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Extracorporeal photopheresis-induced immune tolerance: a focus on modulation of antigen-presenting cells and induction of regulatory T cells by apoptotic cells

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Abstract

Purpose of review—This review is intended to introduce recent advances in the research surrounding extracorporeal photopheresis (ECP) with a focus on how apoptotic cells modulate antigen-presenting cells and induce regulatory T cells, given that ECP therapy induces apoptosis of leukocytes collected through leukapheresis.

Recent findings—It has been suggested that ECP therapy, unlike other immunosuppressive regimens, does not cause global immunosuppression, but induces immune tolerance. Recent clinical and animal studies demonstrate that ECP therapy induces antigen-specific regulatory T cells, including CD4⁺CD25⁺FoxP3⁺ T cells and IL-10-producing Tr1 cells, that may arise secondarily to the induction of tolerogenic antigen-presenting cells (APCs) by infusion of apoptotic cells. It has also been suggested that ECP therapy may induce IL-10-producing regulatory B cells and regulatory CD8⁺ T cells. Finally, several recent studies, which examined the cellular elements involved in the uptake of apoptotic cells, demonstrated that apoptotic cells modulate APCs through binding to specific receptors, particularly TAM receptors that provide inhibitory signals that block APC activation.

Summary—ECP therapy induces immune tolerance through modulation of antigen-presenting cells as well as induction of regulatory T cells. ECP therapy has great potential in the management of allogeneic transplantation and autoimmune diseases.

Keywords

apoptosis; extracorporeal photopheresis; immune tolerance; regulatory T cell

Introduction

Extracorporeal photopheresis (ECP) is a therapeutic regimen approved for the treatment of the palliative skin manifestations associated with cutaneous T cell lymphoma [1]; however, ECP has proven effective across a variety of clinical indications including solid organ transplantation [2] and hematopoietic stem cell transplantation [2,3], as well as for autoimmune diseases [2]. ECP is an effective approach in steroid-resistant allogeneic transplantation, and induces glucocorticoid or immunosuppressant sparing effects [4]. Because of its efficacy and

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the low incidence of adverse effects [2–4], ECP is highly recommended for preventing graft rejection in heart transplantation [2] and grade I and II acute graft-versus-host disease (GvHD) [2,3]. ECP is also effective in treating chronic GvHD [4]. The mechanisms of action of ECP therapy are uncertain, but accumulating evidence suggests that the immunoregulatory properties of ECP therapy include modulation of antigen-presenting cells (APCs) and induction of regulatory T cells [3,5].

Extracorporeal photopheresis procedure

ECP is a clinical procedure approved by the Food and Drug Administration in the USA, and has been widely used in Europe and North America for a variety of clinical conditions. The device is manufactured by Therakos, Inc. (Raritan, Pennsylvania, USA). The most commonly used device is the second generation, UVAR XTS photopheresis system. During ECP, patient blood is drawn from peripheral venous access into a closed loop system. The patient's blood is separated via centrifugation. Red blood cells are immediately returned to the patient, whereas white blood cells are collected separately into a collection bag. The white blood cells are then mixed outside the body with psoralen (UVADEX), which quickly penetrates the plasma and nuclear membranes and covalently binds to DNA and possibly some proteins (e.g. methoxysalen). The UVADEX-treated cells are exposed to UVA irradiation, which activates the UVA-DEX to crosslink the DNA and induce apoptosis. It has been shown that cells undergo programmed cell death within 24–48 h post-ECP [5]. Normally, the apoptotic cells are processed in a quiescent way by APCs including dendritic cells and macrophages and subsequently modulate immune responses [6].

Extracorporeal photopheresis-induced apoptotic cells modulate antigen-presenting cells

Five to 10% of circulating mononuclear cells are collected then exposed to UVADEX and UVA during an ECP procedure [3]. As mentioned earlier, ECP therapy induces apoptosis of white blood cells collected through leukapheresis. An animal study showed that the majority of the injected ECP-treated cells were localized to the liver and spleen where dendritic cells and macrophages have access to them [7]. The authors of this study discovered that intravenously injected ECP-treated spleen cells were distributed in the marginal zones of spleen where there are abundant APCs (dendritic cells and macrophages) [7]. Although the mechanisms that direct these dying cells to the marginal zone are unclear, this location would offer ample opportunities for APCs that have taken up apoptotic cells to interact with T and B lymphocytes. Given that apoptotic cells modulate APCs in a manner that would promote tolerance, for example, increased IL-10 production, the marginal zone may be the central anatomical location for tolerance induction elicited by these dying cells. Clinical studies also demonstrated that APCs from ECP-treated patients are phenotypically and functionally modulated with tolerogenic characteristics [8–11].

