Extracorporeal Photopheresis: A Case of Immunotherapy Ahead of Its Time

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Abstract

Extracorporeal photopheresis (ECP) is a cell-based immunotherapy that involves the reinfusion of autologous leukocytes after exposure to psoralen and UVA. The treatment has been used for over 30 years, at first on patients with cutaneous T-cell lymphoma (CTCL) and later for the management of patients with graft-versus-host disease (GvHD), sclerosing disorders, atopic dermatitis, and other diseases that may share the common driving factor of a pathogenic T-cell clone or clones in blood circulation. Patients with clinical improvement mount an antigen-specific immune response that may have tolerance traits in the case of GvHD or anticlonal cytotoxic characteristics in the case of CTCL. The exact mechanisms that dictate one response or the other are not fully understood, but the evidence accumulated so far indicates that multiple events occur simultaneously and consequentially contribute to the end result. These include contact of cells with the outside (plastics and tubing of the ECP apparatus), exposure to psoralen and UVA that activates platelets, monocytes, and other myeloid cells, the release of damage-associated molecular patterns, differentiation of monocytes into dendritic cells, and generation and successive presentation of numerous antigens after the phagocytosis of apoptotic cells. Once reintroduced, the ECP product increases the frequency and activity of regulatory T cells (Tregs), shifts the systemic cytokine balance, and promotes extravasation of immune cells that together shape the effects of this treatment. In this review, we summarize the seminal work and most recent literature of the therapeutic mechanisms and reflect on future avenues of improvements and applications of ECP.

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Background

In the first few lines of the 1987 seminal paper on extracorporeal photopheresis (ECP), Edelson et al. [1] laid out the principle of their invention. After reflecting on the work of the group of Irun Cohen, who by those days had reported that the infusion of lethally damaged autoreactive T-cell clones triggered a mechanism of resistance to subsequent elicitation of experimental autoimmune disease [2]. They realized that the work carried out by the group of Cohen demonstrated that the pathogenic activity of an aberrant population of T cells can be counteracted by an anti-clone-specific immune response and hy-

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pothesized that a similar phenomenon may take place in humans [2]. Naturally, their impulse was to test their hypothesis in a clinical setup [1]. At that time, psoralen plus UVA (PUVA) was already widely used in the management of psoriasis and cutaneous T-cell lymphoma (CTCL) patients. It offered a way to induce extensive cellular damage with UVA irradiation and primarily target cells that have incorporated 8-methoxypsoralen (8-MOP) while sparing those not exposed to the drug. 8-MOP is a naturally occurring inert compound that, after irradiation with UVA, becomes a highly reactive molecule that binds covalently DNA, cell membranes, and proteins that together lead to cell death and other cytotoxic effects. Despite it not being formally proven, they suspected that PUVA contributed to the generation of numerous new antigens that primed a protective T-cell response in a mechanism with the characteristics envisioned by the group of Cohen [2]. Not until nearly a decade later did the group of Edelson report that B-lymphoblastoid cells increase the expression of class I MHC up to 3-fold as a result of the degradation of cytoplasmic proteins upon exposure to photoactivated 8-MOP [3].

The first ECP trial [1] was a prospective study with Sézary syndrome patients, a variant of CTCL in which malignant cells not only infiltrate the skin but also circulate in the blood stream. An apheresis device with a circuitry of compartments for the exposure of leukocytes to UVA was used in 41 patients. Thirty-seven patients completed the study, 27 of whom presented clinical improvement, with an average reduction of 64% in skin score severity and a mean time to response of 22 weeks. Six patients failed to respond and 4 patients had no change and continued treatment. Twenty-eight patients participating in the study had a previous failed response to systemic chemotherapy, but 20 of them responded to ECP. At the end of the study, exfoliative erythroderma and focal hyperkeratosis improved significantly or were cleared [1]. In the following years, the evidence on the efficacy of ECP in Sézary syndrome was expanded.

