Immunologic Mechanisms of Extracorporeal Photochemotherapy (ECP) in Chronic Graft-Versus Host Disease

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ABSTRACT

Extracorporeal photochemotherapy (ECP) has been shown to be an effective therapy for patients with acute and chronic graft-vs-host disease (GVHD) following allogeneic bone marrow transplantation, but its biological mechanism is not understood. We reported that clinical response to ECP was associated not only with normalization of skewed CD4/CD8 ratios, but also with an increase in CD3/CD56+ NK cells and decrease in the number of CD80+ and CD123+ circulating dendritic cells (DC). To further elucidate the effects of ECP on activated lymphocyte subpopulations and interaction between effector lymphocytes and antigen-presenting dendritic cells, we isolated and characterized DC populations from patients with cGVHD undergoing ECP therapy. Antigen presenting activity of DC, measured as proliferation of antigen-stimulated autologous and allogeneic T cells by mixed lymphocyte reaction (MLR). In MLR assays the proliferation of T cells was decreased in all 10 patients by a mean of 84% (range 75-95%, P≤0.002) after a two day cycle of ECP and longitudinally over the 12 month course of therapy. Immunophenotypic analysis of DC populations revealed a preponderance of DC1 monocytic dendritic cells in all patients prior to initiation of ECP. Nine of 10 patients demonstrated a shift from DC1 to DC2, as well as a concordant shift from a predominantly Th1 (IL-2, IFNγ) to Th2 (IL-4, IL-10) cytokine profile after ECP, and 8 of 10 had a clinical response to ECP. Our results suggest that ECP alters alloreactivity by affecting both allo-targeted effector T-cells as well as antigen presenting dendritic cells.
INTRODUCTION

Acute and chronic graft-versus-host disease (aGVHD and cGVHD) represent a major cause of morbidity and mortality following allogeneic bone marrow transplantation (BMT). The incidence of aGVHD ranges from 20-50% and is dependent on factors related to the graft as well as the effects of conditioning regimens on host tissue. Chronic GVHD affects 30-50% of successfully engrafted patients and is thought to be related to ongoing alloreactivity against minor HLA-antigen mismatch between the donor and recipient.

Extracorporeal photochemotherapy (photopheresis, ECP) involves ex vivo exposure of leukapheresed peripheral blood mononuclear cells to ultraviolet A light in the presence of a DNA-intercalating agent, 8-methoxypsoralen, with subsequent reinfusion of the treated cells. The total number of lymphocytes treated ex vivo per cycle have been estimated to be between 5-15% of the total circulating lymphocytes, and the total energy delivered via UVA light is estimated to be 2J/cm²/lymphocyte. ECP has demonstrated efficacy in the treatment of cutaneous T-cell lymphoma and recently in the management of T-cell mediated diseases such as scleroderma and alloreactivity in the setting of solid organ and bone marrow allografts.

The immunomodulatory effects of ECP have been explored most extensively in the context of cutaneous T-cell lymphoma, where induction of tumor-specific CD8⁺ effector cells have been demonstrated. Induction of apoptosis in circulating tumor cells exposed to UVA and 8-MOP is believed to prime antigen presenting dendritic cells with processed tumor antigens capable of inducing an anti-tumor immune response. In the context of autoimmune diseases and GVHD, ECP appears to induce tolerance to alloreactive or autoreactive antigen-generated T-cell responses but the immunomodulatory effects of ECP in autoimmune disorders is unclear. In scleroderma, identification of autoreactive T-cell clones supports a similar mechanism as in CTCL.
Clinical responses to ECP were demonstrated in patients with chronic GVHD initially by Rosetti et al., who demonstrated a selective effect of ECP on cytotoxic effector CD8⁺ T-cells with no change in CD4⁺ T-helper populations in 5 children with cGVHD, suggesting a differential effect on T-cell subsets without a clear explanation of how this effect was manifest.¹ Recently, we demonstrated not only an effect of ECP on T-cell subsets in cGVHD patients, but also a direct effect on circulating antigen presenting dendritic cells and natural killer cell populations in patients with cGVHD who had a clinical response to ECP, and we hypothesize that the mechanism of action of ECP in cGVHD and, perhaps in autoimmune diseases, may be related to the effects of the treatment on antigen presentation rather than on effector T-cell populations.⁶

