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

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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 graft-versus-host disease (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.

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

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...
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 allogenetic 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 phagocytosis of 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 administered 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
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 [63]. No significant toxicity has been reported in 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 involved in the immunomodulatory effects of apoptotic cells. 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 involved in the immunomodulatory effects of apoptotic cells. The authors declare no conflicts of interest.

References


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Conflict of Interest

The authors declare no conflicts of interest.


70. http://clinicaltrials.gov/


