

Can Allogeneic Hematopoietic Cell Transplantation Outcome be Improved by Intravenous Apoptotic Cell Infusion?

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Abstract

Cell-based therapy approaches have been shown to improve allogeneic hematopoietic cell transplantation (AHCT) outcome by reducing its severe toxic side effects, including graft rejection, acute and chronic graft-versus-host disease (GvHD) or delayed/impaired immune reconstitution. Here, we discuss the use of intravenous apoptotic leukocyte infusion to improve AHCT outcome. In experimental AHCT models, we demonstrated that intravenous apoptotic leukocyte infusion, simultaneously to allogeneic bone marrow grafts, favors hematopoietic engraftment, prevents allo-immunization and delays acute GvHD onset. Here, we review the different mechanisms and the potential beneficial effects associated with the immunomodulatory properties of apoptotic cells in the AHCT setting.

Keywords: Apoptotic cells; Cell therapy; Hematopoietic cell transplantation; Graft-versus-host disease; Regulatory T cells; TGF- β ; Macrophage

Introduction

Cell based-therapy is a dynamic area of research that has made significant advances in recent times. Different cell-based therapies have been proposed in clinical trials to improve allogeneic hematopoietic cell transplantation outcome, including, for instance, mesenchymal stem cells [1,2] or regulatory T cells (Treg) [3,4]. In this review, after a brief description of the immune mechanisms involved in graft rejection and in acute GvHD, we will discuss the potential improvements that can be provided by intravenous apoptotic cell infusion. The immune mechanisms used by this cell-based therapy approach and the settings for a clinical application will be also reviewed. While we will not detail all the immunomodulatory properties of apoptotic cells (for this please refer to [5-7]), apoptotic cells have been used as a cell-based therapy product in several experimental models (Table 1). This supports the development of upcoming clinical trials.

Significant advances have been made in allogeneic hematopoietic cell transplantation (AHCT) over the past decades [8] and now, this therapeutic approach is widely used. In 2010, 1,671 patients received an allograft in France [9]. Among the different advances in AHCT [10], we will focus on 3 that support the use of intravenous apoptotic leukocyte infusion. These advances are the following: successful transplantation from human leukocyte antigen (HLA)-mismatched and unrelated donors [10], successful cord blood transplantation [11], especially in adult patients [12-16] and introduction of the so-called reduced-intensity conditioning regimens (RIC) [17] (for a definition of RIC, please refer to [18]). In parallel to the clinical improvements, therapeutic indications of AHCT have been extended to several pathologies from high risk hematological malignancies and severe acquired immune deficiencies to some solid tumors as well as non malignant disorders [10]. The introduction of RIC regimens a decade ago [17] allowed to treat elderly patients [19,20] or patients presenting single organ comorbidity involving liver, lung, heart, or kidney before transplantation [21], as well as to propose combined hematopoietic cell/organ transplantation in order to achieve immune tolerance [22]. Despite understanding the immune mechanisms involved in the beneficial and deleterious effects of AHCT and identifying criteria for better patients' selection, several severe complications persist leading to a high rate of morbidity and mortality. The main complications

include acute and chronic graft-versus-host disease (GvHD), prolonged immune deficiency due to delayed/impaired immune reconstitution or to graft rejection/failure. This immunodeficient state –aggravated by the administration of non specific immunosuppressive drugs–exposes patients to life-threatening opportunistic infections. All of the latter complications justify the search for new strategies to improve hematopoietic engraftment, reduce non specific immunosuppression, accelerate immune reconstitution and improve GvHD control.

Prevention of Graft Rejection by Intravenous Apoptotic Cell Infusion: Rationale, Experimental Data and Design of Future Clinical Trials

Rationale: Clinical situations where hematopoietic graft rejection/failure is observed. Engraftment is not often a matter of concern after AHCT [10]. However, the introduction of RIC or nonmyeloablative conditioning regimens –that do not aim to completely eliminate host-derived immune cells– and the use of cord blood as an alternative stem cell source, may be associated with a significantly delayed engraftment. Initially, a high rate of graft rejection was observed after RIC [19,23]. However, significant improvement in immunosuppressive strategies has reduced graft rejection rates, notably by the use of fludarabine [24] or anti-thymocyte globulin (ATG) [25]. Nevertheless, the use of these immunosuppressive agents may be associated with an increased rate of severe infections post-transplantation [26]. Moreover, immune reconstitution is delayed favoring more opportunistic infections in the long term [26,27]. Graft rejection is also observed after cord blood transplantation (mainly in adult patients) [12-16] due to the limited number of hematopoietic stem cells contained in the graft [23,28]. Cost analysis of umbilical

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	References
Chronic inflammatory autoimmune diseases	
Diabetes	Xia, 2007 [81]
Experimental Autoimmune Encephalomyelitis	Miyake, 2007 [82]; Qiu, 2009 [83]
Arthritis	Gray, 2007 [84]; Perruche, 2009 [53]; Notley, 2011 [85]
Acute inflammatory diseases	
Sepsis	Huynh, 2002 [40]; Ren, 2008 [86]
Fulminant hepatitis	Zhang, 2011 [87]
Contact hypersensitivity	Griffith, 2007 [88]
Transplantation	
Cardiac allograft	
Acute rejection	Sun, 2004 [89]; Wang, 2006 [90]
Chronic rejection	Wang, 2009 [91]
Hematopoietic cell transplantation	
Hematopoietic engraftment	Bittencourt, 2001 [35]; Kleinclauss, 2006 [36]; Perruche, 2004 [37]
Graft-versus-host disease	Kleinclauss, 2006 [36]
Allo-antibodies after graft rejection	Perruche, 2004 [37]
Acute myocardial infarction	Ankersmith, 2009 [92]; Lichtenauer, 2011 [93]

Modified and updated from [74].