Dendritic cells (DC) are highly potent antigen presenting cells of bone marrow origin which have been shown to stimulate both primary and secondary T- and B-cell responses.⁷ DCs are strategically distributed in tissues, they are constitutively rich in MHC class II molecules and can be readily induced to express the costimulatory molecules necessary for activation of naive or resting T cells. To elicit an immune response, DCs must undergo a maturation process, which is initiated by inflammatory signals and is completed after contact with T cells. Maturation enables DCs to migrate from peripheral tissues to lymphoid organs and to acquire a very potent Ag-presenting capacity. In addition, mature DCs are the most relevant and initial source of cytokines that govern the development of Th1 response.⁸

Two distinct types of DC precursors have been identified: myeloid monocytes (pre-DC1) and plasmacytoid DC precursors (pre-DC2). In mouse spleen, CD8⁺ 'lymphoid' and CD8⁻ 'myeloid' DC subsets have been identified. Whereas myeloid DC1s derived from monocytes produce a large amount of IL-12 and induce T-helper cells to differentiate into Th1 cells, lymphoid DC2s derived from plasmacytoid precursors produce lower amounts of IL-12 and induce a Th2 cytokine profile.⁹ Recent studies have shown that CD1d-expressing DCs stimulate murine and human natural killer T-cells (NKT cells). Thus, the type of DCs may also influence NKT cell differentiation into NKT1 or NKT2 cells.¹⁰
To further elucidate the functional effects of ECP on alloantigen presentation and cytokine production by effector T-cells in cGVHD, we examined DC function and helper T cell (Th1/Th2) differentiation. We demonstrate that prior to ECP, DC from patients with cGVHD were capable of inducing brisk autologous and allogeneic lymphocyte proliferation in 7/10 patients, whereas after in vivo exposure to ECP, both autologous and allogeneic T-cell proliferation was significantly attenuated. Further, there was a significant decrease in DC1 (CD80+; CD123+) compared to DC2 (CD83+, CD86+) dendritic cells, with a resulting shift from a predominantly Th1 (IFNγ) to Th2 (IL-4, IL-10) cytokine profile as early as 3 months after initiation of ECP, which was maintained during the 12 months of ECP therapy. Our results suggest that ECP may induce tolerance to alloantigens in cGVHD by inducing a shift in the DC1/DC2 balance toward DC2, thus attenuating ongoing alloreactivity which is mediated, in part, by Th1 cytokine secretion.

MATERIALS and METHODS

Reagents
Tetanus toxoid (TT) was provided by Massachusetts public health biological laboratories (Boston, MA); Staphylococcal enterotoxin B and phorbol-12-myristate 13-acetate (PMA) were purchased from Sigma Chemical Co (St.Louis, MO); GM-CSF, TNFα and IL-4 from R&D Systems. (Minneapolis, MN). AIM-V medium and human AB serum were obtained from Life Tech (Rockville, MD).

Patients
Patients had a diagnosis of extensive cGVHD, refractory to standard therapy (at least 4 weeks of prednisone 1mg/kg or equivalent), with therapeutic levels of cyclosporin A (CSA). All patients had progressive or symptomatic, non-responsive cGVHD. Five patients had failed mycophenolic acid on an institutional Phase II study, and two failed tacrolimus. All patients had extensive sclerodermatous skin changes. All patients had
ECOG performance status $\leq 3$. Patients with history of photosensitive disease, psoralen allergy, or active uncontrolled infection were ineligible. All patients signed informed consent conforming to an institutional review board-approved protocol. ECP was performed on 2 consecutive days every 2 weeks as previously described. Toxicity and response was assessed on day 0 and at the beginning of each 2-week treatment cycle using the NCI common toxicity criteria. Patients continued their immunosuppressive agents, which were adjusted for the activity of their cGVHD.