At first a report on the case of a 43-year-old woman suffering graft-versus-host disease (GvHD) after allogenic bone marrow transplantation for chronic myeloid leukemia showed that, despite rapid deterioration of joints and muscles and liver damage, ECP was a viable option for successful treatment [4]. Soon after, a large prospective multicenter study with 95 chronic GvHD patients receiving standard steroid therapy alone ($n = 47$) or together with ECP ($n = 48$) was conducted. It revealed that, after 12 weeks of treatment, the skin score of patients on ECP had a median improvement of 14.5 versus 8.5% in the steroid only group and the ECP-treated patients experienced a substantial reduction in the requirement of corticosteroids [5]. Over the years, ECP has been introduced as new therapeutic option for a large number of other conditions, including sclerosing disorders [6–8], atopic dermatitis [9], and erythroderma of other origin [10, 11], and investigated as a preventive measure to reduce risk of heart [12], lung [13], and kidney [14] transplant rejection.

The therapeutic mechanisms triggered by ECP involve immunostimulatory, immunosuppressive, and immunotolerizing effects. These effects may at least to some extent depend on the type of disease (as is the case for conventional photo[chemo]therapy) [15, 16] and in one way or another contribute to the clinical improvement experienced by patients. We herein review the literature on the immunomodulatory effects of ECP and speculate about the future and innovations to come with this phototherapeutic modality.

Apoptotic Fate of ECP-Exposed Cells

Nearly 10% of total blood circulating mononuclear cells are drawn and exposed to 8-MOP and UVA during a regular ECP procedure [17]. Since then, in vitro studies have shown that treated cells follow different fates, e.g., immediate apoptosis of up to 15% with a flip-flop of phosphatidylserine to the outer membrane [18], cell cycle arrest observable from 24 h after exposure [19], and a second wave of apoptosis (driven by multiple factors) that culminates with absolute killing of exposed cells on in vitro cultures between 48 and 72 h after ECP [20–22] (Fig. 1). The susceptibility to ECP-induced apoptosis varies from cell type to cell type. In a recent study with healthy volunteers, B cells and NK cells were severely affected 24 h after treatment, with over 70% of annexin V+ cells increasing up to 90% after 48 h [21]. T lymphocytes are also highly susceptible to 8-MOP/UVA exposure likely due to a rapid downregulation of STAT5, a downstream kinase of IL-2 that coregulates the balance of Bcl-2 family proteins and activation of caspases [23, 24]. In contrast, regulatory T cells (Tregs) are more resilient to ECP, with apoptosis levels below 30% at 24 h and levels of 30–65% 48 h after treatment [21]. Monocytes reached levels of apoptosis above 90% 72 h after treatment [21]. However, an independent study showed that 8-MOP/UVA-exposed monocytes retain their capacity to secrete proinflammatory cytokines in response to LPS even after they initiate apoptosis, and those who survive are able to differentiate into dendritic cells (DC) when cultured with IL-4 and GM-CSF [25].

