			↓ (humans) ↓ (rodents)	↓ (humans)	↑ (humans)
	MHC class I- and class II-restricted presentation of antigen	↓ (humans)				
DC maturation following stimulation	Expression of co-stimulation markers	↓ (humans) ↓ (rodents)	↓ (rodents)	↓ (rodents)	↓ (humans) ↓ (rodents)	↓ (humans)
	Secretion of proinflammatory cytokines	↓ (humans) ↓ (rodents)	↓ (rodents)	↑ (humans) ↓ (rodents)	↓ (humans) ↓ (rodents)	↓ (humans)
T-cell proliferation/ activation induced by DCs	T-cell proliferation	↓ (humans) ↓ (rodents)	↓ (rodents)	↓ (humans) ↓ (rodents)	↓ (humans) ↓ (rodents)	↓ (humans)
	T-cell activation	↓ (humans) ↓ (rodents)	↓ (rodents)			
	Secretion of proinflammatory cytokines by T cells	↓ (humans) ↓ (rodents)	↓ (rodents)	↑ (humans)		
In vivo model of transplantation	Allograft survival			↑ (rodents)	↑ (rodents)	↑ (rodents)
References		[60–72]	[50,61,73]	[51,61,70,74–78]	[79–81]	[82–85]

Clinical trials with dendritic cells

Clinical protocols of immunogenic DCs have been tested in cancer therapy to obtain tumor antigen presentation, allowing clinically effective antitumor immunity (mature DCs vaccine clinical trial) [114–116]. Mature DC antigen-loaded antiviral immunotherapy was also used in a phase I clinical trial [117].

Conversely, in autoimmune diseases or transplantation settings, dendritic cells pushed toward their tolerizing capabilities were tested. Since the beginning of this century, the capacity of human DCs to induce Ag-specific tolerance *in vivo* has been reported in healthy volunteers [118,119]. In two healthy subjects injected with immature DCs pulsed with keyhole limpet hemocyanin (KLH) and influenza matrix peptide (MP), Dhodapkar *et al.* [118] demonstrated the inhibition of MP-specific-$CD8^+$ T-cell function and the appearance of MP-specific IL-10-producing cells. These data made obvious the feasibility of inhibition of T-cell function with cell therapy (negative cellular vaccines) in humans. TolDCs were also tested against autoimmunity (induction of tolerance toward self-antigens). Autologous TolDCs have been administered intradermally in patients with juvenile type I diabetes [120] and intraperitoneally in patients suffering from Crohn's disease [121] without any toxicities reported. In another clinical trial, no adverse effects were observed in patients suffering from rheumatoid arthritis and who received intradermal TolDCs modified with NF-κB inhibitor and pulsed with citrullinated peptides [122]. Moreover, recently, a clinical trial using an intra-articular route of TolDC administration in the context of rheumatoid and inflammatory arthritis therapy was published [123]. Thus far, there have been no reports of clinical trials using TolDCs in transplantation, even if studies performed in rodents ensured the efficient use of TolDCs in this context. The goal today is to transfer these results to humans. To this end, our team is part of a European consortium (the ONE Study). In our center, we are currently evaluating the safety and feasibility (phase I/II clinical trial) of cell therapy using autologous TolDCs, named ATDCs (autologous tolerogenic DCs), in living-donor kidney transplantation (NCT02252055) [124]. Thomson *et al.* [125] also recently proposed to perform a phase I/II safety study in which the effect of donor-derived DCregs combined with conventional immunosuppression on kidney rejection will be studied.

Following cell therapy, the issue of weaning immunosuppression concerns only patients with no clinical alloimmune response, such as acute or chronic rejection, no DSA, no active infection, no proteinuria, and conserved graft histology. Also, there is no absolute biological assessment of true tolerance; the identification of

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concerned patients would be assessed by a monitoring of the immune response including leukocyte profiling as described by Streitz *et al.* [126], DSA monitoring, and IFNγ ELISPOT analysis in patients receiving TolDC therapy. Further trials will be necessary to precise the protocol of ATDC administration and the protocol of immunosuppression. Subsequently, patients requesting reduced immunosuppression, such as those with past history of cancer, could be also considered.

Origin, dose, and route of administration of human TolDCs as cell-based medical products in kidney transplant

Prior to TolDC injection in humans, the parameters of the GMP preparation of the cells have to be considered. For example, the optimal cell culture conditions must be reached with adequate cytokines and medium, clinical grade reagents, and closed culture systems, as well as standardization and quality controls. Furthermore, GMP preparation should be performed with the simplest protocol to avoid various factors of contamination.