Table 1 : Beneficial effects of apoptotic cell infusion in experimental disease models.

cord blood transplantation identifies graft failure/rejection as the major expense [29]. The authors of this study suggest that strategies to enhance hematopoietic engraftment will decrease the cost of cord blood transplantation [29]. The last situation associated with a high rate of graft rejection is when donor T cells are depleted from the graft [30,31]. The use of this latter approach is limited due to an increased risk of relapse incidence [30]. However, this can be used in many centers when the risk of severe GvHD is too high (i.e., HLA-mismatched donor grafts, elderly patients). Recently, a clinical trial reported the use of Treg in T cell-depleted haploidentical allografts [4]. An interesting point of this study is that immunosuppressive drugs can be avoided in the setting of T cell depletion and that immunomodulatory cell-based therapy products (Treg) can replace immunosuppressive regimens in AHCT [4]. Thus, therapeutic approaches are needed to limit excessive immunosuppression and favor hematopoietic engraftment in these clinical situations.

Mechanisms used by Intravenous Apoptotic Cell Infusion to Favor Hematopoietic Engraftment: The major mechanisms involved in graft rejection are: i) recipient alloreactive T and NK cells that resist to conditioning regimen [32] (this is particularly true for RIC) as well as ii) pre-graft donor-specific antibodies [33,34] when patients are allo-immunized by repeated blood transfusions prior to AHCT or in multiparous women. Thus, the three barriers –namely, host NK and T cells as well as allo-antibodies– have to be overcome to favor engraftment (Figure 1). Until now, we focused on the inhibition of host NK and T cell-mediated rejection. In different experimental AHCT models using RIC regimens, we reported that intravenous apoptotic leukocyte infusion, simultaneously to allogeneic bone marrow grafts favors hematopoietic engraftment [35]. This effect is observed whatever the origin of apoptotic cells (i.e., donor, recipient, third party or even xenogeneic) and the apoptotic stimulus used (i.e., Fas antibody, γ - or UVB-irradiation) [35]. Then, we explored the immune mechanisms involved in this beneficial effect of intravenous apoptotic cell infusion (Figure 1). We identified several critical steps for apoptotic cell-induced engraftment, including: host splenic macrophage [36], TGF- β secretion [36,37] and donor graft-derived plasmacytoid dendritic cells (PDC) [38]. In contrast, depletion of host conventional dendritic cells (cDC) using CD11c/Diphtheria Toxin Receptor/green fluorescent protein transgenic mice and diphtheria toxin administration did not alter

hematopoietic engraftment after intravenous apoptotic cell infusion [36,38], suggesting that cDC did not play a major role. Despite the fact that we did not directly demonstrate a link between macrophage and TGF- β secretion, we may speculate –from the literature [39,40] and from our data showing in vivo apoptotic cell uptake by macrophages [36]– that TGF- β is secreted by splenic macrophages phagocytosing infused apoptotic cells. Thus, we propose the following scenario: after intravenous infusion, apoptotic cells are eliminated by host splenic macrophages that in turn release TGF- β . Splenic macrophages are known to be specialized in the daily elimination of blood-borne leukocytes and in the control of immune responses against apoptotic cell-derived antigens [41,42]. TGF- β is an immunosuppressive cytokine that neutralizes NK cell cytotoxicity [41,42], and so, may prevent hematopoietic graft rejection mediated by host NK cells. In addition, we have shown that TGF- β secretion and host macrophages are required for apoptotic cell-induced Treg increase observed 6-8 days after infusion [36]. Whether this Treg increase after intravenous apoptotic cell infusion is necessary for apoptotic cell-induced engraftment remains to be determined. However, Treg are able to inhibit NK cell-mediated cytotoxicity [43,44] and have been reported to favor allogeneic hematopoietic engraftment in experimental mouse model [45], but also in a clinical trial after cord blood transplantation [3]. We have also shown that apoptotic cell-induced Treg are mainly from recipient origin [36] and may result from the differentiation into Treg of naive CD4⁺ T cells persisting to RIC. Moreover, PDC, but not cDC, were identified as the main antigen-presenting cells (APC) required for Treg commitment in this transient immunosuppressive environment generated by apoptotic cells [38]. This data fits with the requirement of PDC for Treg induction after donor specific transfusion (DST) and CD40/CD40L blockade in a cardiac allograft model [46]. Some of the immune mechanisms of DST are suspected to be related to the presence of apoptotic cells in blood products [6,47]. We also reported that infused apoptotic cells did not directly interact with PDC, but that PDC require macrophages phagocytosing apoptotic cells and the subsequent TGF- β production [38]. We suspect that, in addition to TGF- β , another factor secreted by macrophages uptaking apoptotic cells is required for the generation of Treg by PDC since PDC exposed in vitro to recombinant TGF- β favors the generation of IL-17-producing CD4⁺ T cells (TH17) but not Treg [48]. Experiments are ongoing to identify such factor produced by macrophages uptaking

apoptotic cells and “conditioning” PDC to generate Treg. Altogether, intravenous apoptotic cell infusion may neutralize host immune cells involved in graft rejection through TGF- β secretion and Treg induction (Figure 1). In this setting, the origin of apoptotic cells (i.e., antigen specificity) does not matter since apoptotic cells are only necessary to trigger TGF- β secretion by phagocytosing macrophages.