Mitochondrial Alterations and Bcl-2 Family Proteins

Used alone, 8-MOP increases the ratio of Bax/Bcl-2 in hepatocellular carcinoma (HepG2) cells by decreasing the expression of DEC1 and effectively affecting cell via-
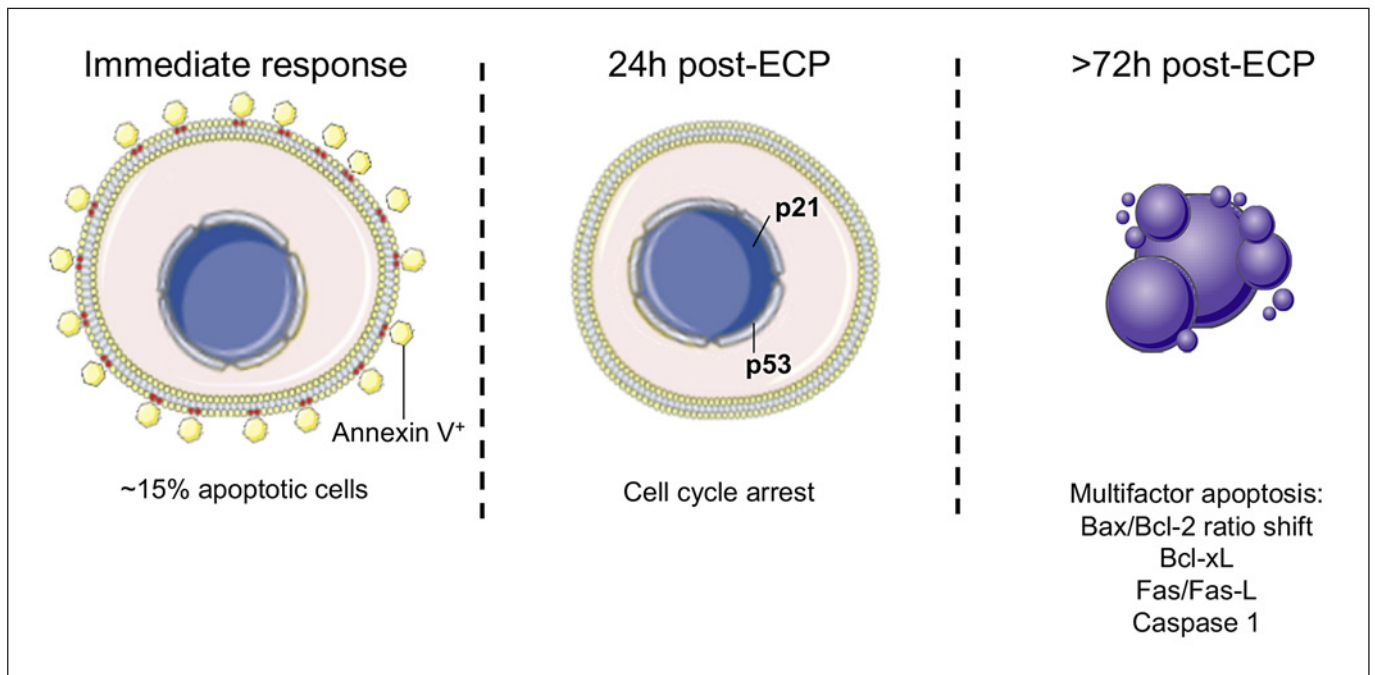
Fig. 1. Cell cycle arrest and commitment to apoptosis of ECP-exposed cells. Upon exposure to ECP cells that suffer extensive DNA damage, fail to repair it or bear severe mitochondrial alterations initiate a gradual process of apoptosis. Severely affected cells have an immediate flip-flow of phosphatidylserine, others halt cell cycle via activation of p21 and p53, and apoptotic bodies appear as early as 72 h after treatment as a result of activation of both intrinsic and extrinsic apoptosis pathways.

bility [26]. The Bcl-2 family proteins are central regulators of mitochondrial dependent apoptosis. The increase in Bax induces apoptosis commitment by the formation of oligomers that compromise mitochondrial membrane integrity, the translocation of apoptotic proteins such as AIF, cytochrome C, and the activation of caspases that lead to activation of the intrinsic pathway of apoptosis [27]. Exposure to 8-MOP/UVA also disrupts the opening regulation of permeability transition pores, causing a disturbance of membrane potential, liberation of ROS, and generation of psoralen photoproducts [28]. Once these photoproducts (a group of photodegraded intermediaries of psoralen) are released, they exert a potent cytotoxic activity given their high reactivity with proteins and unsaturated lipids [29].

Histological analysis of psoriatic lesions treated with PUVA and normal skin of patients have shown a prominent reduction of Bcl-2 expression in lymphocytes and keratinocytes together with a high score of apoptosis [30]. A similar reduction of Bcl-2 expression has been found comparing lymphocytes prior ECP treatment and immediately after irradiation (before reinfusion into the patient), altering the Bcl-2/Bax ratio [23]. Evidence suggests that the induction of apoptosis observed 24 h after ECP may be mediated by independent but at the same time synergizing mechanisms. In fact, 2 studies carried out in patients with systemic scleroderma showed that blood drawn 1 day after ECP had a high frequency of CD4 T cells with a high expression of CD95, a major regulator of extrinsic apoptosis [31, 32]. In vitro studies suggest that CD95 can cooperate with Bcl-xL and Bcl-2 to mobilize Ca2+ inside the mitochondria [33]. In mouse epidermal cells, exposure to 8-MOP/UVA results in p53 stabilization and nuclear translocation that triggers CD95 expression as early as 48 h after irradiation [34, 35].

