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Concise Review: Mechanisms behind apoptotic cell-based therapies against transplant rejection and graft versus host disease

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ABSTRACT

The main limitations to the success of transplantation are the antigraft response developed by the recipient immune system, and the adverse side effects of chronic immunosuppression. Graft-versus-host disease (GVHD) triggered by donor-derived T lymphocytes against the recipient tissues is another serious obstacle in the field of hematopoietic stem cell transplantation. Several laboratories have tested the possibility of promoting antigen (Ag)-specific tolerance for therapy of graft rejection, GVHD, and autoimmune disorders, by developing methodologies that mimic the mechanisms by which the immune system maintains peripheral tolerance in the steady state. It has been long recognized that the silent clearance of cells undergoing apoptosis exerts potent immune-regulatory effects and provides apoptotic cell-derived Ags to those Ag-presenting cells (APCs) that internalize them, in particular macrophages and dendritic cells. Therefore, in situ-targeting of recipient APCs by systemic administration of leukocytes in early apoptosis and bearing donor Ags represents a relatively simple approach to control the antidonor response against allografts. Here, we review the mechanisms by which apoptotic cells are silently cleared by phagocytes, and how such phenomenon leads to down-regulation of the innate and adaptive immunity. We discuss the evolution of apoptotic cell-based therapies from murine models of organ/tissue transplantation and GVHD, to clinical trials. We make emphasis on potential limitations and areas of concern of apoptotic cell-based therapies, and on how other immune-suppressive therapies used in the clinics or tested experimentally likely also function through the silent clearance of apoptotic cells by the immune system. STEM CELLS *2016; 00:000—000*

SIGNIFICANCE STATEMENT

This review analyzes the mechanisms by which the silent internalization of dying cells by leukocytes of the immune system, contributes to prevent immune responses against self-tissues. Importantly, it describes how such mechanisms of apoptotic cell-clearance are currently being implemented for development of novel therapies for treatment of transplant rejection and graft-versus-host disease.

INTRODUCTION

In steady-state conditions, programmed cell death by apoptosis and apoptotic cell clearance by motile professional phagocytes and neighboring tissue cells are critical during embryogenesis, tissue remodeling and homeostasis, and deletion of autoreactive lymphocytes [1–3]. Recognition and phagocytosis of cells undergoing apoptosis, termed efferocytosis, exert immune-regulatory effects on leukocytes, in particular antigen (Ag)-presenting cells (APCs). Numerous laboratories have attempted to harness the immune-suppressive potential of cells undergoing early apoptosis for therapeutic purposes in transplant rejection, autoimmune diseases, and chronic inflammatory disorders. This review describes the mechanisms by which apoptotic cell clearance regulates the immune response, and how these principles are being applied for the development of apoptotic cell-based therapies for treatment of transplant rejection and graft-versus-host disease (GVHD). It also explains how other therapies already in use may also function through the silent removal of apoptotic cells.

Apoptotic Cells as Regulators of the Immune Response

It was initially considered that the rapid removal of apoptotic cells by phagocytes before the escape of intracellular toxic

mediators due to apoptotic cell membrane leakage, was the main reason cells undergoing apoptosis do not elicit inflammatory or immune responses. In 1997, Voll and colleagues demonstrated that phagocytosis of apoptotic cells by monocytes stimulates secretion of interleukin (IL)-10 and decrease release of proinflammatory cytokines including Tumor Necrosis Factor (TNF)-α, IL-1β, and IL-12 [4]. Since then, numerous studies have shown that apoptotic cells control the function not only of macrophages [5], but also dendritic cells (DCs), the latter the most potent APCs. Interaction of apoptotic cells with immature DCs restricts up-regulation of DC-activation markers (i.e., MHC molecules, CD40, CD80, CD86, and CD83), secretion of proinflammatory mediators, and its T cell stimulatory ability, all effects resistant to challenge with DC-activating mediators [6–15] (Fig. 1). Other effects associated with apoptotic cell uptake include enhanced capability of the target DCs of inducing $CD4^+$ $FoxP3^+$ regulatory T cells (Treg), increased secretion of TGF-β and IL-10, and IFN-γ-induced release of indoleamine 2,3-dioxygenase (IDO), the latter an enzyme that catabolizes tryptophan generating metabolites that inhibit T cell function [11, 13, 16–19]. Macrophages that recognize apoptotic cells down-regulate innate and acquired immunity by releasing of IL-10, TGF-β, and PGE_2 [4, 5]. Following interaction with apoptotic cells, macrophages and DCs secrete an inactive (latent) form of TGF-β. Interestingly, the $\alpha_v\beta_6$ and $\alpha_v\beta_8$ integrins expressed by DCs activate locally latent TGF-β released in areas of phagocytosis of apoptotic cells [20, 21]. Efferocytosis-induced TGF-β secretion, acting in an autocrine/paracrine fashion, down-regulates synthesis of proinflammatory eicosanoids and nitric oxide in activated macrophages, the latter by down-regulating expression of inducible nitric oxide synthase [22]. Molecules involved in binding and internalization of apoptotic cells also down-regulate inducible nitric oxide synthase in microglial cells, which is critical during silent removal of apoptotic neurons during development and aging [23, 24]. By contrast, others have shown that following efferocytosis, macrophages and activated DCs increase production of nitric oxide, which suppresses inflammation and T cell immunity [25, 26].