A Preclinical Study to Test Immunosuppressive Drugs: We also studied the impact of clinically relevant immunosuppressive drugs on apoptotic cell-induced hematopoietic cell engraftment. We showed that ciclosporin (CsA) prevented apoptotic cell-induced engraftment, while mycophenolate mofetil (MMF) did not affect apoptotic cell-induced engraftment and sirolimus (SRL) had synergistic effects [49]. Moreover, in contrast to MMF or SRL, CsA inhibited donor

apoptotic cell-induced Treg [49]. As reported by others for conversion of naive CD4⁺ T cells into peripheral Treg [50], our data suggest that apoptotic cell-induced Treg commitment required NFAT signaling. Furthermore, we demonstrated in an experimental model of specific CD3 antibody-induced T cell apoptosis that T cell receptor (TCR) signaling is mandatory for Treg generation [51]. We propose that engagement of TCR simultaneously to apoptotic cell induction/infusion allows to distinguish transient immunosuppression (linked to immunosuppressive cytokine secretion) [39,52] from tolerance induction (i.e., Treg induction). Transient local immunosuppression may be sufficient to limit or resolve inflammation [40,53] and maybe favor engraftment. However, Treg induction has the advantage to export tolerance to other sites than the site where cells are dying, as well as to remain for a certain time period according to apoptotic cell-induced Treg persistence. This Treg induction may be beneficial for the control of GvHD (please see below).

Design of Future Clinical Trials: For a transfer to a clinical trial, our findings with immunosuppressive drugs represent a step forward toward the use of intravenous donor apoptotic cell infusion to enhance engraftment in various clinical settings. Most of the immunosuppressive regimens usually used in RIC transplantation associate CsA and MMF with or without ATG [19,24,26,27]. Although CsA may exert a beneficial effect on GvHD, it is likely that CsA should be avoided with apoptotic cell infusion, as it may abrogate its favorable effect. Alternatively, MMF, which is commonly administrated in clinical practice for calcineurin inhibitor-intolerant hematopoietic cell recipients [54], can be used without interference with the action of apoptotic cells. Most interestingly, and despite some severe adverse effects [55,56], SRL-based immunosuppressive regimens that are increasingly used in the solid transplant field (such as kidney transplantation) can probably represent an attractive setting for the design of cell-based therapies using donor apoptotic cell infusion. Recent publications report clinical studies using SRL alone as immunosuppressive regimen after RIC [57,58]. Lastly, as proposed for Treg-based therapy [4], infusion of apoptotic cells may be used without any immunosuppressive drugs for instance in the settings of T cell-depleted grafts. We already reported that donor apoptotic cell infusion favors engraftment of enriched hematopoietic stem cell allografts [36].

Control of Acute Graft-Versus-Host Disease by Intravenous Donor Apoptotic Cell Infusion: During the last years, acute GvHD physiopathology understanding has been significantly improved mainly due to different animal AHCT models. Acute GvHD is an uncontrolled inflammatory disease associated with a “cytokine storm”. Schematically, acute GvHD involves three successive steps: i) activation of host innate immune cells by the conditioning regimen [59]; ii) donor CD4⁺ T cell differentiation into Th1 cells (the major pathological CD4⁺ T cell subset in acute GvHD); iii) destruction of healthy tissues (e.g., liver, gastrointestinal tract, or skin) by donor-derived cytotoxic T lymphocytes and soluble inflammatory factors (i.e., TNF- α) [60-62]. When considering these successive steps, blockade at the initial step (i.e., host APC activation) seems to be the best way to prevent the “vicious circle” of GvHD. We hypothesized that through targeting host APC and modulating their functional properties, apoptotic cell infusion may dampen GvHD. Indeed, donor apoptotic cell infusion in recipient mice delays acute GvHD lethality in a dose-dependent manner [36]. In contrast, recipient apoptotic cell infusion exacerbates GvHD, while third party apoptotic cells do not affect GvHD onset (Perruche S & Saas P, unpublished results). Moreover, CD25⁺ cell depletion at day 3 and day 6 after transplantation can prevent the favorable effect of donor apoptotic cell infusion on GvHD [36]. Administration of