P53 and Alteration of Cell Signaling

PUVA-induced p53 activation is preceded by increased expression of p21, a protein associated with cell cycle arrest in the G1 phase and subsequent apoptosis [36]. Additionally, p21 is a potent suppressor of the PI3K pathway during cell cycle arrest [37]. In fact, hitting PI3K may be a prominent factor in the therapeutic effect of ECP as the PI3K pathway is often involved in oncogenesis of CTCL patients and the severity of GvHD in animal models [38, 39]. 8-MOP/UVA exposure decreases the decoding of signal transduction by inducing changes in the cell membrane as a consequence of 8-MOP intercalation and covalent binding to phospholipids [40]. This inhibits the recruitment of effector kinases including Akt and Btk, affecting the PI3K pathway and rendering T cells unresponsive to IL-2 and TCR stimulus [41].
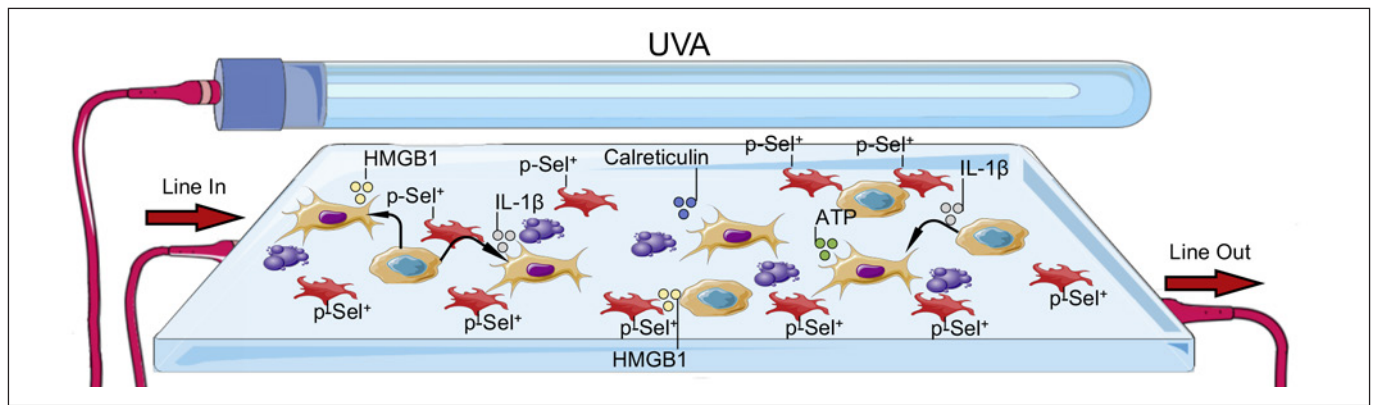
Vieyra-Garcia/Wolf

Fig. 2. Activated platelets contribute to APC activation. In parallel to the induction of apoptosis, the exposure to 8-MOP/UVA and the contact with tubing and plastics of the ECP apparatus induce changes in multiple blood components. Platelets are activated, adhere to the walls of the irradiation chamber, and express p-selecting. Extracellular ATP, IL-1β, HGMB1, and other DAMP are secreted and collectively promote monocyte differentiation into DC. A prolonged incubation in the irradiation chamber seems to promote phagocytosis of apoptotic cells and presentation of new antigens in a process referred as transimmunization.