DCs that internalize apoptotic cells restrain the function of CD8 T cells by cross-presenting apoptotic cell-derived peptides loaded MHC-I molecules [27]. The inhibitory effects are restricted to those DCs that phagocytose the apoptotic cells, whereas the bystander DCs are unaffected [9]. Importantly, DCs that internalize apoptotic cells down-regulate the chemokine receptor CCR5, and upregulate or maintain CCR7 expression, resulting in DC migration from periphery to secondary lymphoid organs, where DCs down-regulate T cell immunity and maintain T cell peripheral tolerance [10, 28]. Unlike conventional DCs and in vitro-generated myeloid DCs, plasmacytoid DCs (pDCs) are not well equipped to internalize apoptotic cells [14, 29]. Although pDCs do not seem to interact directly with apoptotic cells, soluble factors released by macrophages previously incubated with apoptotic cells promote immune-regulatory functions on pDCs [30].

Increasing evidence indicates that apoptotic cells induce a regulatory B-cell (Breg) phenotype. Mouse splenic marginal zone ($CD1d^{hi}$) B cells, and to a less extent follicular B cells, increase IL-10 secretion when exposed to apoptotic cells [31, 32]. This result has been confirmed in human B cells [32]. The increase in IL-10 production by B cells requires the interaction of CpG DNA motifs -contained in chromatin complexes translocated to the apoptotic cell surface, with Toll-like receptor (TLR)-9 expressed in the phagosome membrane of B cells. In a mouse transgenic T cell receptor (TCR) system against OVA, the IL-10 released by B cells exposed to apoptotic cells promoted differentiation of IL-10-secreting CD4 T cells [31]. Thus, the B cell—apoptotic cell interaction leads to differentiation of Breg-like cells that, as APCs, stimulate differentiation of IL-10-secreting CD4 T cells.

Interaction with apoptotic cells not always suppresses immunity. Phagocytosis of stressed, activated, tumor, or infected cells undergoing apoptosis promotes DC maturation and immunity, [6, 7, 17, 33–38] and proinflammatory apoptotic cells have been tested to boost immunity for vaccination against cancer and pathogens [39–41]. Besides the intrinsic properties of the apoptotic cells, the rate of apoptotic cell clearance, the microenvironment where it occurs, and the opsonins deposited on the apoptotic cells are factors that influence the impact of apoptotic cells on the target APCs and therefore, the consequent ability of the APCs to stimulate or suppress immunity [42–44].

Apoptotic Cell Clearance and the Immune Response

Cells undergoing apoptosis release "find me" signals that act as chemotactic factors for phagocytes [45–49]. Once the phagocytes reach the apoptotic cells, the latter are recognized through its Apoptotic-Cell-Associated Molecular Patterns (ACAMPs) that function as "eat me" signals for Pattern-Recognition Receptors (PRRs) expressed by the phagocytes (Fig. 1). During apoptosis, phosphatidylserine (PtdSer), calreticulin, the annexins A5 and A13, and DNA are some of the intracellular molecules that become exposed on the apoptotic cell surface as ACAMPs. PtdSer, and the annexins A5 and A13 externalized on the apoptotic cell membrane exert potent immune-regulatory effects on target leukocytes [50–53]. Apoptotic cells also suppress the immune response through the release of soluble mediators, including IL-10, TGF-β, and annexin A1 [54–56] (Fig. 1).