In contrast to live cells, apoptotic cells display a unique feature with phosphatidylserine flipping from the inner leaflet to the outer leaflet of the plasma membrane. Although it is still uncertain whether apoptotic cells silence or activate APCs [12–14], the consensus in the field is that steady-state apoptotic cells silence APCs and render them tolerogenic [6,12,15]. It has been shown that dendritic cells in the process of phagocytosing apoptotic cells downregulate expression of costimulatory molecules such as CD40, CD80 and CD86 [8,11,16]. Intriguingly, those dendritic cells produce heightened levels of the anti-inflammatory cytokine IL-10 [8], a characteristic of tolerogenic dendritic cells. Apoptotic cells can also trigger APCs to secrete TGF- β [17], another anti-inflammatory cytokine that was recently documented to be a critical factor for differentiation of adaptive regulatory T cells in the periphery [18]. In addition to promoting IL-10 and TGF- β , apoptotic cells may downregulate proinflammatory factors. Kim

et al. [19] reported that apoptotic cells suppressed IL-12 production by macrophages through cell–cell contact. They found that the signal for IL-12 suppression induced by apoptotic cells was through the interaction between phosphatidylserine on apoptotic cells and its receptors on macrophages, because blockade of this interaction abrogated the apoptotic cell-induced IL-12 suppression. This suppression was not ascribed to autocrine and paracrine IL-10 and TGF- β [19].

Many receptors on APCs have been identified with the capacity to bind apoptotic cells, of which TAM receptors have drawn much attention because of their important roles in inhibiting inflammation and promoting phagocytosis of dendritic cells and macrophages [20]. TAM receptors, expressed on dendritic cell and macrophages, include Tyro-3, Axl and Mer, which are members of a distinct receptor protein tyrosine kinase subfamily [21,22]. A recent report by Sen *et al.* [23] demonstrated that apoptotic cells suppressed dendritic cell NF- κ B activation induced by LPS stimulation through activation of the Mer tyrosine kinase pathway. They found that blockade of Mer tyrosine kinase abolished apoptotic cell-induced NF- κ B suppression and led to enhanced TNF- α production in response to LPS challenge. TAM receptor signaling has also been found to suppress TLR-induced production of proinflammatory cytokines including TNF, IL-6, IL-12 and type 1 interferons (IFNs) through inducing the suppressor of cytokine signaling (SOCS) proteins SOCS1 and SOCS3 [24].

Programmed cell death is a physiological process and the apoptotic cells need to be quickly cleared up to maintain homeostasis, otherwise late dying cells in the apoptotic process may activate the immune system, potentially causing immune responses to self, for example, autoimmune diseases. Indeed, recent studies suggest that deficient clearance of apoptotic cells is central to the pathogenesis of systemic lupus erythematosus (SLE) [25–27]. When the cells are undergoing apoptosis, they release some metabolic materials, such as lysophosphatidylcholine (LPC), which has a potent chemotactic effect on APCs [28]. Phagocytes can interact with apoptotic cells through direct receptor binding, or through soluble ‘bridging’ molecules, such as mobile fat globulin (MFG)-E8, plasma protein S and growth-arrest-specific 6 (GAS6) [12,15,20]. Recently, two groups independently reported another important phosphatidylserine receptor on APCs, TIM4 (T cell immunoglobulin mucin 4) playing a critical role in mediating phagocytosis of apoptotic cells [29,30]. Blocking TIM4 *in vivo* using anti-TIM4 antibodies diminishes the clearance of apoptotic cells and consequently causes autoantibody development [30].

One of the suggested important differences between apoptosis and necrosis is that apoptotic cells do not release the potent proinflammatory molecule, high mobility group box protein 1 (HMGB1) whereas necrotic cells do [31^{**},32–34]. However, recent studies contradict this finding and suggest that apoptotic cells also release HMGB1 [31^{**},35,36]. However, it appears that activation of caspases on mitochondria in cells undergoing apoptosis leads to production of reactive oxygen species (ROS), which subsequently oxidize HMGB1 such that it loses its inflammatory effect [31^{**}]. Thus, the apoptotic cells, in contrast to necrotic cells, will not be able to activate APCs.