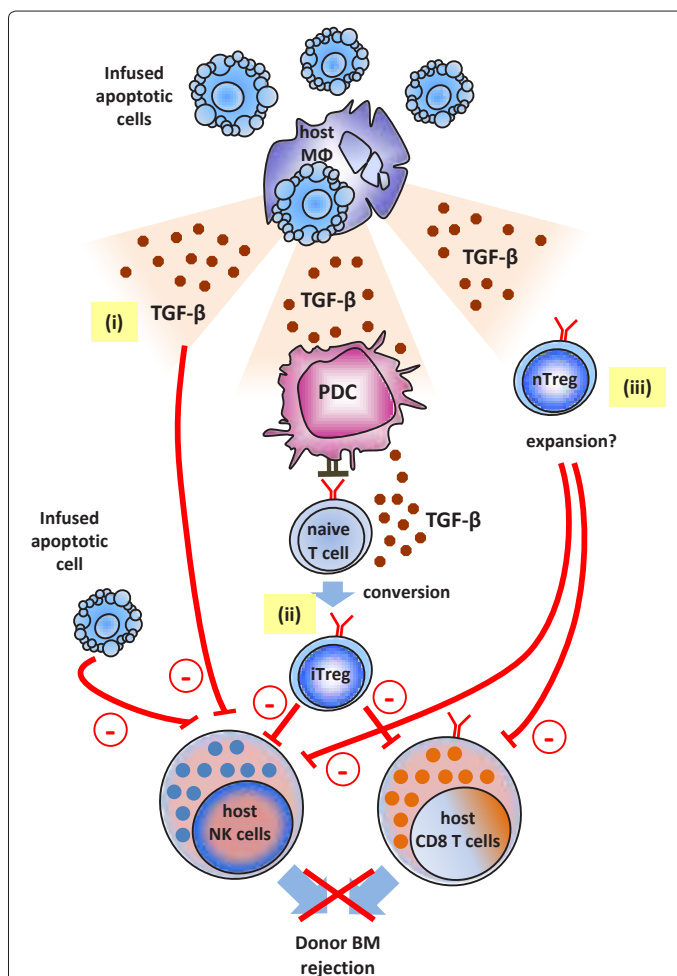


Figure 1: Immune mechanisms involved in the graft-facilitating effect of intravenous apoptotic cell infusion. Graft rejection is mediated mainly by host NK and CD8⁺ T cells that resist to conditioning regimen [32]. Intravenous apoptotic cell infusion may neutralize these effectors of graft rejection by several mechanisms, including: (i) secretion of TGF- β after elimination of infused apoptotic cells by host splenic macrophages [36] and (ii) generation of induced Treg (iTreg) by conversion from naive CD4⁺ T cells after interaction with graft-derived donor PDC in a TGF- β -dependent manner [38]. (iii) TGF- β secretion may also participate to the expansion of natural Treg (nTreg). We hypothesize that TGF- β neutralizes NK cell-mediated rejection [41,42], while Treg (i.e., iTreg and nTreg) inhibits both NK and CD8⁺ T cell cytotoxicity [43,44,94]. Moreover, apoptotic cells may interact directly with NK cells and alter their cytotoxicity [95]. Abbreviations used: BM, bone marrow; iTreg, induced regulatory CD4⁺ T cells; MΦ, macrophage; nTreg, natural regulatory CD4⁺ T cells; PDC, plasmacytoid dendritic cell.

CD25 monoclonal antibody in lymphopenic hosts selectively depletes Treg [63]. This suggests that Treg induced by intravenous donor apoptotic cell infusion may be implicated in this effect. In contrast to what we observed in graft rejection models where the beneficial effect of intravenous apoptotic cell infusion is independent of the origin of apoptotic cells (donor, recipient, third party or even xenogeneic), the protective effect on GvHD occurrence and severity was found only using donor apoptotic cells. This suggests that there is a requirement for antigen specificity and this needs to be explored in the future. Several experimental studies have demonstrated the protective role on GvHD of donor Treg administration simultaneously to bone marrow grafts [64-69] and two clinical trials report the feasibility of such approach [3,4]. Despite these promising effects of donor apoptotic cell infusion on GvHD, it is still needed to establish whether this cell-based therapy does not also affect or abrogate the immune graft-versus-leukemia effect (GvL) that is usually linked to GvHD. This has to be evaluated in the future. Nevertheless, a phase I/II clinical trial using donor apoptotic cells has started in Israel (ClinicalTrials.gov Identifier #NCT00524784 [70]) and preliminary data were presented in the last European Bone Marrow Transplantation (EBMT) meeting in Paris with no toxicity reported [71]. In our experimental model, we did not find any signs of autoimmune disease in mice receiving apoptotic cell infusion simultaneously with their BM grafts [72]. Intravenous infusion of apoptotic cells may mimic other therapeutic approaches generating intravenous apoptotic leukocytes [73,74]. This can be the case of extracorporeal photochemotherapy (ECP), a therapeutic approach already used to treat patients suffering from severe chronic or acute GvHD [75,76]. Indeed, significant numbers of apoptotic leukocytes are generated post-ECP prior to their reinfusion [6]. No significant toxicity has been reported for this approach. In addition, Treg have been shown to be induced after ECP in two different murine models [77,78] confirming the link between apoptotic cells and Treg [36,51,77,79]. Finally, a clinical study has reported the intramuscular injection of syngeneic apoptotic cells in more than thousand patients suffering from chronic heart failure [80]. Despite a limited beneficial effect and an arguable method to induce apoptosis, no significant adverse effects were reported [80]. Altogether, this suggests that clinical studies can be envisaged.

Conclusion

In conclusion, despite significant improvements over the past decades performed in transplantation practice and patient care that significantly have been reducing patient mortality [8], GvHD (in its acute or chronic form) still occurs at higher rate and remains the most life-threatening complication after AHCT. Furthermore, in certain clinical situations (i.e., RIC regimen, T cell depletion or cord blood transplantation), graft rejection/failure may occur. Our data obtained in experimental models have demonstrated that intravenous apoptotic cell infusion may be used to encompass these deleterious effects. Immune mechanisms used by apoptotic cell infusion to control acute GvHD and favor engraftment are not completely identified, but APC might be the targets of apoptotic cell-induced tolerance mechanisms as well as induction of Treg. Furthermore, interactions of the cell-based therapy approach with clinically relevant immunosuppressive drugs have been defined. The safety of this approach is sustained by preliminary data obtained in a phase I/II study [71]. Thus, we believe that intravenous apoptotic cell infusion is a promising approach to be implemented clinically for the prevention of graft failure and GVHD.

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Conflict of Interest

The authors declare no conflicts of interest.