**Generation of dendritic cells from monocytes**

Fresh peripheral blood mononuclear cells (PBMC) from patients were isolated by ficoll-hypaque (Amersham-Pharmacia Biotech, Upsala, Sweden) gradient centrifugation. Non-adherent cells were removed by incubation of cells 16 h on culture flasks in complete medium (RPMI-1640 medium including 10% human AB serum, 50µg/ml glutathione, 50µg/ml streptomycin and 50µg/ml penicillin) at 5% CO$_2$ and 37°C. Non-adherent cells were frozen at -80°C.

To induce DC differentiation monocytes were cultured in 24-well plates (Costar, Cambridge, MA) at $5 \times 10^5$ cells/well in AIM V supplemented with 3% heat-inactivated human AB serum, 1000IU/ml IL-4, and 1000IU/ml GM-CSF at 37 °C in 5% CO$_2$ for 4 days. DCs were pulsed once with TT concentration at 0.01Lf/ml on day 3. Ag was removed washing the cells and renewing the supplemented medium. The maturation-inducing agent TNF$\alpha$ was added to the supplemented culture medium at day 4 (20ng/ml) and at day 6 (10ng/ml). DCs were stimulated with PMA (10ng/ml) at day 6 for 2 days. Cells were collected at the end of the culture (day 8) by incubating with PBS containing 0.2mmol/L EDTA for 5 minutes.

**Flow cytometry**

Cell staining was performed on $1 \times 10^5$ cells/ml with the following phycoerythrin (PE) or FITC-conjugated monoclonal antibodies: CD3 FITC, CD4 FITC, CD8 PE, CD25 PE, CD28 PE, CD56 PE, CD69 FITC, CD80 PE (B7-1), CD83 PE, CD86 PE (B7-2) and
CD123 PE (Becton Dickinson, San Jose, CA). Cells were stained for 30 minutes in PBS with 0.05% FBS, 2mmol/L EDTA and 0.01% sodium azide. Cells were washed twice for 5 minutes in PBS with 0.05% FBS, 2mmol/L EDTA and 0.01% sodium azide and fixed with 1% paraformaldehyde in PBS. 10000 cells were analyzed by means of a FACScan, Becton Dickinson equipped with 488nm-argon laser. All isotype controls were set to be less than 2% positive for statistical analysis.

**Mixed lymphocyte reaction**

Autologous lymphocytes from GVHD patients treated with ECP or allogeneic lymphocytes from normal volunteers were cultured at $10^3$ cells/well in 96-well plates (Costar) in AIM V supplemented with 3% heat-inactivated human AB serum with increasing numbers of irradiated DCs (30 Gy from a $^{137}$Cs source). Cells were pulsed with TT. Thymidine incorporation was measured in triplicate on day 6 by an 18 hour pulse with $[^3]$H-Thymidine (1uCi/well) (NEN, Boston, MA). Cells were harvested and $[^3]$H-Thymidine incorporation was measured by β-liquid scintillation counter.

**Ag presentation**

Isolated pre and post ECP autologous lymphocytes were cultured at $10^3$ cells/well in 96-well plates in AIM V, 3% heat inactivated human AB serum. Autologous DCs were irradiated and added $1x10^4$, $5x10^3$, $2.5x10^3$ and $1.25x10^3$ cells per well to obtain T/DC ratios of 10/1, 20/1, 40/1 and 60/1, respectively. Proliferation of Ag-specific lymphocytes was evaluated in triplicate after 6 days exposure to tetanus toxoid (TT) by measuring $[^3]$H-Thymidine uptake during last 18 hours.