As mentioned earlier, apoptotic cells modify APC in such a manner that they would be likely to induce tolerance. However, some caution needs to be taken as apoptotic cell therapy has the potential to induce inflammation in settings where apoptotic cell numbers outstrip the capacity of APC to ingest them, potentially leading to secondary cell necrosis, exposure to active forms of HMGB1 and other inflammatory factors, such as heat shock proteins [37]. Recently, it was demonstrated that HMGB1 significantly blocked the phagocytosis of apoptotic cells by macrophages [38^{*}]. Another recent report illustrated that late apoptotic cells released HMGB1-nucleosome complexes, which were easily detected in the plasma of SLE patients because of the impaired clearance of apoptotic cells. These HMGB1–nucleosome complexes trigger

dendritic cells to secrete the proinflammatory cytokines IL-1 β , IL-6 and TNF- α and upregulate costimulatory molecules on macrophages through binding to Toll-like receptor (TLR)-2 [39] thus activating APCs that would probably induce immune responses. Nevertheless, circumstances that lead to situations in which large numbers of cells undergo death rarely lead to clinical autoimmune disease, for example, ischemia reperfusion injury. Furthermore, there are no reports that ECP therapy induces autoimmune disorders [2,8]. Our unpublished data also showed that apoptotic cell injection did not induce autoantibodies against DNA and histone in a murine model of type 1 diabetes. In addition, clinical findings further support that ECP therapy does not induce inflammatory responses, but suppresses dendritic cell maturation and proinflammatory cytokine production [2,8,10,40]. However, it remains to be definitively shown if APCs exposed to apoptotic cells directly participate in tolerance induction *in vivo*, which can be addressed by adoptive transfer of apoptotic cell-experienced dendritic cells to syngeneic naive mice to evaluate whether the form of tolerance is transferable.

Additionally, Gray, *et al.* [41] reported recently that apoptotic cell treatment significantly protected mice from collagen-induced autoimmune arthritis through the generation of CD19⁺ regulatory B cells. These regulatory B cells secrete high levels of IL-10, significantly suppress effector T cells and induce IL-10-producing regulatory T cells.

Extracorporeal photopheresis therapy-induced apoptotic cells promote regulatory T cell differentiation

Regulatory T cells are regarded as key players in the maintenance of immune tolerance [18]. There are different subsets of regulatory T cells including CD4⁺CD25⁺FoxP3⁺ regulatory T cells, IL-10-producing Tr1 cells and TGF- β -producing Th3 cells. Approaches that induce regulatory T cells are thought to be beneficial to the generation of allogeneic tolerance in organ transplantation [42,43]. ECP therapy differs from other immunosuppressive agents in that it uses apoptotic leukocytes to suppress alloantigen-responding T cells, and to induce immune tolerance to allogeneic grafts, whereas immunosuppressive agents globally suppress the immune system [2,3].

We believe that regulatory T cells result from the intravenous administration of apoptotic white blood cells generated through ECP therapy. It has been demonstrated that APCs that have phagocytosed apoptotic cells secrete TGF- β [17], an essential cytokine for differentiation of adaptive CD4⁺CD25⁺FoxP3⁺ regulatory T cells from naive CD4⁺ T cell population [18]. Indeed, studies show that intravenous infusion of apoptotic cells induces TGF- β -dependent regulatory T cell expansion [44]. For a few decades, anti-CD3 therapy has been commonly utilized to generate clinical immunosuppression in transplantation and autoimmune diseases. It was initially thought that anti-CD3 therapy worked by simply depleting T cells through antibody-dependent complement-mediated cytotoxicity. However, recent studies demonstrated that anti-CD3 therapy induced immune tolerance by promoting differentiation of regulatory T cells [45,46,47^{**}]. Perruche *et al.* [47^{**}] provided further evidence that anti-CD3 therapy induced generation of regulatory T cells and that its effects were associated with TGF- β secreted by APCs that were involved in the clearance of apoptotic T cells resulting from the action of the anti-CD3 antibodies. Similarly, it has been speculated that the effect of ECP therapy involves tolerance induction facilitated by physiological, steady-state processes that normally handle apoptotic cell removal by creating a tolerogenic microenvironment.

Increasing in-vivo evidence shows that ECP therapy induces regulatory T cells. Maeda *et al.* [48] reported that an antigen challenge along with infusion of ECP-treated syngeneic splenocytes successfully induced antigen-specific immune tolerance. This immune tolerance could be transferred to syngeneic naive mice by adoptive transfer of splenocytes from treated mice, suggesting a cell-mediated effect, most likely involving regulatory T cells. Further