References

1. Pontikoglou C, Deschaseaux F, Sensebe L, Papadaki HA (2011) Bone marrow mesenchymal stem cells: biological properties and their role in hematopoiesis and hematopoietic stem cell transplantation. *Stem Cell Rev* 7: 569-589.
2. Ringden O, Le Blanc K (2011) Mesenchymal stem cells for treatment of acute and chronic graft-versus-host disease, tissue toxicity and hemorrhages. *Best Pract Res Clin Haematol* 24: 65-72.
3. Brunstein CG, Miller JS, Cao Q, McKenna DH, Hippen KL, et al. (2011) Infusion of ex vivo expanded T regulatory cells in adults transplanted with umbilical cord blood: safety profile and detection kinetics. *Blood* 117: 1061-1070.
4. Di Ianni M, Falzetti F, Carotti A, Terenzi A, Castellino F, et al. (2011) Tregs prevent GVHD and promote immune reconstitution in HLA-haploidentical transplantation. *Blood* 117: 3921-3928.
5. Green DR, Ferguson T, Zitvogel L, Kroemer G (2009) Immunogenic and tolerogenic cell death. *Nat Rev Immunol* 9: 353-363.
6. Morelli AE, Larregina AT (2010) Apoptotic cell-based therapies against transplant rejection: role of recipient's dendritic cells. *Apoptosis* 15: 1083-1097.
7. Saas P, Bonnefoy F, Kury-Paulin S, Kleinclauss F, Perruche S (2007) Mediators involved in the immunomodulatory effects of apoptotic cells. *Transplantation* 84: S31-S34.
8. Gooley TA, Chien JW, Pergam SA, Hingorani S, Sorrow ML, et al. (2010) Reduced mortality after allogeneic hematopoietic-cell transplantation. *N Engl J Med* 363: 2091-2101.
9. Rapport annuel de l'Agence de BioMédecine 2010.
10. Appelbaum FR (2007) Hematopoietic-cell transplantation at 50. *N Engl J Med* 357: 1472-1475.
11. Gluckman E, Broxmeyer HA, Auerbach AD, Friedman HS, Douglas GW, et al. (1989) Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical-cord blood from an HLA-identical sibling. *N Engl J Med* 321: 1174-1178.
12. Lekakis L, Giral S, Couriel D, Shpall EJ, Hosing C, et al. (2006) Phase II study of unrelated cord blood transplantation for adults with high-risk hematologic malignancies. *Bone Marrow Transplant* 38: 421-426.
13. Jabbour E, Rondon G, Anderlini P, Giral S, Couriel DR, et al. (2007) Treatment of donor graft failure with nonmyeloablative conditioning of fludarabine, antithymocyte globulin and a second allogeneic hematopoietic transplantation. *Bone Marrow Transplant* 40: 431-435.
14. Mizutani E, Narimatsu H, Murata M, Tomita A, Kiyoi H, et al. (2007) Successful second cord blood transplantation using fludarabine and cyclophosphamide as a preparative regimen for graft rejection following reduced-intensity cord blood transplantation. *Bone Marrow Transplant* 40: 85-87.
15. Nakamura Y, Tanaka Y, Ando T, Sato Y, Yujiri T, et al. (2007) Successful engraftment of the second reduced-intensity conditioning cord blood transplantation (CBT) for a patient who developed graft rejection and infectious complications after the first CBT for AML. *Bone Marrow Transplant* 40: 395-396.
16. Takagi S, Masuoka K, Uchida N, Ishiwata K, Araoka H, et al. (2009) High incidence of haemophagocytic syndrome following umbilical cord blood transplantation for adults. *Br J Haematol* 147: 543-553.
17. Slavin S, Nagler A, Naparstek E, Kapelushnik Y, Aker M, et al. (1998) Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. *Blood* 91: 756-763.
18. Bacigalupo A, Ballen K, Rizzo D, Giral S, Lazarus H, et al. (2009) Defining the intensity of conditioning regimens: working definitions. *Biol Blood Marrow Transplant* 15: 1628-1633.

