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Potential Mechanisms of Photopheresis in Hematopoietic Stem Cell Transplantation

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ABSTRACT

Immune tolerance **describes specific unresponsiveness to antigens. In clinical situations such as graft-versus-host disease it may be useful to capitalize on these pre-existing tolerance mechanisms to treat patients. Extracorporeal photopheresis is a pheresis treatment whereby the approximately 5×10^9 leukocytes are treated with a photoactivatable compound (8-methoxypsoralen) and UVA light, and immediately returned to the patient in a closed-loop, patient-connected system. This therapy induces apoptosis of virtually all the treated leukocytes. There is growing evidence that infusion of apoptotic cells may trigger certain tolerance mechanisms and, thus, be of therapeutic use in graft-versus-host disease. These apoptotic cells are taken up by phagocytes (antigen-presenting cells) in the body of the patient. Apoptotic cell engagement has been reported to induce several changes and functional activities in the engulfing antigen-presenting cell. These antigen-presenting cells: (1) decrease production of proinflammatory cytokines; (2) increase production of anti-inflammatory cytokines; (3) lower ability to stimulate T-cell responses; (4) delete CD8 T effector cells; and (5) induce regulatory T cells. Any and all of these mechanisms could explain the noted effect in graft-versus-host disease. It is still unclear which one or ones are truly responsible. Ongoing studies in animals and human trials will ultimately unravel these details.**

KEY WORDS

Photopheresis • Graft versus host disease • Regulatory T cells • Immune tolerance

INTRODUCTION

The immune system is a collection of organs, cells, and molecules that work in concert to protect higher-order animals from a wide diversity of pathogens such as bacteria, viruses, and parasites. A major component in the initiation of immune responses is the requirement for "danger" signals derived from injured tissue and pathogens [1]. Ways of minimizing nonfunctional or autoreactive activities also evolved to prevent destruction of healthy tissue by immune overreaction. Central mechanisms of tolerance, which occur in the bone marrow and thymus, lead to deletion of autoreactive B and T cells whereas peripheral mechanisms of tolerance can lead to T-cell skewing (e.g. T-helper 1, T-helper 2), suppression (e.g. anti-inflammatory cytokines, regulatory cells), and peripheral deletion (e.g. activation-induced cell death, veto cells). In the majority of cases these tolerance mechanisms help maintain balance between sufficient immunity to fight pathogens and overreaction to self (Figure 1). However, these mechanisms of tolerance are usually not sufficient to overcome complications caused by allogeneic hematopoietic stem cell transplantation.

Graft-versus-host disease (GVHD) is a serious complication of hematopoietic stem cell transplantation leading to significant morbidity and mortality. Current treatment (e.g. steroids, immunosuppressants, T-cell depletion) is based primarily on inhibition of the effector arm of the immune system responds believed to be responsible for the development of GVHD. Although these therapies have decreased the incidence and severity of GVHD, toxic side effects have limited their use. To expand the use of hematopoietic stem cell transplantation, less toxic and more effective therapies for GVHD need to be developed. Recent reports of using tolerance-boosting mechanisms such as adding regulatory T cells or tolerogenic antigen-presenting cells (APCs) have produced promising results in animal models [2-5]. This brief review will discuss one such tolerance-inducing therapy: the

