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Extracorporeal photopheresis in refractory chronic graft-versus-host disease: The influence on peripheral blood T cell subpopulations. A study by the Hellenic Association of Hematology

Panagiotis Tsirigotis [a,*], Violetta Kapsimalli [b], Ioannis Baltadakis [c], Panayotis Kaloyannidis [d], Dimitrios Karakasis [c], Apostolia Papalexandri [d], Ekaterini Psarra [b], Efi Nosi [b], Evi Konsta [a], Myrofora Vikentiou [a], Sotirios Papageorgiou [a], Ioanna Sakellari [d], Vassiliki Pappa [a], Nikolaos Harhalakis [c], Achilles Anagnostopoulos [d], John Dervenoulas [a]

[a] 2nd Department of Internal Medicine, "ATTIKON" General University Hospital, Medical School, University of Athens, Greece
[b] Department of Immunology, "EVAGELISMOS" General Hospital, Athens, Greece
[c] Department of Hematology and Bone Marrow Transplantation, "EVAGELISMOS" General Hospital, Athens, Greece
[d] Department of Hematology and Bone Marrow Transplantation, "PAPANICOLAOU" General Hospital, Thessaloniki, Greece

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ABSTRACT

Extracorporeal photopheresis (ECP) has been established as an effective treatment modality for patients with chronic extensive graft-versus host disease (GVHD). In the present study, we evaluated the influence of ECP on the numbers of CD4+, CD8+, CD20+, CD56+ cells, and on T-regulatory (Tregs), as well as on the numbers of naïve, central memory (CM), and effector memory (EM) T-cells in patients treated for refractory chronic GVHD. Flow cytometric analysis of peripheral blood lymphocytes was performed for the calculation of the different T-cell subsets. Patients with GVHD had a higher percentage of EM-CD4+ cells in comparison with healthy donors ($p = 0.046$). The percentages of naïve-CD8+, naïve-CD4+, CM-CD8+, CM-CD4+, EM-CD8+, and Tregs were not different between patients with GVHD and healthy donors. Similarly there was no statistical difference in the percentages of naïve, CM, and EM CD4+ and CD8+ cells before and after 3 months of treatment with ECP. However, in the subset of Tregs a statistically significant increase was observed after 3 months of treatment with ECP ($p = 0.015$). Responders to ECP had statistically significantly higher absolute numbers of CD4+, and CD8+ cells, in comparison with non-responders. These data further support the concept that ECP does not cause immune-suppression, but should be better considered as an immune-modulating treatment.

1. Introduction

Allogeneic stem cell transplantation (Allo-SCT) remains the only therapeutic modality with a curative potential for various hematological malignancies refractory to standard chemotherapy regimens. Despite its therapeutic activity, Allo-SCT is associated with significant morbidity and mortality. Chronic graft-versus-host disease (cGVHD) represents the most serious late complication after Allo-SCT, with a significant negative impact on quality of life, and long-term survival. Immunosuppressive treatment with corticosteroids in combination with calcineurin inhibitors is currently the standard of care for patients with cGVHD [1]. However, for the vast majority of patients with extensive cGVHD, treatment needs to be prolonged over

* Corresponding author. Address: 2nd Department of Internal Medicine, Hematology Division, "ATTIKON" General University Hospital, Rimini-1, Haidari, P.O. Box 12462, Athens, Greece.
E-mail address: panagtsirigotis@gmail.com (P. Tsirigotis).

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many months or even years, resulting in impaired immune reconstitution and increased incidence of life-threatening infections. Extracorporeal photopheresis (ECP) was introduced into the clinical practice almost two decades ago, as an approved treatment for patients with cutaneous T-cell lymphomas [2]. During the previous years, a large number of non-randomized trials showed the effectiveness of ECP in the treatment of steroid-refractory cGVHD [3]. Recently, in a prospective randomized trial conducted by Flowers et al. ECP showed significant activity in the treatment of cGVHD, especially in patients with skin disease [4]. Moreover, it seems that the efficacy of ECP is mediated through an immune-modulating effect, since ECP does not cause immune-suppression, and it is not associated with increased incidence of infections or relapse rates. Despite the proven activity of ECP in the treatment of GVHD, much less is known about its mechanisms of action.

In the present study, we evaluated the influence of ECP on the numbers of various lymphocyte subsets such as: CD3+/CD4+, CD3+/CD8+, B-cells, and natural killer cells (NK) as well as the influence of ECP on various T-cell subsets such as: T-regulatory, naive, central memory, and effector memory T-cells in patients treated for extensive chronic GVHD.

2. Materials and methods

2.1. Patients

We examined the influence of ECP in two different cohorts of patients with chronic GVHD.

Cohort 1 served as the study group for evaluation of the influence of ECP on the following lymphocyte subpopulations: CD4+, CD8+, B-cells, and NK-cells. Cohort 1 consisted of 39 consecutive patients with chronic GVHD treated with ECP in the same institution (Thessaloniki).

Cohort 2 served as the study group for evaluation of the influence of ECP on the following T-cell subsets: T-regulatory (Tregs), naive, central memory (CM), and effector memory (EM) T-cells. Cohort 2 consisted of eight consecutive patients with various hematological malignancies who underwent Allo-SCT and subsequently developed chronic GVHD refractory to standard immunosuppression. All eight patients treated with ECP in the same institution (Athens). All patients in both cohorts gave informed consent before treatment.