**Separation of Th cell subpopulations**

PBMCs were pulsed to Staphylococcal enterotoxin-B protein (10ug/ml) for 16 hours in complete medium. $1x10^6$ cells were washed with PBS supplemented with 0.5% bovine serum albumin (BSA) and 2mM EDTA. Cells were incubated with cytokine-catch reagent (IFN$_\gamma$, IL-4 or IL-10) in complete medium for 45 minutes at 37 °C. Cells were labelled with PE or FITC conjugated cytokine detection antibodies CD8 PE/IFN$_\gamma$ FITC, CD4
FITC/IL-4 PE and CD4 FITC/IL-10 PE (Miltenyi Biotech, Auburn, CA)\textsuperscript{11} for 10 minutes. Cells were washed twice with PBS supplemented with 0.5\%BSA and 2mM EDTA. Cytokine secreted T cell subsets were detected by flow cytometry using a 388nm argon laser. Datas were analysed with student T-test and shown as mean with standard error. \( P \) values were detected as \( \leq 0.05 \).

**RESULTS**

Ten consecutive patients undergoing ECP for cGVHD after allogeneic bone marrow transplantation were studied. As we previously reported, 9 of 10 responded to treatment with improvement in sclerodermatous skin changes and in oral, musculoskeletal, hepatic, and lung involvement. Patients received two consecutive days of ECP every 2 weeks. Lymphocyte and dendritic cell subsets and functional analysis was performed prior to initiation and at the completion of day 2 (post-photopheresis) of each photopheresis cycle. Patients were treated for a minimum of 6 months and until response plateau. Peripheral blood dendritic cells were differentiated from monocytes and stem cells in presence of GM-CSF, IL-4 and TNF\( \alpha \) for 8 days. Dendritic cells were harvested and stained for expression of CD 80 (B7.1, preDC1), CD 83 (interdigitating reticulum DC and circulating DC), CD 86 (B7.2, preDC2) and CD 123. Dendritic cell yields and DC1/DC2 distribution were similar among 10 patients, are shown in Table 1. Prior to ECP therapy, the number of CD80\textsuperscript{+} and CD123\textsuperscript{+} DC1 cells in the cGVHD patients was increased compared to CD83\textsuperscript{+} and CD86\textsuperscript{+} DC2 cells. After one cycle of ECP, the number of DC1 cells decreased while DC2 increased, as shown in Figure 1.
Figure 1: Modulation of DC subtypes before and after ECP treatment. (A). At baseline, PBMC were stained with CD80, CD123 (DC1) and CD83, CD86 (DC2). DC subpopulations were gated and analyzed by FACS Scan. Pre ECP (dark filled peaks); post ECP (outlined peaks ). (B). At 6 months of ECP treatment. (C). At last cycle of ECP treatment.

Similar results were obtained at 6 and 12 months of therapy, and the overall change in DC populations during treatment was consistent among patients, as shown in Table 1.
Table 1: Dendritic cell yield (baseline-last ECP cycle day 2).

Cultured monocyte and lymphocyte derived DC subpopulations were stained with CD80, CD123 (DC1) and CD83, CD86 (DC2) and analyzed by flow cytometry at baseline, before ECP treatment and at 12 months after initiation of ECP at the completion of day 2 of the treatment cycle. Results are shown fold difference, with each column demonstrating increase (+) or decrease (-). \((P<0.05)\)