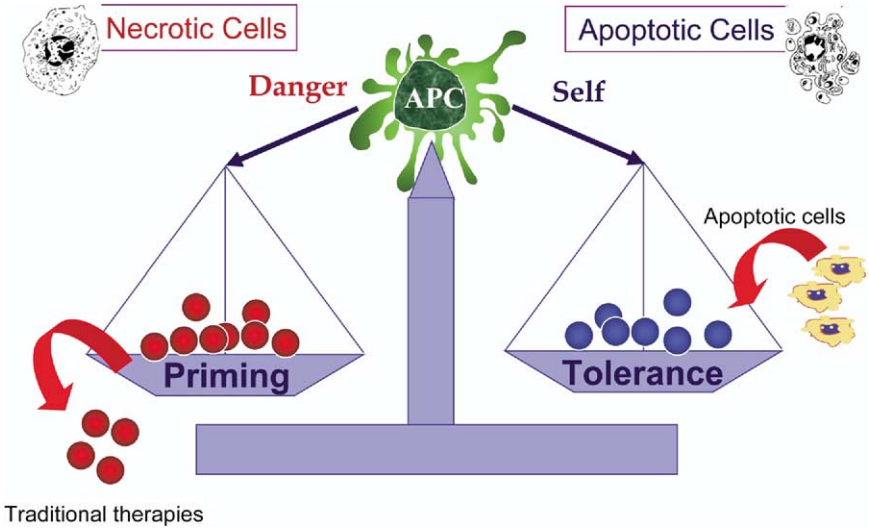

Figure 1. The immune system is a careful balance between priming effector function to fight pathogens and tolerance to inhibit overzealous immune responses. Traditional therapies act primarily via inhibition of the effector arm of the immune response. Therapies such as ECP may add clinical value by targeting the tolerance side of the immune balance, offering a novel way to treat disease and possibly synergize with traditional therapies.

infusion of apoptotic cells (ACs) generated by extracorporeal photopheresis (ECP).

EXTRACORPOREAL PHOTOPHERESIS

Extracorporeal photopheresis has been used clinically for almost 20 years as an approved therapy for the palliative treatment of cutaneous T-cell lymphoma (CTCL). ECP instruments reside in many major academic institutions in Europe and North America. This therapy is an apheresis-based process whereby approximately 5×10^9 autologous leukocytes are treated with a photoactivatible compound, 8-methoxypsoralen, followed by exposure to ~1.5 J/cm^2 of Ultraviolet A light and reinfused. This occurs in a point-of-care, patient-connected, sterile, closed-loop system. As a result of ECPs demonstrated efficacy and safety profile in CTCL, physicians applied ECP treatment to a wide variety of diseases that respond to immunosuppression [6] including GVHD [7-9]. The history of ECPs development for CTCL is well documented [10]. During the last two decades several mechanisms have been proposed as the mode of action of ECP. Unfortunately, none are completely satisfying from a scientific standpoint, especially in the absence of convincing data. From the beginning it was clear that the therapeutic effect of ECP could not be explained solely by destruction of malignant cells, because only a small proportion of the circulating pathogenic T cells are treated during an ECP treatment cycle. This led to the hypothesis that a systemic and specific immune-mediated antitumor activity may be involved in the clinical activity of ECP [11]. The possibility that ECP induces a generalized immunosuppression is unlikely because patients with CTCL undergoing long-term ECP therapy have not demonstrated a higher risk of developing infections or malignancies [12] and respond normally to both novel and recall antigens [13]. This review summarizes the recent work concerning the mechanism of action of AC infusion.

IMMUNE REGULATION BY ECP: AC CLEARANCE

ECP induces the cell death of most of the treated leukocytes within 24 to 48 hours [14-16]. What is the consequence of infusing patients with a bolus of ACs?

It has been known since the time of Metchnicoff that cellular debris is removed from complex organisms by phagocytes. Many cell types in the body can remove apoptotic and cellular debris from tissues; however, the professional phagocyte, or APC, has a higher capacity to do so. The recognition of ACs occurs by a series of evolutionarily conserved, AC-associated molecular-pattern receptors on APCs that recognize and bind corresponding AC-associated molecular patterns found on ACs. These receptors recognize ligands such as phosphotidyl serine and oxidized lipids found on ACs. The full description of the receptor systems involved in AC clearance is beyond the scope of this article and has been reviewed in detail [17,18]. The vast majority of ECP-treated cells are retained in the spleen and liver after infusion where they are presumably engulfed by APCs [14,19].