investigation showed that CD4⁺ CD25⁺ T cells were involved in tolerance as the transfer of splenocytes depleted of either CD4⁺ T cells or CD25⁺ T cells was unable to confer tolerance. Recent data presented by Gatza, *et al.* [49**] showed that ECP therapy was not only able to prevent but also reverse GvHD through induction of CD4⁺CD25⁺ regulatory T cells of donor origin in an allogeneic bone marrow transplantation mouse model. Functionally, these regulatory T cells were able to efficiently suppress CD8⁺ T cells in IFN- γ production. This study provides a mechanistic basis for the clinical findings that ECP therapy ameliorates acute and chronic GvHD in patients who are receiving allogeneic hematopoietic stem cell transplantation (allo-HST) [2,3]. Indeed, in a clinical study, Biagi *et al.* [50] reported that a positive response to ECP therapy in allo-HST patients was accompanied by a significant increase in CD4⁺CD25⁺ regulatory T cells from 8.9 to 29.1% of total CD4⁺ T cells after six treatments. These induced regulatory T cells expressed high levels of CD62L, CD45RO and FoxP3 and displayed cell–cell contact-dependent immune suppression against effector T cells with an approximate 80% reduction in IFN- γ production. In addition, the increases of CD4⁺ CD25⁺ regulatory T cells following ECP therapy were also noted in other allogeneic transplantation settings, such as lung [51] and kidney [52] transplantation. These studies are very encouraging. However, as most published studies examine the number of regulatory T cells, additional studies are needed to demonstrate that these putative regulatory T cells are functional and that their numbers and functions persist. These studies are likely to provide guidance for refinement of ECP therapy with regard to monitoring the potential efficacy of the therapy and suggesting when additional treatments may be required.

Another subset of regulatory T cell is the IL-10-producing Tr1 cell population that plays an important role in tolerance induction [53]. Indeed, our recent studies showed that infusion of apoptotic cells induced by UVB-irradiation significantly induced IL-10-producing regulatory T cells [54,55], which are suggested to be critical in apoptotic cell-induced protection from type 1 diabetes in nonobese diabetic mice in which Th1 cells are pathogenic. Recently, Maeda *et al.* [48,56] discovered that ECP-induced immune tolerance was also associated with IL-10-producing regulatory T cells as neutralization of IL-10 by anti-IL-10 antibodies during tolerance induction by ECP diminished immune tolerance. A further study by the same group demonstrated that IL-10 produced by ECP-induced regulatory T cells played an important role in the suppression of antigen-specific responses during the effector phase of T cell activation [57**]. From these studies, it appears that IL-10-producing Tr1 cells significantly contribute to ECP-induced immune tolerance.

In addition to inducing CD4⁺ regulatory T cell subsets, apoptotic cells may also affect other T cell populations. Recently, Griffith *et al.* [58] reported that injection of apoptotic cells induced antigen-specific immune tolerance through the generation of immunosuppressive regulatory CD8⁺ T cells. These CD8⁺ T cells suppressed the immune response by upregulating TNF-related apoptosis-inducing ligand (TRAIL) and adoptive transfer of the CD8⁺ T cells from the treated animals transferred antigen-specific tolerance. It is not clear if these apoptotic cell-induced regulatory CD8⁺ T cells are the Ts cells described a few decades ago [59–63]. Nonetheless, additional studies are needed to further characterize CD8⁺ T cell responses to ECP therapy.

Conclusion

As illustrated in Fig. 1, ECP therapy induces apoptosis of leukocytes. The apoptotic leukocytes are recognized by APCs (dendritic cells and macrophages) through interaction with specific receptors, especially TAM receptors that promote inhibitory signals that suppress anti-inflammatory cytokine production through blocking NF- κ B activation and promoting the production of anti-inflammatory factors, for example IL-10 and TGF- β . The latter cytokines produced by APCs contribute to the induction of regulatory T cells. APCs interacting with

apoptotic cells also downregulate expression of costimulatory molecules and resist maturation that may additionally induce energy. Finally, ECP therapy may also induce regulatory B cells and/or regulatory CD8⁺ T cells that further reinforce the regulatory T cell network. Given that ECP has shown great promise as a means of modulating immune function in allogeneic transplantation and autoimmune disease, advances of our basic understanding of the mechanism of action of this therapy may allow further refinements of this exciting approach to immune modulation. Furthermore, additional studies need to be undertaken to examine ECP in combination with other immune modulating therapies, for example, based on allo-, autoantigen administration. In the end, through ongoing research we may come to understand that death is indeed a key to life and the maintenance of immune system health.

Acknowledgments

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 449–450).

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Figure 1. ECP therapy modulates APCs and induces regulatory T cells through apoptotic cells
Leukocytes undergo apoptosis after ECP treatment and the apoptotic leukocytes phagocytosed by APCs, including macrophages and dendritic cells, lead to downregulation of costimulatory molecules and proinflammatory cytokines and upregulation of anti-inflammatory cytokines by APCs to create a tolerogenic environment that facilitates the generation of regulatory T cells.