In our center, we derived human ATDCs from monocytes ($CD14^+$) enriched via the leukapheresis of peripheral blood by elutriation. In humans, blood monocytes are the most common source of DCs, and elutriation is a purification technique adapted for GMP facilities. Indeed, elutriation allows the separation of untouched cells based on their size and their density, avoiding the risk of extra contaminant components (i.e., beads). In contrast to most human DC differentiation protocols, where cells are derived in the presence of different cytokines or IS drugs [35,63], ATDCs have been obtained following culture with GM-CSF only. This simple protocol is in accordance with previous rodent and human protocols, where the efficacy of generated TolDCs was demonstrated [64,127–129]. Accordingly, ATDCs display an immature phenotype, preserved upon TLR triggering. This resistance to maturation stimuli suggests that ATDCs will not become immunogenic once they are injected into patients. In transplantation, the issue of whether DCs should be derived from the donor or from the recipient must be taken into consideration. In transplant animal models, most studies explored the effects of donor TolDCs injected at least 1 week before transplantation [50]. In humans, we have chosen to administer unpulsed autologous cells in accordance with our results in rodents [43,108,129–131]. Furthermore, the absence of alloantigen appears to be a safer approach in a context of a clinical trial. The major benefits of using recipient-derived DCs compared with donor-derived DCs are (i) the lower risk of donor sensitization because of the presence of contaminant cell products and (ii) the lack of destruction of the injected cells by non-self-recognition [132]. In the ATDC manufacturing process, contaminant cells, mainly B cells, T cells, and basophils, are estimated to be lower than 3% [63]. Furthermore, autologous TolDCs do not require activation or pulsing to be efficient at migrating to the LN and presenting antigen to T cells. Based on our results in rodents [129], we postulate that injected autologous unpulsed human ATDCs are able to migrate to the graft, where they capture and process donor-derived Ags, leading to Ag-specific regulation. In contrast, it has been demonstrated that donor-derived TolDCs quickly die after *in vivo* injection, suggesting that these cells are unable to directly regulate the immune response [104]. Furthermore, autologous TolDCs are suitable from either living or deceased donors and could be prepared as soon as the patient is waiting for a transplant and preserved frozen. The manufacturing process for ATDC generation fits perfectly with the ONE Study clinical trial, as we validated that ATDCs derived from monocytes of healthy volunteers and patients with renal dysfunction share similar phenotypes and *in vitro* functions.

Regarding the route of delivery, different methods have been used in humans. TolDCs have been administered intraperitoneally in patients suffering from Crohn's disease [121], via arthroscopic injection for patients suffering from rheumatoid and inflammatory arthritis [123] and intradermally in type I diabetes patients [120]. All of these routes of administration were well tolerated without any signs of toxicity. Experiments performed in mice have shown that compared with subcutaneous injection, the optimal route of administration of dexamethasone/lipopolysaccharide-treated BMDCs in the context of cardiac transplantation is intravenous delivery [103]. In nonhuman primates, recent studies reported the safety of TolDCs after their intravenous administration [110,133]. Finally, another issue is to define the time of cell administration. Based on our reports in rodents [43,108,129–131], autologous TolDCs must be injected at the time of the graft to take up donor Ag in the graft. Taking all of these concerns together, patients from the ONE Study ATDC trial received a single intravenous injection of TolDCs (one million/kg body weight) the day before kidney transplantation. These patients also receive minimized IS drugs (tacrolimus, MMF, and prednisolone). At the

time of the phase I/II ONE Study ATDC trial, no toxicity associated with the cell infusion was reported.

Conclusion

The first clinical trials using autologous TolDCs in patients suffering from arthritis, diabetes, or Crohn's disease suggest that the use of these cells appears to be a safe therapy in autoimmune diseases. Furthermore, increased populations of regulatory cells have also been highlighted in the blood of rheumatoid arthritis and type 1 diabetes patients. Our experiments performed in rodents demonstrated that autologous TolDC therapy could efficiently protect from graft rejection, alone or in combination with IS drugs. In the ONE Study ATDC trial, we are testing the safety of ATDC therapy in transplantation in combination with tacrolimus, prednisolone, and MMF. Once the safety status of ATDC therapy is confirmed, further clinical trials will focus on the optimal conditions required to potentiate the protective ability of ATDCs. It will thus be possible to consider some modifications in the pharmacological IS regimen or in the cell injection time (for instance, to wait until a decrease in the immunosuppressive treatment).

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Conflict of interest

The authors declare no conflict of interest.

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