Multiple laboratories using a variety of methods and models both in vitro and in vivo have now reported that AC clearance by APCs regulates immune responses [17]. This immune modulation appears to

occur primarily by an alteration of APC function with several hallmarks of a tolerance-inducing APC. There is confusion about the nature and phenotype of tolerogenic APCs; however, functional characteristics in a variety of systems imply their existence, even if it is transient [20,21]. Clearance of ACs in a noninflammatory situation may be an important natural reminder of self and an ongoing peripheral tolerance mechanism [22,23]. APCs in the periphery take up ACs and transfer them to draining lymph nodes. These tolerogenic APCs do not appropriately stimulate T effector cells and may induce tolerance using several different mechanisms outlined below. Only in the presence of a stimulatory milieu from the tissue will the phagocyte be altered to become immunogenic and stimulate T cells to fight the pathogens. Although signals [1] that activate immune responses have been well defined during the last decade, it has been assumed that the absence of such signals was simply a default leading to no immune response. It has recently been suggested that ACs from normal tissues are one mechanism of active tolerance induction. The cells are taken up by APCs and deliver autoantigens in an anti-inflammatory manner, ensuring self-tolerance [23]. Dendritic cells (DCs) may play an important role in this tolerance as they have all the appropriate receptors, and they are the only APC known to present AC peptides by both HLA antigen class I and class II. The presentation by HLA antigen class I is termed cross-presentation because it "crosses" the classically defined endogenous class I and exogenous class II compartments [24,25]. In a noninflammatory context this presentation by APCs of self-antigens on HLA antigen class I to CD8 T^+ cells is a mechanism to directly tolerize the $CD8^+$ T-cell compartment [26] and has been demonstrated in apoptotic tolerance [27]. The $CD8^+$ T-cell tolerance mechanism may be particularly relevant in GVHD, although other tolerance mechanisms, such as the generation of $CD4^+$ regulatory cells, could also lead to decrease of CD8 responses by indirect mechanisms.

To date, there are 5 mechanisms reported for the effect of ACs on the APCs responsible for their clearance. Upon engagement of ACs, APCs will: (1) produce lower levels of proinflammatory cytokines secretion; (2) secrete anti-inflammatory cytokines; (3) have a diminished effector T-cell stimulation capability; (4) possibly induce accelerated death of effector cells; and (5) stimulate regulatory T-cell generation. These 5 mechanisms (Table 1) may all, theoretically, be relevant for the mechanism of action of ECP in GVHD. Studies are now underway for animals and patients to determine which of these mechanisms may be playing a more relevant clinical role in GVHD.

Table 1. *Potential Mechanisms of Apoptotic Cell Induction of Tolerance in Graft-versus-Host Disease*

1. Inhibition of proinflammatory cytokines
2. Anti-inflammatory cytokine production
3. Decreased stimulation of effector T cells
4. Deletion of effector T cells
5. Stimulation of regulatory cells

Inhibition of Proinflammatory Cytokines

The most well-studied phenomenon in the area of AC-mediated tolerance is the modulation of APC cytokine production in vitro. The decrease in proinflammatory cytokines such as interleukin (IL)-1, IL-6, tumor necrosis factor α, and IL-12 is well documented in several different APC cell types in both mice and human beings [28,29]. Neither the maturation state of the DC [26] nor the type of AC [30-32] appear to matter. Interestingly, the most dramatic decrease has been reported for IL-12 [19,33]. Recently, Kim et al. [34] described a novel zinc finger nuclear factor, named GC binding protein, responsible for the down-regulation of the p35 subunit of IL-12 after incubation with phosphotidyl serine liposomes. The central role of IL-12 in the induction of immune responses is becoming increasingly apparent, and its absence during T-cell stimulation by APCs may lead to regulatory T cells [35-38].

Anti-Inflammatory Cytokine Production

In addition to the suppression of proinflammatory cytokines, there is a well-described phenomenon of increased anti-inflammatory cytokines secretion including transforming growth factor β [29,39] and IL-10 [40]. These cytokines may be important in decreasing immune responses at inflammatory sites. At appropriate concentrations in secondary lymph node organs, these cytokines inhibit stimulation of effector T cells and drive regulatory T-cell generation [41-43].