On the phagocyte side, T cell immunoglobulin domain and mucin domain protein (TIM) 1, 3 and 4, the membrane receptor kinases Mer and Axl, the $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins, lectins, scavenger receptors (i.e., CD14, CD36, CD68, SR-A, and Lox-1), brain-specific angiogenesis inhibitor 1 (BAI-1), stabilin-2, and receptors for α_2 macroglobulin (CD91) and β_2-glycoprotein I, participate in apoptotic cell binding by phagocytes [49, 57–59]. Soluble proteins including the C1q and iC3b complement fragments, milk fat globule protein-E8 or lactadherin, growth arrest specific gene-6 (Gas-6), protein S, collectins (mannose-binding lectin, surfactant proteins A and D), pentraxins (C-reactive protein, serum amyloid P, long pentaxin 3), thrombospondin-1, and serum β_2-glycoprotein opsonize apoptotic cells, forming molecular bridges between ACAMPs on apoptotic cells and PRR on phagocytic cells [49, 57–59] (Fig. 1). Externalized PtdSer is recognized by the phagocytes directly by TIM-1, 3 and 4, CD300a, BAI-1, or stabilin-2, and indirectly through bridge molecules like lactadherin that binds to α_v integrins, or via soluble Gas-6 and protein S, which bind to the membrane receptor kinases Mer and Axl expressed by phagocytes. Different phagocytic cells, in particular macrophages and DCs, use distinct receptors, and the mechanisms

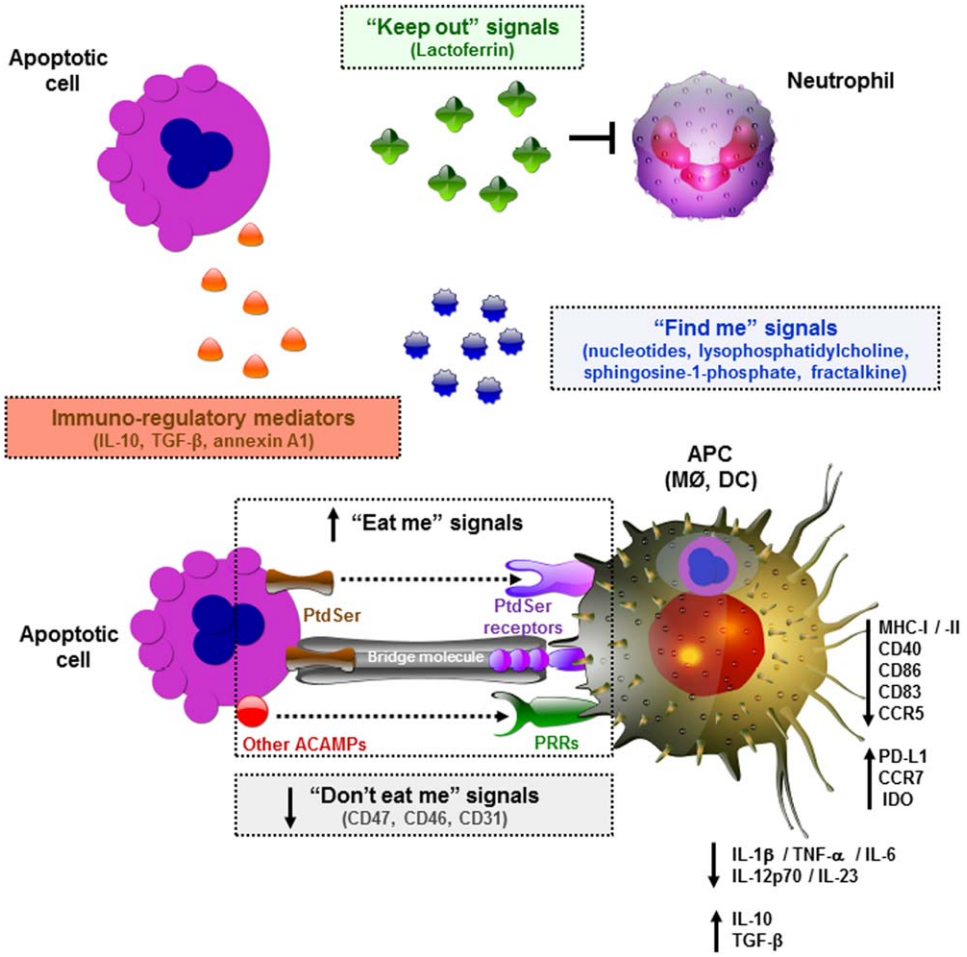

Figure 1. Silent clearance of apoptotic cells. In the steady-state, cells undergoing apoptosis release soluble factors, which include immuno-regulatory mediators, "keep out" signals (that prevent migration of neutrophils), and "find me" signals (that attract professional phagocytes). Apoptotic cells expose PtdSer and other ACAMPs. Externalized PtdSer binds PtdSer receptors directly or through bridge molecules. ACAMPs are recognized directly by PRRs expressed on the phagocyte membrane. Internalization of apoptotic cells exerts immuno-regulatory effects on target APCs, by down-regulating expression of Ag-presenting (MHC class-I and -II) and T cell co-stimulatory molecules (CD80, CD86), augmenting IDO, reducing secretion of proinflammatory cytokines (IL-1β, TNF-α, IL-6, IL-12p70, IL-23), and increasing release of immunosuppressive mediators (IL-10, TGF-β). ACAMPs, Apoptotic-Cell-Associated Molecular Patterns; DC, dendritic cell; MØ, macrophage; PRRs, pattern recognition receptors; PtdSer, phosphatidylserine.