19. McSweeney PA, Niederwieser D, Shizuru JA, Sandmaier BM, Molina AJ, et al. (2001) Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. *Blood* 97: 3390-3400.
20. Majhail NS, Brunstein CG, Tomblyn M, Thomas AJ, Miller JS, et al. (2008) Reduced-intensity allogeneic transplant in patients older than 55 years: unrelated umbilical cord blood is safe and effective for patients without a matched related donor. *Biol Blood Marrow Transplant* 14: 282-289.
21. Sorror ML (2010) Comorbidities and hematopoietic cell transplantation outcomes. *Hematology Am Soc Hematol Educ Program* 2010: 237-247.
22. Fudaba Y, Spitzer TR, Shaffer J, Kawai T, Fehr T, et al. (2006) Myeloma responses and tolerance following combined kidney and nonmyeloablative marrow transplantation: in vivo and in vitro analyses. *Am J Transplant* 6: 2121-2133.
23. Mattsson J, Ringden O, Storb R (2008) Graft failure after allogeneic hematopoietic cell transplantation. *Biol Blood Marrow Transplant* 14: 165-170.
24. Niederwieser D, Maris M, Shizuru JA, Petersdorf E, Hegenbart U, et al. (2003) Low-dose total body irradiation (TBI) and fludarabine followed by hematopoietic cell transplantation (HCT) from HLA-matched or mismatched unrelated donors and postgrafting immunosuppression with cyclosporine and mycophenolate mofetil (MMF) can induce durable complete chimerism and sustained remissions in patients with hematological diseases. *Blood* 101: 1620-1629.
25. Blaise D, Bay JO, Faucher C, Michallet M, Boiron JM, et al. (2004) Reduced-intensity preparative regimen and allogeneic stem cell transplantation for advanced solid tumors. *Blood* 103: 435-441.
26. Maris M, Boeckh M, Storer B, Dawson M, White K, et al. (2003) Immunologic recovery after hematopoietic cell transplantation with nonmyeloablative conditioning. *Exp Hematol* 31: 941-952.
27. Maris MB, Niederwieser D, Sandmaier BM, Storer B, Stuart M, et al. (2003) HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative conditioning for patients with hematologic malignancies. *Blood* 102: 2021-2030.
28. Laughlin MJ, Barker J, Bambach B, Koc ON, Rizzieri DA, et al. (2001) Hematopoietic engraftment and survival in adult recipients of umbilical-cord blood from unrelated donors. *N Engl J Med* 344: 1815-1822.
29. Majhail NS, Mothukuri JM, Brunstein CG, Weisdorf DJ (2009) Costs of hematopoietic cell transplantation: comparison of umbilical cord blood and matched related donor transplantation and the impact of posttransplant complications. *Biol Blood Marrow Transplant* 15: 564-573.
30. Maraninchi D, Gluckman E, Blaise D, Guyotat D, Rio B, et al. (1987) Impact of T-cell depletion on outcome of allogeneic bone-marrow transplantation for standard-risk leukaemias. *Lancet* 2: 175-178.
31. Marmont AM, Horowitz MM, Gale RP, Sobocinski K, Ash RC, et al. (1991) T-cell depletion of HLA-identical transplants in leukemia. *Blood* 78: 2120-2130.
32. Martin PJ (2000) Winning the battle of graft versus host. *Nat Med* 6: 18-19.
33. Taylor PA, Ehrhardt MJ, Roforth MM, Swedin JM, Panoskaltis-Mortari A, et al. (2007) Preformed antibody, not primed T cells, is the initial and major barrier to bone marrow engraftment in allosensitized recipients. *Blood* 109: 1307-1315.
34. Spellman S, Bray R, Rosen-Bronson S, Haagenson M, Klein J, et al. (2010) The detection of donor-directed, HLA-specific alloantibodies in recipients of unrelated hematopoietic cell transplantation is predictive of graft failure. *Blood* 115: 2704-2708.
35. Bittencourt MC, Perruche S, Contassot E, Fresnay S, Baron MH, et al. (2001) Intravenous injection of apoptotic leukocytes enhances bone marrow engraftment across major histocompatibility barriers. *Blood* 98: 224-230.
36. Kleinclauss F, Perruche S, Masson E, de Carvalho Bittencourt M, Biichle S, et al. (2006) Intravenous apoptotic spleen cell infusion induces a TGF-beta-dependent regulatory T-cell expansion. *Cell Death Differ* 13: 41-52.
37. Perruche S, Kleinclauss F, Bittencourt Mde C, Paris D, Tiberghien P, et al. (2004) Intravenous infusion of apoptotic cells simultaneously with allogeneic hematopoietic grafts alters anti-donor humoral immune responses. *Am J Transplant* 4: 1361-1365.