Immunomodulatory Effects of ECP

Once reinfused, the ECP product represents only a minor fraction of the cells in peripheral circulation. Multiple interactions between apoptotic bodies and exposed and nonexposed cells initiate a cascade of immunological changes that cement the core elements of the ECP therapeutic mechanism. As patients benefit from a response with anticlonal and/or immunotolerance characteristics and they do not enter into a generalized immunosuppression state (opportunistic infections are rarely seen in ECP-treated patients and they have a normal response to vaccination) [42, 43], it is reasonable to infer that ECP provides a pool of antigens and immunomodulatory mediators that resettle the immune system. This, however, challenges one of the long-standing assumptions that apoptosis is a silent modality of cell death that prevents the elicitation of autoimmunity [44]. The evidence suggests that this is not always the case. Indeed, it has been shown that keratinocytes in apoptosis often display antigen determinants that are likely generated after caspases carry out degradation of endogenous proteins [45]. The numerous photoadducts formed between UVA-activated psoralen and amino acids may also modify the enzymatic processing of proteins and generate neo-antigens that increase the immunogenicity of irradiated cells [46]. Consequently, with this hypothesis, 8-MOP/UVA exposure leads to a substantial increase of MHC class I expression and a higher density display of peptides in transformed lymphocytes as early as 20 h after treatment [3]. In comparison with 8-MOP/UVA, no other agents including UVB, mitomycin C, and γ-irradiation had such potency of cytoplasmic protein degradation and presentation of antigenic peptides [3, 47].

Platelet Activation and Differentiation of Monocytes into DC

As outlined, not all cells are equally susceptible to ECP. Monocytes are highly resistant to 8-MOP/UVA exposure and increase phagocytosis of apoptotic cells in response to irradiation [48]. Parallel to that, platelets are activated, their adherence is increased, and they engage with monocytes promoting their differentiation into DC in a process mediated by p-selectin [49, 50] (Fig. 2). An ingenious modeling work of ECP in mice confirmed that monocyte-to-DC differentiation takes place soon after 8-MOP/UVA exposure and demonstrated that antigens from melanoma can be processed and presented by freshly differentiated DC [51]. The tumor antigens loaded in these cells makes them effective vaccine agents to prevent tumor development as long as their viability is not compromised by ECP [51]. Interestingly, it seems that the characteristics of these ECP-induced DC make them superior initiators of T-cell responses in contrast to DC generated by other methods that employ cytokine cocktails ex vivo at nonphysiological levels [52]. Such methods take several days to select positively cell populations that presumably once reintroduced in the patient do not find adequate levels of cytokines for their survival and have limited clinical performance when used in cancer immunotherapy [53, 54]. In contrast to that, ECP may be one of the most practical approaches to load tumor antigens into potent antigen-presenting cells (APC) as evidenced in a study on CTCL patients in which the addition of overnight incubation of ECP-treated cells prior reinfusion promoted the processing and presentation of antigens from tumor cells by freshly differentiated monocytes in a process described as "transimmunization" [55]. A transcriptomic analysis of monocytes 18 h after exposure to ECP showed a
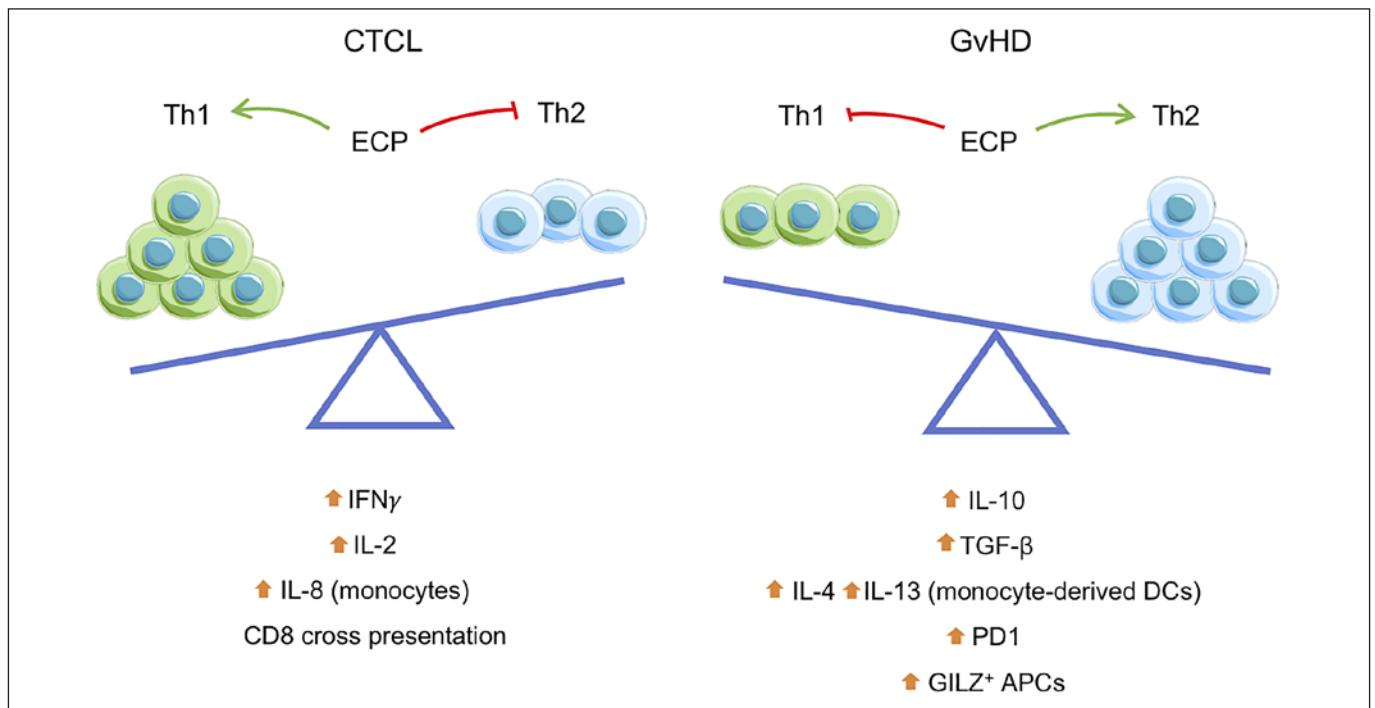
Fig. 3. Dual effect of ECP on antigen-specific immune and clinical responses. Upon reintroduction of treated cells, patients experience a shift in Th1 and Th2 balance closely associated with the type of disease. Whereas CTCL frequently respond to ECP with a shift toward a Th1 type response and cytotoxic CD8 T cells are primed by cross-presentation, GvHD patients often transit to a Th2 type response with immunomodulatory cytokines such as IL-10 and TGF-β closely related to the upregulation of PD1 and expression of GILZ in APC.