employed for phagocytosis of apoptotic cells differ according to the tissues.

Some of these contacts only "tether" apoptotic cells to phagocytes, whereas others also trigger efferocytosis—"tether and tickle" effect. The multiple signaling pathways by which apoptotic cells down-regulate the proinflammatory and—immune functions of APCs have been reviewed elsewhere [60]. The redundancy in the mechanisms of apoptotic cell recognition makes apoptotic cell clearance a very efficient process. Elegant studies in mice with defects in molecules involved in recognition, internalization, and lysosomal degradation of apoptotic cells, have shown that accumulation of apoptotic cells in peripheral tissues generates danger signals that cause inflammation, autoimmunity, or chronic inflammatory disorders [2, 3, 49, 59–68]. In the clinics, genetic defects in complement factors that opsonize apoptotic cells, and in apoptotic cell clearance by phagocytes have been detected in patients with systemic lupus erythematous [2, 69–75].

The discovery that, under certain conditions, cells undergoing apoptosis down-regulate the proinflammatory/proimmunogenic properties of APCs, plus the fact that apoptotic cells are a natural source of foreign or autologous Ags for APCs, led to the idea of testing apoptotic cell-based therapies for induction of Ag-specific immuno-suppression in T cell-mediated disorders in the fields of transplantation [76, 77] and autoimmunity [31, 32, 78–82].

Apoptotic Cell-Based Therapies in Organ/Tissue Transplantation

In murine transplant models, systemic administration of donor splenocytes undergoing early apoptosis-induced by UV-B- or γ-irradiation, has been proven to promote donor-specific immunosuppression, and to prolong survival of heterotopic (abdomen) heart allografts [76, 77]. Once injected i.v., the blood-borne donor apoptotic leukocytes are rapidly phagocytosed by macrophages and DCs in the spleen, liver and lung [11, 14, 77]. In mice, the subset of CD8α^+ CD103$^+$ DCs of the splenic marginal zone selectively internalize blood-borne apoptotic cells, and then present the apoptotic cell-associated Ags in a protolerogenic fashion to T cells [83] (Fig. 2).

Importantly, systemic injection of apoptotic leukocytes induces TGF-β and IL-10 expression by splenic phagocytes [84, 85]. Internalization of the donor splenocytes in early apoptosis before transplantation restricts activation of the acceptor APCs in vivo [77]. As a consequence, presentation of apoptotic cell-derived donor allo-Ags by recipient *quiescent* APCs - expressing a low ratio of T cell co-stimulatory vs. regulatory signals, promotes deficient activation followed by transient proliferation and deletion of antidonor T cells, increasing the percentage of donor-specific CD4 Treg [14, 77, 86, 87] (Fig. 2). This inhibitory effect of apoptotic cell clearance on CD80 and CD86 expression by recipient APCs could enhance CTLA4-Ig (betalacept) therapy, which blocks CD80 and CD86 already externalized on the APC surface. I.v. infused apoptotic leukocytes also down-regulate the T cell response by promoting T cell anergy, and inducing "CD4 T cell helpless" CD8 T cells that secrete the proapoptotic molecule TRAIL [88].

Systemic injection of donor apoptotic splenocytes before heart transplantation also decreases the titer of donor-specific antibodies (Abs) in serum, likely due deficient T-B cell help caused by the immune-regulatory effect of the apoptotic cell-therapy on donor-specific CD4 T cells [77] (Fig. 2). Alternatively the donor apoptotic cells could regulate directly the function of donor-reactive B cells[31].