Decreased Stimulation of Effector T Cells

Engagement of ACs by APCs decreases their ability to stimulate a functional effector immune response [44]. There are several reported reasons for this decrease in stimulatory activity. The APCs have decreased levels of costimulatory surface molecules, such as CD86, along with decreased levels of secondary cytokine signals such as IL-12 [33]. In addition, ACs can inhibit maturation of DCs by CD36 ligation [45], and it has been suggested that immature DCs may not be as efficient as mature DCs at stimulating an immune response [46]. Finally, in the draining lymph node, which harbors ACs, the local milieu of anti-inflammatory molecules such as transforming growth factor β, IL-10, prostaglandin E_2, and platelet-activating factor may all act in a paracrine fashion to diminish effector T-cell stimulation [29].

Deletion of Effector T Cells

There are reports that delivery of ACs in vivo leads to tolerance by deletion of the effector T cells. The exact mechanism of this deletion is still not clear. Steinman et al. [22] suggested that the tolerogenic APCs improperly stimulate the effector T cells so that they divide several times and then undergo an activation-induced cell death. Herndon et al. [47] recently published results using a hapten-based model in which they found induction of anti-idiotypic cytotoxic lymphocytes that killed effector T cells in a manner reminiscent of veto cells.

Stimulation of Regulatory T Cells

Regulatory T cells comprise a heterogeneous group of T lymphocytes, which actively inhibit immune responses [43,48,49]. They have been recognized to play an important role in GVHD [5,50]. Thus, there is great excitement about the potential to develop regulatory T cell–based therapies.

One way to generate regulatory T cells in vivo is by the infusion of ACs. There is evidence from both animal models and human treatments that AC infusion, such as during ECP, induces regulatory T cells [14,16,51-53]. Maeda et al. [14] found that the immune modulation of ECP is mediated by regulatory cells in a murine model of contact hypersensitivity. Deletion of either CD4 or CD25 from the transferred population led to a loss of transferred protection suggesting the cells with protective activity express CD4 and CD25. They also showed that the tolerance was antigen-specific, suggesting T-cell involvement. Kleinclauss et al. [54] has recently reported in an animal model of GVHD that the induction of regulatory T cells by AC infusion is through the generation of transforming growth factor β by macrophages in the spleen of these animals. In human beings, Lamioni et al. [16] showed that an up-regulation of $CD4^+CD25^+$ T cells follows ECP treatment in solid organ transplantation patients.

In recently published AC trafficking experiments, we were unable to find ECP-treated cells in the ears of hapten-sensitized animals [14]. We presume that the mode of action is in the secondary lymphoid organs such as the spleen. We surmise that, as with effector responses, antigen and APCs draining from the inflammatory site meet with T and B cells in secondary lymph node organs. In the presence of ACs, an anti-inflammatory environment is created, limiting the stimulation of effector T cells and possibly generating regulatory T cells that may traffic to the site of inflammation. Indeed, in the contact hypersensitivity model others have shown elevated levels of IL-10 message, presumably derived from infiltrating regulatory cells, after UVB treatment [55].

ECP IN GVHD

Most of the mechanistic studies cited above are in vitro or in a variety of different animal models and diseases. It is not known which of the immune-modulating mechanisms are most relevant to GVHD. Ongoing murine studies and clinical substudies in the context of controlled clinical trials will hopefully address this issue.

Many commercially available drugs control immune responses by suppression of the effector arm of the immune system. However, their toxicity, the profound immunosuppression that they cause, and their limited efficacy call for more effective therapies. Tolerogenic mechanisms of controlling disease are novel and hold great promise. Even more exciting is the possibility of combining tolerance and effector suppression therapies. Theoretically, combinations therapies may act in concert or even synergistically, to significantly improve clinical outcomes by increasing the effectiveness of therapy and by allowing a reduction in doses of toxic drugs. Ongoing murine and human substudies will hopefully shed light on both the relevant mechanisms of immune tolerance in GVHD and mechanism of action of ECP. This will in turn increase the effective use of ECP, and hopefully provide us with another therapeutic strategy to control GVHD.

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