38. Bonnefoy F, Perruche S, Couturier M, Sedrati A, Sun Y, et al. (2011) Plasmacytoid dendritic cells play a major role in apoptotic leukocyte-induced immune modulation. *J Immunol* 186: 5696-5705.
39. Fadok VA, Bratton DL, Konowal A, Freed PW, Westcott JY, et al. (1998) Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF. *J Clin Invest* 101: 890-898.
40. Huynh ML, Fadok VA, Henson PM (2002) Phosphatidylserine-dependent ingestion of apoptotic cells promotes TGF-beta1 secretion and the resolution of inflammation. *J Clin Invest* 109: 41-50.
41. Li MO, Wan YY, Sanjabi S, Robertson AK, Flavell RA (2006) Transforming growth factor-beta regulation of immune responses. *Annu Rev Immunol* 24: 99-146.
42. Trotta R, Col JD, Yu J, Ciarlariello D, Thomas B, et al. (2008) TGF-beta utilizes SMAD3 to inhibit CD16-mediated IFN-gamma production and antibody-dependent cellular cytotoxicity in human NK cells. *J Immunol* 181: 3784-3792.
43. Ghiringhelli F, Menard C, Terme M, Flament C, Taieb J, et al. (2005) CD4+CD25+ regulatory T cells inhibit natural killer cell functions in a transforming growth factor-beta-dependent manner. *J Exp Med* 202: 1075-1085.
44. Smyth MJ, Teng MW, Swann J, Kyriakopoulos K, Godfrey DI, et al. (2006) CD4+CD25+ T regulatory cells suppress NK cell-mediated immunotherapy of cancer. *J Immunol* 176: 1582-1587.
45. Hanash AM, Levy RB (2005) Donor CD4+CD25+ T cells promote engraftment and tolerance following MHC-mismatched hematopoietic cell transplantation. *Blood* 105: 1828-1836.
46. Ochando JC, Homma C, Yang Y, Hidalgo A, Garin A, et al. (2006) Alloantigen-presenting plasmacytoid dendritic cells mediate tolerance to vascularized grafts. *Nat Immunol* 7: 652-662.
47. Kleinclauss F, Perruche S, Cahn JY, Tiberghien P, Saas P (2003) Administration of donor apoptotic cells: an alternative cell-based therapy to induce tolerance? *Transplantation* 75: 43S-45S.
48. Bonnefoy F, Couturier M, Clauzon A, Remy-Martin JP, Gaugler B, et al. (2011) TGF-beta-exposed plasmacytoid dendritic cells participate in Th17 commitment. *J Immunol* 186: 6157-6164.
49. Bonnefoy F, Masson E, Perruche S, Marandin A, Borg C, et al. (2008) Sirolimus enhances the effect of apoptotic cell infusion on hematopoietic engraftment and tolerance induction. *Leukemia* 22: 1430-1434.
50. Wu Y, Borde M, Heissmeyer V, Feuerer M, Lapan AD, et al. (2006) FOXP3 controls regulatory T cell function through cooperation with NFAT. *Cell* 126: 375-387.
51. Perruche S, Zhang P, Liu Y, Saas P, Bluestone JA, et al. (2008) CD3-specific antibody-induced immune tolerance involves transforming growth factor-beta from phagocytes digesting apoptotic T cells. *Nat Med* 14: 528-535.
52. Voll RE, Herrmann M, Roth EA, Stach C, Kalden JR, et al. (1997) Immunosuppressive effects of apoptotic cells. *Nature* 390: 350-351.
53. Perruche S, Saas P, Chen W (2009) Apoptotic cell-mediated suppression of streptococcal cell wall-induced arthritis is associated with alteration of macrophage function and local regulatory T-cell increase: a potential cell-based therapy? *Arthritis Res Ther* 11: R104.
54. Dvorak CC, Callard E, Agarwal R (2006) Use of intravenous mycophenolate mofetil for graft-versus-host disease prophylaxis in an allogeneic hematopoietic stem cell transplant recipient with an allergic reaction to cyclosporine and tacrolimus. *Bone Marrow Transplant* 38: 253-254.
55. Cutler C, Henry NL, Magee C, Li S, Kim HT, et al. (2005) Sirolimus and thrombotic microangiopathy after allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 11: 551-557.
56. Busca A, Locatelli F, Moscato D, Falda M (2005) Sirolimus-related toxicity in stem cell transplantation. *Biol Blood Marrow Transplant* 11: 647-649.
57. Schleuning M, Judith D, Jedlickova Z, Stubig T, Heshmat M, et al. (2009) Calcineurin inhibitor-free GVHD prophylaxis with sirolimus, mycophenolate mofetil and ATG in Allo-SCT for leukemia patients with high relapse risk: an observational cohort study. *Bone Marrow Transplant* 43: 717-723.
58. Hsieh MM, Kang EM, Fitzhugh CD, Link MB, Bolan CD, et al. (2009) Allogeneic hematopoietic stem-cell transplantation for sickle cell disease. *N Engl J Med* 361: 2309-2317.
59. Zhang Y, Shlomchik WD, Joe G, Louboutin JP, Zhu J, et al. (2002) APCs in the