The immune-regulatory effect of donor apoptotic cells on the antidonor T cell response is mediated through macrophages and conventional $CD11c^{high}$ $CD8\alpha^{+}$ DCs of the recipient [76, 77, 86, 89]. Indeed, $CD169^{+}$ metalophillic macrophages and $MARCO^{+}$ macrophages of the splenic marginal zone are critical for the immuno-suppressive effect of i.v. injected apoptotic cells [78, 90]. Both subsets of specialized macrophages regulate engulfment by DCs of blood-borne apoptotic cells entering the spleen [78, 90]. Following systemic challenge with apoptotic leukocytes in mice, metalophillic macrophages secrete CCL22, a chemokine that promotes accumulation of $FoxP3^{+}$ Tregs and DCs in the splenic follicles [84] (Fig. 2). I.v. infusion of apoptotic splenocytes up-regulates expression of the immune-regulatory molecule PD-L1/2 by splenic macrophages and DCs in mice [14, 85, 86]. Although apoptotic cells injected i.v. exert multiple regulatory effects on target APCs, splenic DCs present allopeptides derived from i.v. injected donor splenocytes for a limited time-span, which reaches a plateau 3 days after apoptotic cell infusion [14]. This could explain why, in the absence of pharmacological immunosuppression, a single dose of donor apoptotic splenocytes although effective, only prolongs transiently cardiac allograft survival in murine models.

The beneficial effects of donor apoptotic splenocytes on heart allograft survival are donor-specific, take place in different murine strain combinations, and depend on the intrinsic properties of the donor leukocytes in early apoptosis [76, 77]. The therapeutic effect of donor apoptotic splenocytes on heart allograft survival depends to a great extent on the interaction of externalized PtdSer with PRRs expressed by recipient APCs [76]. The beneficial effect also relies on the timing of administration of the donor apoptotic splenocytes, with optimal results when the apoptotic cells are injected i.v. 7 days before transplantation [76, 77]. This 7-day time-window represents a problem for the potential implementation of donor apoptotic cell-based therapies with deceased donors, as occurs in cardiac transplantation. Administration of donor apoptotic splenocytes in combination with a suboptimal dose of anti-CD154 (CD40 ligand) blocking Ab (to inhibit the stimulatory effect of CD40-signaling on recipient APCs), prolonged indefinitely heart allograft survival in mice [77]. The accepted allografts showed minimal parenchymal damage, and were infiltrated by $CD4^{+}$ $FoxP3^{+}$ Tregs containing IL-10 and TGF-β [77]. Thus, apoptotic cell-based therapies could reduce the doses of pharmacological immune-suppressants and costimulation blockade currently used and therefore, ameliorate its harmful side-effects.

Donor splenocytes made apoptotic by γ-radiation, when administered i.v. as a single dose (7 days before transplantation), prolonged survival of pancreatic islet allografts in streptozotocin-treated diabetic mice, with a broad range of allograft survival depending on the study [91, 92]. By contrast, repeated infusions (7 days before and 1 day after transplantation) of donor splenocytes rendered apoptotic by incubation with the cross-linker ethylene carbodiimide (ECDI), induced donor-specific tolerance and long-term survival of islet allografts in a different donor-recipient mouse strain combination [93]. In such studies, the effect of the donor apoptotic leukocytes on islet allograft survival was prevented by depletion of conventional DCs, it was mediated via PD-1/PD-L1-dependent effector T cell down-regulation and Treg expansion, and required the presence of Tregs during tolerance induction [91–93]. In another nonvascularized allograft, male apoptotic thymocytes or female apoptotic leukocytes ECDI-coupled with the male CD4 T cell epitope Dby, injected i.v. 7 days before transplant, prolonged survival of minor-mismatch male skin grafts in female mice [84, 94].

The initial studies on apoptotic cell-based therapies in transplantation models were focused on prevention, delay or regulation of the immune response that leads to acute rejection [76, 77]. Nevertheless, with the existing success rate of current immunosuppressive therapies, chronic rejection represents a major clinical problem that present-day immuno-suppressants fail to avert. One of the features of chronic rejection is the development of chronic allograft vasculopathy (CAV), a pathology that results from immune and non-immune mechanisms, and that consists of intimal thickening, endothelialitis, elastic fiber disruption, adventitial fibrosis, and leukocyte infiltration of arteries in the organ graft. CAV leads to progressive reduction of the lumen of graft vessels, which become prone to thrombosis. The T cell response against donor-derived peptides presented by self (recipient) MHC molecules—also known as indirect allorecognition-, and the generation of donor-specific Abs are considered the immunological factors behind CAV. Interestingly, systemic administration of donor apoptotic splenocytes, 7 days before transplantation, reduced CAV in mouse aortic allografts—a model of CAV [14].