shift of over 1,100 genes including 20 genes associated with DC function and 60 genes participating in transmembrane signaling [56]. ECP-generated DC exceled at stimulating cytotoxic CD8 T cells with melanoma tumor antigens via MHC class I cross-presentation in a mouse model [57]. A recent report suggests that the potency of ECP-generated DC relays on the emission of damage-associated molecular patterns (DAMP) that augment phagocytic, chemotactic, and activation potential [58]. In particular, calreticulin exposure stimulates phagocytic activity. HMGB1 is a nuclear protein with adjuvant properties mediated by TLR4 binding, and extracellular ATP secreted during autophagy promotes chemotaxis via P2Y2 receptor [58, 59]. An in vitro study modeling GvHD indicated that pathogenic T cells express calreticulin and release HMGB1 after 8-MOP/UVA exposure, substantially increasing their phagocytosis by DC [60]. IL-1β is a potent inflammatory cytokine that synergizes with IL-2 to promote antitumoral cytotoxic activity in CD8+ T cells, inhibits the formation of Tregs, and plays a pathogenic role through the interactions of DC and T cells in inflammatory diseases such as rheumatoid arthritis [61–63]. A recent report with data from a mouse model and ECP-treated patients indicates that 8-MOP/UVA exposure has prominent immunostimulatory consequences closely associated with the release of IL-1β [64]. Monocytes and myeloid DC produced IL-1β after ECP in a mechanism pre-

dominantly independent of caspase-1 activation and in vitro culture of these cells showed a robust survival of professional APC for up to 7 days [64] (Fig. 2). Altogether these findings collectively indicate that ECP can in fact provide the third signal for T-cell priming through the production of soluble immunostimulatory factors.