- liver and spleen recruit activated allogeneic CD8⁺ T cells to elicit hepatic graft-versus-host disease. *J Immunol* 169: 7111-7118.
60. Socie G, Blazar BR (2009) Acute graft-versus-host disease: from the bench to the bedside. *Blood* 114: 4327-4336.
61. Shlomchik WD (2007) Graft-versus-host disease. *Nat Rev Immunol* 7: 340-352.
62. Ferrara JLM, Levine JE, Reddy P, Holler E (2009) Graft-versus-host disease. *Lancet* 373: 1550-1561.
63. Mitchell DA, Cui X, Schmittling RJ, Sanchez-Perez L, Snyder DJ, et al. (2011) Monoclonal antibody blockade of IL-2 receptor α during lymphopenia selectively depletes regulatory T cells in mice and humans. *Blood* 118: 3003-3012.
64. Cohen JL, Trenado A, Vasey D, Klatzmann D, Salomon BL (2002) CD4(+)CD25(+) immunoregulatory T Cells: new therapeutics for graft-versus-host disease. *J Exp Med* 196: 401-406.
65. Hoffmann P, Ermann J, Edinger M, Fathman CG, Strober S (2002) Donor-type CD4(+)CD25(+) regulatory T cells suppress lethal acute graft-versus-host disease after allogeneic bone marrow transplantation. *J Exp Med* 196: 389-399.
66. Ermann J, Hoffmann P, Edinger M, Dutt S, Blankenberg FG, et al. (2005) Only the CD62L⁺ subpopulation of CD4⁺CD25⁺ regulatory T cells protects from lethal acute GVHD. *Blood* 105: 2220-2226.
67. Taylor PA, Lees CJ, Blazar BR (2002) The infusion of ex vivo activated and expanded CD4(+)CD25(+) immune regulatory cells inhibits graft-versus-host disease lethality. *Blood* 99: 3493-3499.
68. Taylor PA, Panoskaltis-Mortari A, Swedin JM, Lucas PJ, Gress RE, et al. (2004) L-Selectin(hi) but not the L-selectin(lo) CD4⁺25⁺ T-regulatory cells are potent inhibitors of GVHD and BM graft rejection. *Blood* 104: 3804-3812.
69. Edinger M, Hoffmann P, Ermann J, Drago K, Fathman CG, et al. (2003) CD4⁺CD25⁺ regulatory T cells preserve graft-versus-tumor activity while inhibiting graft-versus-host disease after bone marrow transplantation. *Nat Med* 9: 1144-1150.
70. <http://clinicaltrials.gov/>
71. Mevorach DV, Zuckerman T, Zazon J, Grau A, Rowe JM (2011) Early apoptotic cells cellular treatment in allogeneic haematopoietic cell transplantation. *Bone Marrow Transplant* 46: S312-S313.
72. Perruche S, Kleinclauss F, Angonin R, Cahn JY, Deconinck E, et al. (2003) A single intravenous infusion of apoptotic cells, an alternative cell-based therapy approach facilitating hematopoietic cell engraftment, do not induce autoimmunity. *J Hematother Stem Cell Res* 12: 451-459.
73. Griffith TS, Ferguson TA (2011) Cell death in the maintenance and abrogation of tolerance: the five Ws of dying cells. *Immunity* 35: 456-466.
74. Saas P, Gaugler B, Perruche S (2010) Intravenous apoptotic cell infusion as a cell-based therapy toward improving hematopoietic cell transplantation outcome. *Ann N Y Acad Sci* 1209: 118-126.
75. Paczesny S, Choi SW, Ferrara JL (2009) Acute graft-versus-host disease: new treatment strategies. *Curr Opin Hematol* 16: 427-436.
76. Flowers ME, Apperley JF, van Besien K, Elmaagacli A, Grigg A, et al. (2008) A multicenter prospective phase 2 randomized study of extracorporeal photopheresis for treatment of chronic graft-versus-host disease. *Blood* 112: 2667-2674.
77. Maeda A, Schwarz A, Kernebeck K, Gross N, Aragane Y, et al. (2005) Intravenous infusion of syngeneic apoptotic cells by photopheresis induces antigen-specific regulatory T cells. *J Immunol* 174: 5968-5976.
78. Gatzka E, Rogers CE, Clouthier SG, Lowler KP, Tawara I, et al. (2008) Extracorporeal photopheresis reverses experimental graft-versus-host disease through regulatory T cells. *Blood* 112: 1515-1521.
79. Torchinsky MB, Garaude J, Martin AP, Blander JM (2009) Innate immune recognition of infected apoptotic cells directs T(H)17 cell differentiation. *Nature* 458: 78-82.
80. Torre-Amione G, Anker SD, Bourge RC, Colucci WS, Greenberg BH, et al. (2008) Results of a non-specific immunomodulation therapy in chronic heart failure (ACCLAIM trial): a placebo-controlled randomised trial. *Lancet* 371: 228-236.
81. Xia CQ, Peng R, Qiu Y, Annamalai M, Gordon D, et al. (2007) Transfusion of apoptotic beta-cells induces immune tolerance to beta-cell antigens and prevents type 1 diabetes in NOD mice. *Diabetes* 56: 2116-2123.
82. Miyake Y, Asano K, Kaise H, Uemura M, Nakayama M, et al. (2007) Critical role of macrophages in the marginal zone in the suppression of immune responses to apoptotic cell-associated antigens. *J Clin Invest* 117: 2268-2278.
83. Qiu CH, Miyake Y, Kaise H, Kitamura H, Ohara O, et al. (2009) Novel subset of CD8 α ⁺ dendritic cells localized in the marginal zone is responsible for tolerance to cell-associated antigens. *J Immunol* 182: 4127-4136.
84. Gray M, Miles K, Salter D, Gray D, Savill J (2007) Apoptotic cells protect mice from autoimmune inflammation by the induction of regulatory B cells. *Proc Natl Acad Sci USA* 104: 14080-14085.
85. Notley CA, Brown MA, Wright GP, Ehrenstein MR (2011) Natural IgM is required for suppression of inflammatory arthritis by apoptotic cells. *J Immunol* 186: 4967-4972.
86. Ren Y, Xie Y, Jiang G, Fan J, Yeung J, et al. (2008) Apoptotic cells protect mice against lipopolysaccharide-induced shock. *J Immunol* 180: 4978-4985.
87. Zhang M, Xu S, Han Y, Cao X (2011) Apoptotic cells attenuate fulminant hepatitis by priming Kupffer cells to produce interleukin-10 through membrane-bound TGF- β . *Hepatology* 53: 306-316.
88. Griffith TS, Kazama H, VanOosten RL, Earle JK Jr, Herndon JM, et al. (2007) Apoptotic cells induce tolerance by generating helpless CD8⁺ T cells that produce TRAIL. *J Immunol* 178: 2679-2687.
89. Sun E, Gao Y, Chen J, Roberts AI, Wang X, et al. (2004) Allograft tolerance induced by donor apoptotic lymphocytes requires phagocytosis in the recipient. *Cell Death Differ* 11: 1258-1264.
90. Wang Z, Larregina AT, Shufesky WJ, Perone MJ, Montecalvo A, et al. (2006) Use of the inhibitory effect of apoptotic cells on dendritic cells for graft survival via T-cell deletion and regulatory T cells. *Am J Transplant* 6: 1297-1311.
91. Wang Z, Shufesky WJ, Montecalvo A, Divito SJ, Larregina AT, et al. (2009) In situ-targeting of dendritic cells with donor-derived apoptotic cells restrains indirect allorecognition and ameliorates allograft vasculopathy. *PLoS One* 4: e4940.
92. Ankersmit HJ, Hoetzenecker K, Dietl W, Soleiman A, Horvat R, et al. (2009) Irradiated cultured apoptotic peripheral blood mononuclear cells regenerate infarcted myocardium. *Eur J Clin Invest* 39: 445-456.
93. Lichtenauer M, Mildner M, Baumgartner A, Hasun M, Werba G, et al. (2011) Intravenous and intramyocardial injection of apoptotic white blood cell suspensions prevents ventricular remodelling by increasing elastin expression in cardiac scar tissue after myocardial infarction. *Basic Res Cardiol* 106: 645-655.
94. Vignali DA, Collison LW, Workman CJ (2008) How regulatory T cells work. *Nat Rev Immunol* 8: 523-32.
95. Chong WP, Zhou J, Law HK, Tu W, Lau YL (2010) Natural killer cells become tolerogenic after interaction with apoptotic cells. *Eur J Immunol* 40: 1718-27.

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