Apoptotic Cell-Based Therapies in Hematopoietic Engraftment and GVHD

As in any other allograft, the level of engraftment of bone marrow (BM) or hematopoietic stem cell (HSC) allografts depends to a great extent on the quantity, allospecificity and function of the remaining recipient T and NK cells that resisted the conditioning regimen, and capable of rejecting the transplanted BM cells. But for those allografts that contain donor-derived passenger T cells like the BM, HSC, liver, or

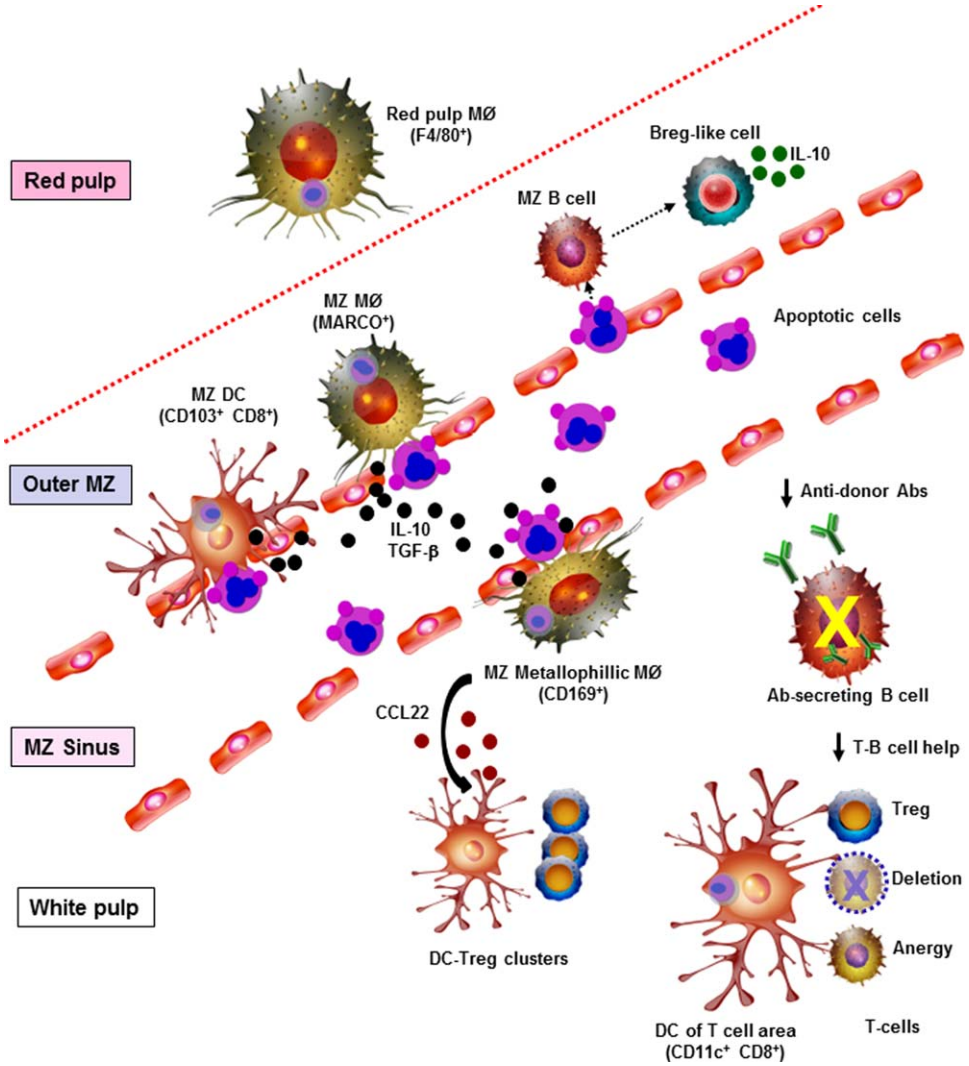

Figure 2. Immuno-suppressive effects of apoptotic cell-based therapies. In mice, once apoptotic leukocytes are injected i.v., they are rapidly ingested by marginal zone (MZ) DCs, MZ macrophages (MØ), MZ metallophillic MØ, and red pulp MØ in the spleen. MZ MØ and MZ metallophillic MØ regulate the phagocytosis of circulating apoptotic cells by MZ DCs. MZ metallophillic MØ that have phagocytosed apoptotic cells secrete CCL22, a chemokine that attracts Treg and DCs [84]. Following contact with apoptotic cells, MZ B cells and to a less extent follicular B cells acquire a Breg-like phenotype and secrete IL-10. The splenic phagocytes that engulf apoptotic cells release IL-10 and TGF-β into the systemic circulation. In the splenic white pulp, those DCs that have interacted with apoptotic cells present apoptotic cell-derived Ag to T cells in a protolerogenic manner, by promoting CD4 Treg expansion, T cell deletion, and T cell anergy. The consequent reduction in the T-B cell help needed for differentiation of Ab-secreting B cells is likely one of the reasons donor apoptotic cell-based therapies decrease the titer of donor-specific Abs in serum.