A study on leukemic (L-CTCL) and GvHD patients demonstrated how the effect of ECP is translated into frequency changes of DC subpopulations in the periphery by monitoring the rate of CD123+ plasmacytoid DC (pDC) and CD11c+ myeloid DC (mDC). It showed that both pDC and mDC were normalized and further increased in GvHD patients, while in L-CTCL patients pDC remained unaltered and mDC were substantially augmented over a 3- to 6-month period of ECP treatment [65]. In addition to that, the majority of responding L-CTCL patients increased the expression of HLA-DR in both populations of DC, reinforcing the association of DC maturation to the therapeutic mechanism of ECP [65].

Modulation of Antigen-Specific T-Cell Responses

Reports on the effects of ECP in the production of inflammatory mediators are controversial. Whereas post-ECP monocytes in culture upregulate TNF-α and IL-6

Vieyra-Garcia/Wolf

production, serum levels of TNF-α tend to decrease and normalize in responding GvHD patients [66, 67]. A cytokine profiling study of CTCL patients revealed that, after 8-MOP/UVA exposure, peripheral blood mononuclear cells exhibit enhancement of IFN-γ and IL-2 production together with a decline of IL-4 and IL-10 [68]. This occurred not at the expense of Th2 cells converting into Th1; instead, the overall number of Th1 cells increased together with their cell-to-cell interactions, initiating feedback mechanisms that stimulated the production of IL-8 in monocytes [68]. In fact, it is generally understood that CTCL patients experience a transition after ECP to Th1 restoration and a lower production of pathogenic Th2 cytokines [69, 70].

In contrast to the elusive switch from a predominant Th2 to a Th1 cytokine microenvironment harnessed by ECP, studies addressing the secretory capacity of peripheral blood mononuclear cells of GvHD patients and in vitro models of allogenic reactions revealed that ECP exposure can increase the production of Th2 cytokines including IL-10 and TGF-β [71–73] (Fig. 3). Indeed, GvHD patients benefit from an induction of Th2 polarization after multiple rounds of ECP [71, 74]. Monocyte-derived DC exposed to 8-MOP/UVA skew naive T cells towards a Th2 like response with increased production of IL-4, IL-10, and IL-13, while the production of Th1 cytokines is substantially suppressed [75, 76]. The internalization of apoptotic cells may decrease the inflammatory reaction of phagocytes, inhibit the transcription of IL-12 in nonirradiated cells, and induce antigen-specific immunotolerance [77–79]. In vitro priming of T cells with ECP-exposed monocytes increases IFN-γ and IL-17 production but reduces the proliferation capacity of T cells in response to TCR stimulation in a cell contact mechanism regulated by transient PD-1 interactions [80]. Expression of the glucocorticoid-induced leucine zipper (GILZ) (an inhibitor of multiple pathways including NF-κB) is a distinctive characteristic of tolerogenic DC and it is increased by 8-MOP/UVA exposure [81, 82]. As a consequence, these cells downregulate CD80 and CD86, become unresponsive to TLR-induced maturation, and have a higher production of IL-10 and lower IL-12p70 levels [82]. The tolerogenic profile of DC is gradually induced by ECP, and in fact the same study suggests that minimal exposure to 8-MOP/UVA enhances immunogenic properties of myeloid cells in an invitro assay [82].

Activation of Tregs

Mouse models of contact hypersensitivity have shown that the infusion of 8-MOP/UVA-irradiated cells from sensitized mice inhibit the immune response once injected into naive animals [83]. Depletion of the CD11c+ and not the T-cell fraction prior to infusion reverses the inhibitory effect of 8-MOP/UVA mediated by the elicitation of antigen-specific CD4+ CD25+ T cells and the production of IL-10 in the sensitization and effector phase [83, 84]. The magnitude of the immunomodulatory effect of ECP during the effector phase can be observed in a mouse model of GvHD where treated cells from the donor were able to revert the already established disease and depletion of donor Tregs proved that these cells played a major role in decreasing disease severity [85].