Dendritic cell functional activity before and after ECP was measured by antigen presentation to autologous and allogeneic lymphocytes in MLR assays. DCs generated from PBMCs both pre and post ECP at three different times over the course of one year of treatment were irradiated and incubated with autologous and allogeneic lymphocytes in variable T/DC numbers in presence of tetanus toxoid for 6 days. Cell proliferation was measured by \([^3H]\)-Thymidine uptake. Prior to ECP treatment, brisk proliferation of autologous and allogeneic T-cells was noted. Interestingly, proliferation of autologous T-cells was greater than allogeneic in 7 of 10 patients. (Table 2) Of the three patients in whom there was greater stimulation with allogeneic T-cells, two responded to ECP and one had no response but had received a donor lymphocyte infusion (DLI) for recurrent lymphoma.
<table>
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<tr>
<th>PATIENT NUMBER</th>
<th>ORGAN INVOLVEMENT</th>
<th>DRUGS AT START OF ECP</th>
<th>DRUGS AT END OF TRIAL</th>
<th>ONSET OF ECP FROM TRANSPLANT (DAYS)</th>
<th>MONTHS of ECP</th>
<th>RESPONSE</th>
<th>MLR Allo-vs-Auto</th>
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<td>Skin, joints, mouth</td>
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<td>PR</td>
<td>Autologus</td>
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<td>Prednisone 3.5mg/d, Cellcept 1000BID, Tacrolimus DIC</td>
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<td>9</td>
<td>PR</td>
<td>Allogeneic skin improved, lungs unchanged</td>
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*DLI for recurrent lymphoma after 3rd cycle of ECP

**Table 2: ECP effects on antigen presentation function of dendritic cells.**
MLR data refer to maximal proliferation (pre vs post 8-MOP/UVA exposure in cycle 3 of therapy, autologous vs allogeneic lymphocytes)
At the completion of the first ECP treatment, allogeneic and autologous lymphocyte proliferation were significantly blunted in all 10 patients, as shown in Figure 2, demonstrating an effect of ECP on antigen presentation by DC.
Because the observed effects of ECP on DC subpopulations would be anticipated to result in functional skewing of cytokine secretion from Th1 to Th2 cytokines, we evaluated Th1/Th2 ratios before and after ECP using cytokine capture assays. After antigen stimulation of peripheral blood lymphocytes with *Staphylococcus* enterotoxin B, IFNγ secreting Th1 cells decreased 1-3 fold and IL-4 and IL-10 secreting Th2 cells increased 1-3 and 1-4 fold, respectively, from baseline to last cycle of ECP after the initiation of therapy, as shown in Table 3. Similar proportional changes in cytokine profiles were seen before and after each cycle of ECP and were consistent at initiation of therapy and at 6 and 12 months or last cycle of ECP, as shown in Figure 3.
### Table 3: Modulation of Th cell (Th1/Th2) subpopulations by ECP.

Th1 and Th2 cells were detected by cytokine secretion. Captured cytokines were analyzed by flow cytometry and results are shown fold difference between baseline and last cycle of ECP (increased (+) or decreased (-)). ($P<.005$)

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<th>Patients</th>
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<tr>
<td>10</td>
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Figure 3: Changes in Th cell (Th1/Th2) subtypes during ECP treatment.

Dot plots of secreted IFN-γ (Th1); IL-4, IL-10 (Th2) captured by cytokine catching molecule and detected by FACS Scan. (A). Th1/Th2 subpopulations of PBMC from patients with cGVHD at first cycle of ECP. (B). Th subpopulations after 6 months ECP treatment. (C). Th subpopulations after last cycle of ECP treatment.
DISCUSSION

The pathogenesis of cGVHD is controversial and may be related either to an extension of the acute alloreactivity seen in aGVHD or a manifestation of dysfunctional immune reconstitution with generation of tissue auto-reactive T-cell clones and dysregulation of CD4⁺CD25⁺ immune modulating T-cells. In murine GVHD models, donor CD4-enriched cells of TH2 phenotype have been shown to prevent GVHD without affecting engraftment, suggesting that they play a role in downregulating the TH1 response. Similarly, in chronic GVHD, upregulation of TH1 cytokines IFNγ and IL-12 in peripheral blood mononuclear cells implicates in part a TH1-driven mechanism.