intestine, the donor T cells adoptively transferred with the grafts can attack the recipient (host) tissues (i.e., skin, gut, liver), eliciting acute or chronic GVHD [95]. Activation of the host innate immune system by the conditioning regimen followed by differentiation of donor-derived effector T cells against the host tissues causes GVHD [96]. Thus, targeting host APCs and down-regulating its immunogenic functions by i.v. injected apoptotic cells may control GVHD [96]. Indeed, donor apoptotic cells have been proven to decrease BM rejection—therefore increasing BM engraftment—, and to ameliorate GVHD in mice.

In a restrictive BM engraftment model, coadministration (i.v.) of a single bolus of donor apoptotic splenocytes together with the BM allograft facilitated BM engraftment and prevented GVHD in different host mouse strains [97, 98]. The beneficial effect of the apoptotic cells did not depend on the stimulus used to trigger cell death, since apoptotic splenocytes generated by γ- or UVB-irradiation, or by Ab-mediated CD95-signaling exerted similar effects [97]. Interestingly, administration donor-, host-, or third-party-derived, and even xenogeneic (human) apoptotic leukocytes promoted BM engraftment without GVHD in mice [97, 98]. This seemingly MHC-independent effect of the injected apoptotic cells can be attributed to the coadministration of the BM allograft and apoptotic leukocytes, a delivery strategy that simultaneously provided the recipient APCs with donor-Ag from the BM inoculum, and immune-regulatory signals from the apoptotic leukocytes regardless its MHC haplotype.

In mice, the beneficial effect of apoptotic cell-based therapy on BM engraftment correlated with increased numbers

FoxP3$^+$ Treg of donor origin, which inhibited proliferation of donor-reactive T cells from the host via a cell contact-mediated mechanism, independent of IL-10 or TGF-β [99]. Expansion of the donor-derived Treg required TGF-β, likely secreted by the recipient phagocytes that internalized the apoptotic cells injected i.v. [99]. Apoptotic splenocyte infusion along with the BM allograft prevented, via a TGF-β-dependent mechanism, development of cytotoxic Abs against the donor BM, an effect that also favored BM engraftment [98]. In the same mouse model, coadministration of donor apoptotic splenocytes with the BM allograft correlated with induction of donor-derived Treg that restrained the anti-host response, which may explain the preventive effect of the apoptotic cell-based therapy on GVHD [99].

Donor-derived pDC precursors seem to constitute a percentage of the "CD8$^+$ TCR^{neg} facilitating cells" that promote HSC engraftment and prevent GVHD [100]. Interestingly, i.v. injection of donor pDCs, in combination with donor apoptotic splenocytes and the BM allograft, enhanced the beneficial effect of the apoptotic cells on BM engraftment in mice [30]. By contrast, depletion of pDCs from BM allografts prevented the increase in BM engraftment induced by co-administration of donor apoptotic splenocytes in the same model [30]. In this scenario, the immune-suppressive effect of the donor pDCs contained in the BM inoculum was induced by soluble factors released by those splenic phagocytes that interacted with the apoptotic cells injected i.v. [30].

Before apoptotic cell-based therapies can be implemented in HSC transplantation in humans, it is key to understand how the current conditioning and maintenance immunosuppressive regimens interfere with the regulatory effects of the systemically administered apoptotic leukocytes. In mice, although cyclosporine A antagonized the beneficial effect of i.v. injected apoptotic splenocytes on BM engraftment, rapamycin worked in a synergistic fashion with the apoptotic cell-based therapy [101]. In a recently completed phase I/IIa clinical trial, 13 patients receiving myeloablative HLA-matched allogeneic HSC transplants were treated 1 day before the transplant with a single i.v. infusion of donor leukocytes in early apoptosis, as prophylaxis for GVHD [102]. In such study, no adverse effects associated to the exogenous apoptotic cells were detected, and although the apoptotic cell-based therapy did not enhance BM engraftment, the incidence of acute grade II to IV GVHD was lower than the one previously reported and was zero in the six patients that received the higher doses of apoptotic cells [102].