A follow-up study in GvHD patients under ECP showed that, after 3 months and up to 1 year of treatment, CD4+, CD8+, and Tregs in circulating blood increased in frequency but the CD4+/Tregs ratio remained unaltered [86, 87]. Tregs in the circulation of patients under ECP expressed high levels of CD62L, CD45RO, and Foxp3 and had a potent suppressive function on allogenic effector T cells, reducing the IFN-γ secretion mediated by cell contact interactions [88]. Although no gains in the production of IL-10 and TGF-β were found in ECP-induced Tregs [88], a functional study in GvHD patients with cells obtained 48 h after treatment showed that CD39 (an ectonuclotidase responsible of adenosine production) is upregulated in Tregs and participates in the conversion of ATP into adenosine in the extracellular medium [89]. Adenosine production is a known immunosuppressive mechanism that involves the coordinated expression of CD39 and CD73 in Tregs to deliver a signal to effector cells through stimulation of the adenosine A2A receptor [90]. Although these findings suggest a role of Tregs in the therapeutic effect of ECP, a recent prospective study in 32 chronic GvHD patients showed a significant increase in Tregs during the first 6 months of treatment but no statistical association between Tregs and skin or steroid response; there was also a large variability in the absolute count and frequency of these cells [91]. In the last few years, experimental models of ECP have shown that classical Tregs are not the only subpopulation of cells with IL-10-mediated immunomodulatory properties. A mouse model of skin allograft showed that the infusion of splenocytes exposed to 8-MOP/UVA increased the number of IL-10+ regulatory B cells in circulation and promoted survival of the graft [92]. In contrast to that, a study in chronic GvHD patients under ECP revealed that serum levels of B-cell activating factor may be a biomarker of negative treatment outcomes as high levels of B-cell activating factor (BAFF) were associated with a worse median skin score after 6 months of treatment, whereas patients with low levels (<4 ng/ml) exhibited substantial skin improvement and had a high rate (>50%) of complete resolution [93]. Moreover, a recent report suggests that ECP also induces the formation of a CD8 T-cell subset with a suppressive capacity on CD8 effector cells via an indirect mechanism that involves the inhibition of

priming functions and migration out of the skin of APC [94]. Interestingly, these CD8 cells with a suppressive function did not show characteristics of regulatory CD8 cells including IL-10-mediated suppression or expression of Foxp3.

Concluding Remarks

ECP is an immunotherapy that arose way ahead of its time, proving that observations in basic research can quickly translate into principles for clinical application. Today, with better insight into the critical phenomena occurring during 8-MOP/UVA exposure, contact with non-self-materials of the ECP devices, and reinfusion into the blood stream, improvements in treatment protocols and further applications of ECP as a DC modifier therapy are imminent. It is clear that the immunomodulatory effect of ECP is primarily antigen specific and the dual pro/anti-inflammatory effect relies on interactions of cells that have initiated apoptosis with 2 kinds of APC, i.e., phagocytosis by nonexposed APC that result in immunoregulation and phagocytosis by ECP-activated myeloid cells in the presence of proinflammatory mediators such as IL-1β that result in immunostimulation.

As the immunomodulatory effects of ECP have direct and indirect reach across multiple cells in circulation, understanding the factors that determine the type of response and their role in therapeutic mechanisms is a challenging task. However, disruptive technologies such as scRNA-seq, ChIP-seq, and CyTOF have become available to address complex immune networks. Indeed, we live in times when we can look at transcriptional programs and cell interactions with incredible detail. With those tools at hand, further work is yet to be carried out to understand clearly how to procure a specific effect for the desired purpose by engineering systems that optimize platelet activation and DC differentiation as well as offline devices that facilitate customizable times of incubation prior to reinfusion of cells.

Author Contributions

P.A.V.-G. and P.W. conceived the ideas. P.A.V.-G. drafted this paper and created the figures, modifying graphic material from Servier licensed under a Creative Commons Attribution 3.0 Unported License. Both authors revised and approved the final version of this paper for publication.

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