Dendritic cells are capable of presenting antigens to helper T cells, including naive T cells, and are likely important for inducing T cell responses to foreign (allogeneic) MHC molecules in tissue allografts. The MLR is a useful in vitro model of direct T cell recognition of allogeneic MHC gene products and is used as a predictive test of cell mediated graft rejection. Both alloreactive CD4⁺ and CD8⁺ T cells are stimulated during an allogeneic MLR. The CD8⁺ T cells differentiate into CTLs and are indistinguishable from self-class I MHC-restricted CTLs specific for foreign protein antigens. The CD4⁺ T cells differentiate into both Th1 and Th2 cells.

Both donor and recipient APCs are likely to be involved in the process of graft rejection. The most important APCs stimulating an antigraft response are most likely to be dendritic cells, either of donor origin and resident in the interstitium of the graft or of recipient origin and entering the graft through the blood supply. These APCs may stimulate recipient T cells within the graft. It is also possible that donor APCs migrate from the graft into draining lymph nodes, where they activate naive alloreactive T cells by the direct pathway.

We demonstrate here that ECP alters host (autologous) lymphocyte proliferation in MLR by modulating DC antigen presentation and differentiation of DC subtypes, favoring decrease in DC1 and increase in DC2 cells (Figure 4). The CD123⁺ lymphoid DC1 dendritic cells produce large amounts of IFNα. Although these cells might also process
and present antigen, they are postulated to enter lymph nodes through the high endothelial venule. Previous studies with low dose cutaneous ultraviolet B radiation in patients with skin manifestations of cGVHD have demonstrated an effect on intradermal antigen-presenting Langerhans cells to preferentially activate CD4+ Th2 cells. UVB irradiation has been shown to down-regulate Class II expression on APC and eliminate costimulatory signals involving cytokine secretion (IL-2 and IL-6) required for signal transmission from APC to T cells. UVB irradiated APC’s included in the donor bone marrow may present host antigen by the alternate pathway to donor lymphocytes.

While the paradigm of GVHD is based on alloreactive donor T cells recognizing foreign histocompatibility antigens of the host, there is now substantial experimental and clinical evidence to implicate a dysregulation of cytokine networks as a primary cause for the induction and maintenance of GVHD. The balance between Th1 cytokines (IL-2, IFNγ) and Th2 cytokines (IL-4, IL-10) governs the extent to which a cell-mediated immune response or a systemic inflammatory response develops. Because Th2 cytokines can inhibit the production of the proinflammatory cytokines IL-1 and tumor necrosis factor-alpha, a type 1 to type 2 shift in the initial response of donor T cells to host alloantigens may interrupt the cytokine cascade after allogeneic BMT and may offer a new approach to the prevention and treatment of acute GVHD.

In order to elucidate ECP effects on helper T cell subsets in cGVHD, we evaluated the helper T cell subsets by their specific cytokine profile. We observed that Th1 cells are decreased by ECP whereas Th2 cells are increased over the 12 month treatment. Current models of alloreactivity in acute GVHD implicate Th1 cells in the afferent arm, and cytokine production by monocytes and macrophages in the efferent arm. In addition, Th2 cells have been used experimentally to prevent GVHD in a murine model. Th1 cells induce the activation of macrophages resulting in delayed-type hypersensitivity responses and the killing of intracellular parasites. In contrast, Th2 cells control humoral responses, including the production of Ig E associated eosinophilia. An important feature of Th1 and Th2 cells is the ability of one subset to regulate the activity of the
other. This occurs at the level of the effector cells triggered by these subsets, as indicated by the inhibitory effects of IFNγ / IL-4-induced B cell activation or those of IL-4 on IL-2-induced T and B lymphocyte proliferation. It also occurs directly at the level of these subsets, as the products of one subset can antagonize the activation of the other: IFNγ inhibits proliferation of Th2 cells24,25, whereas IL-4 inhibits cytokine production by Th1 cells.26