Therapies Dependent on Apoptotic Cell Clearance

Other cell- or Ab-based approaches used to promote immune-suppression for therapy of transplant rejection, GVHD, or auto-immune disorders also function through generation of apoptotic leukocytes. The apoptotic cells generated in vivo are rapidly internalized by host APCs, which receive immune-regulatory signals and in some cases the pathogenic Ag(s), via the apoptotic leukocytes [60].

In extracorporeal photopheresis (ECP), mononuclear cell-enriched plasma separated by apheresis is exposed to 8-methoxypsoralen and UV-A radiation, which prime the leukocytes for apoptosis. The leukocytes are then reinfused into the patient. Supplementation of pharmacological immunosuppression with ECP has been proven to decrease the risk of heart, kidney, lung, and liver transplant rejection [103–106]. In combination with standard therapy, ECP is also indicated for chronic GVHD and steroid-refractory acute GVHD, although the latter requires treatment over a long period of time [107, 108]. Importantly, not all GVHD patients respond to ECP and frequent ECP cycles are required, which is a burden for patients with severe acute GVHD.

The effect of ECP on GVHD is probably not caused by induction of apoptosis of host-reactive T cells in peripheral blood, since less than 10% of peripheral T cells are eliminated by a single ECP procedure. Several studies have found that the effects of ECP are associated with induction of host/recipient immunosuppressive DCs by the clearance of the ECP-treated apoptotic cells, and with increased number or function of CD4 Tregs [109–115]. Interestingly, ECP does not cause generalized immunosuppression, and the presence of host-reactive T cells in the ECP-treated inoculum seems to correlate directly with the ECP efficacy in GVHD [116–118]. Based on these observations, D. Hannani [119] has raised the hypothesis that during ECP, reinfusion of host-reactive T cells undergoing immunogenic cell death provides Ags (host-reactive TCR-derived peptides) plus APC-activating signals to host APCs, which then generate anticlonotypic CD8 T cells that specifically eliminate the pathogenic T cells responsible for GVHD. However, in patients with chronic GVHD, French et al. [117] did not detect reduction of the previously expanded T cell clones in peripheral blood after successful ECP treatment.

In the blood transfusion field, alloimmunity against red blood cells is an adverse effect in less than 10% of blood recipients. In mice, Vallion et al. [120] has demonstrated that leukocytes undergoing apoptosis in short-term stored blood, release TGF-β that down-regulates the risk of red blood cell alloimmunization. By contrast, in long-term stored blood, leukocytes become predominately necrotic, increasing the probability of red blood cell alloimmunization [120].

Randomly selected, haplotype-shared, or donor-specific transfusion (DST) plus standard immunosuppression has been employed to promote donor-specific immunosuppression. The use of DST has been discontinued due to the risk of allosensitization and the arrival of new immunosuppressants. More recently, donor (or recipient)—derived DCs, rendered tolerogenic by pharmacological or genetic manipulation, have been used to control alloimmunity. The prevailing idea on the mechanism of action of DST and tolerogenic DC-based therapies is that the i.v. injected leukocytes and DCs rapidly undergo apoptosis (due to short life spam or attack by recipient NK and cytotoxic T cells), and therefore serve as a source of donor peptides and immune-regulatory signals for recipient quiescent APCs, which in turn restrain the antidonor response [121–123].

Administration of T cell-depleting Abs (i.e., CD3 Ab) has been used to treat autoimmunity and transplant rejection. The immune-suppression mediated by CD3 Abs is caused indirectly through release of TGF-β by macrophages and DCs that internalize the apoptotic T cells [124].

Summary

In the clinics, apoptotic cell-based therapies through administration of donor apoptotic leukocytes or apoptotic cell mimics

could reduce the use of pharmacological immunosuppression and therefore, its harmful side effects. Different laboratories have proved in murine models that optimal immunosuppression by apoptotic cell administration is achieved with the use of nonstressed leukocytes undergoing early apoptosis, administered i.v. days before or by the time of transplantation, and in quantities that do not overload the mechanisms of apoptotic cell clearance. However, (i) the capacity of the injected apoptotic cells to modulate alloimmunity in recipients with alloreactive memory T cells, (ii) the shelf-life of apoptotic cells generated in vitro for therapeutic applications, (iii) the stability of the immunosuppression induced by short-lived apoptotic cells -administered once or in multiple doses, and (iv) the risk of allosensitization after apoptotic cell infusion, represent critical issues to be addressed in the laboratory or clinical trials, before embarking in the implementation of apoptotic cell-based therapies in transplantation in humans.

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Author Contributions

A.E.M and A.T.L. contribute equally to the conception and writing of the manuscript.

Disclosure of Potential Conflicts of Interest

The authors declare no competing financial interests.

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