Antigen specific T cell responses are characterized by distinct profiles of secreted cytokines. Rissoan et al27 have demonstrated that polarization of the T cell response into Th1 or Th2 depends on the type of antigen presenting cell: DC1 induce Th1 responses whereas DC2 induce Th2 responses.9 It has been proposed that DC2 might be responsible for maintaining peripheral T cell tolerance to self-antigens. Therefore, inducing proliferation of antigen specific T cells might be obligatory for DC1 but not DC2.28 During inflammatory or immune responses initiated by macrophages and DC1, however, the secretion of TNFα and the expression of CD40 ligand induce full activation of DC2. Under these circumstances, DC2 cells initiate the proliferation of antigen specific T cells, leading to the expansion of Th2 clones and secretion of IL-4 and IL-10. These cytokines produce a negative feedback on Th1 differentiation and terminate the immune and inflammatory responses.29 In several experimental models, donor Th2 cells have a decreased potential to induce acute GVHD.19,21,30,31 It is also possible that donor DC2 contribute to the pathogenesis of GVHD after transfer to the recipient through the indirect presentation of host antigen to donor T cells and the induction of Th2 responses. Interestingly, umbilical cord blood, another source of allogeneic stem cells for transplantation associated with relatively low incidence of acute GVHD, contains DC2 but not DC1 cells.31

Direct presentation of donor alloantigens by resident host DC, however, is likely to exert a dominant effect on the activation of donor T cells, resulting in both acute and chronic GVHD.32 Donor DC engraftment in recipients of allogeneic transplants has been associated with prolonged organ transplant survival.33,34 Donor DC1 would activate
allogeneic host T cells to produce IFNγ and generate cytotoxic responses against the graft, favoring rejection. Donor DC2 may induce host T cells to produce IL-4 and IL-10 which suppress Th1 and cytotoxic responses, favoring engraftment.35,36 Thus, manipulation of DC subsets may be useful in stem cell therapy for the induction of tolerance to hematopoietic cells or solid organ allografts. It has been reported that DC2 might be capturing alloantigen and undergo maturation after transfer into the host. These DC2 might be presenting alloantigen to donor T cells, inducing them to undergo Th2 differentiation, which would attenuate the Th1 driven mechanisms inducing aGVHD.10,37

Modulation of DC subpopulations correlated with clinical response. Of 9 patients who demonstrated a decrease in DC1 and increase in DC2 cells, 8 had a response to ECP in at least one target organ. Of two patients with lung involvement, neither demonstrated a response in the lung. One had early relapse of his lymphoma and received DLI, while the other had a response in skin GHVD but no change in pulmonary function. The one patient who had no response in any target organ (patient 10) demonstrated a decrease in both DC1 and DC2 populations and therefore demonstrated no selective effect of ECP. Interestingly, this patient demonstrated similar changes in T-cell cytokine profile with attenuation of Th1 activity. These results suggest that selective changes in DC subsets might predict for response to ECP, but these observations need to be confirmed in larger numbers of patients.

In summary, our data suggests that ECP has a direct effect to modulate APC function in patients with cGVHD. The net inhibitory effect of ECP on both activated T-cells and antigen presenting cells favors attenuation of ongoing Th1 mediated events, thereby removing the inhibition to Th2 cytokine secretion and restoring balance between Th1 and Th2 cytokines. We have recently initiated a clinical trial to longitudinally examine the biomodulatory effects of ECP on lymphocyte and dendritic cell function in patients with cGVHD randomized to either ECP or standard immunosuppressive therapy.
Figure 4: Modulation of dendritic cell function by ECP

ECP modulates dendritic cell populations, resulting in an increase in CD83⁺, CD86⁺ plasmacytoid DC2 cells with a concordant decrease in CD80⁺, CD123⁺ monocytoid DC1 cells. These stimulate Th2 T-helper cells to secrete Th2 cytokines, which indirectly inhibit Th1-mediated alloreactivity.
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Immunologic mechanisms of extracorporeal photochemotherapy (ECP) in chronic graft-versus host disease

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