2.2. Diagnosis and staging of GVHD

Diagnosis and staging of chronic GVHD was performed using the global scoring system for chronic GVHD, proposed by the National Health Institute Working Group for GVHD. Briefly, organs or anatomical sites considered for scoring included skin, mouth, eyes, gastrointestinal tract, liver, lungs, joints, fascia, and female genital tract. The severity of GVHD in each organ or site was scored according to a four-point scale, from 0 (no involvement) to 3 (severe involvement) [5].

2.3. Treatment schedule

ECP was performed, using the device THERAKOS-UVAR XTS (Johnson & Johnson) according to standard protocols [6].

2.3.1. Cohort 1

All patients were treated with ECP until maximal response or progression of GVHD. Treatment schedule was as follows. First month of treatment: two consecutive ECP-procedures every week for a total of eight procedures. Thereafter two consecutive ECP – procedures were performed every 2 or 4 weeks according to clinical response at the discretion of the responsible physician. Treatment with ECP was discontinued in the following circumstances: GVHD progression, relapse of malignancy, 1–2 months beyond the achievement of maximal response.

2.3.2. Cohort 2

All patients were treated with ECP for at least 6 months. Treatment schedule was as follows. First month of treatment: two consecutive ECP-procedures every week for a total of eight procedures. Second and third month of treatment: Two consecutive ECP – procedures every second week for a total of four procedures per month. Fourth, fifth, and sixth month of treatment: two consecutive ECP-procedures every month.

2.4. Response to treatment

Response to treatment was evaluated using the provisional response criteria proposed by the National Health Institute Working Group for response in chronic GVHD. In more detail, for an objective estimation of response we used the "Chronic GVHD Data Collection Forms" (http://www.asbmt.org/GvHDForms) [7].

2.5. Flow cytometry

2.5.1. Cohort 1

Peripheral blood (PB) samples were taken from patients at the onset, and 3, 6, 9, and 12 months after the onset of treatment with ECP. Evaluation of the absolute numbers of CD3+/CD4+, CD3+/CD8+, CD20+, and CD56+ was performed using flow cytometry.

2.5.2. Cohort 2

In order to examine the influence of ECP on T-cell subsets, PB was collected before and 3 months after treatment with ECP. PB was also collected from 29 healthy volunteer donors who served as the control group. All healthy volunteer donors gave informed consent.

A standardized methodology was used for the preparation of specimens. EDTA anticoagulated PB specimens were collected and processed within 4–6 h using (a) Immunoprep™ Reagent System [Beckman Coulter (BC), Miami] for red cell lysing and surface markers staining and (b) IntraPrep™ Permeabilisation Reagent (BC) and (c) FoxP3 Staining Set (eBioscience, San Diego) for cytoplasmic markers staining. Three- and four-color MFC was performed on an EPICS Coulter XL-MCL™ Flow Cytometer (BC) using

commercially available reagents and the appropriate staining procedures recommended by the manufacturing company. The panel of monoclonal antibodies included: (a) fluorescein isothiocyanate (FITC): CD25, CD45RO, and CCR7 (b) phycoerythrin (PE): CD45RA, CD127, FoxP3, and CD62L (c) PE-Texas Red-x (ECD): CD4 and (d) PE-Cyanin 5 (PC5): CD4 and CD25. FoxP3 molecules were detected by intracellular staining. All the other molecules were detected by surface staining.

The following phenotypic expression profiles were used for the calculation of various T-cell subpopulations in patients and healthy volunteer donors. (1) CD3+ cells, CD3+/CD4+ cells, and CD3+/CD8+ cells. (2) Naïve-CD4 (RA+, CCR7+, CD62L^{high}), central memory CD4 (CM-CD4) (RA-, CCR7+, CD62L^{high}), and effector memory CD4+ cells (EM-CD4) (RA-, CCR7-, CD62L^{low}). The results were given as absolute numbers as well as percentage of the total CD4+ cells. (3) Naïve-CD8 (RA+, CCR7+, CD62L^{high}), central memory (CM-CD8) (RA-, CCR7+, CD62L^{high}), and effector memory CD8+ cells (EM-CD8) (RA-, CCR7-, CD62L^{low}). The results were given as absolute numbers as well as percentage of the total CD8+ cells. (4) T-regulatory cells (Tregs). The results were given as percentage of the total CD4+ cells. Two different phenotypic expression profiles were used for the estimation of Tregs: (a) Tregs1 (CD4+, CD25^{strong}+, CD127^{low}), and (b) Tregs2 (CD4+, CD25^{strong}+, FoxP3+).

2.6. Statistical analysis

A two-sided Mann–Whitney *U* test was used for comparison between non-paired samples. Wilcoxon test was used for comparison between paired samples. Fisher's exact test was used for comparison between categorical variables. All comparisons were two-tailed. *P* values less than 0.05 were considered statistically significant.

3. Results

3.1. Response to treatment

3.1.1. Cohort 1

Four patients died before completion of treatment with ECP. Twenty-five patients showed an objective response to ECP, five patients progressed while on treatment with ECP, while five patients had no response. The median time to response was 8 weeks (range, 2–16 weeks). The cumulative incidence of overall response to ECP was 75.4% (CI 95%, 61.9–91.9%) (Fig. 1).

3.1.2. Cohort 2

One patient discontinued treatment with ECP due to early relapse of the malignant disease. Treatment with ECP was effective in two out of seven patients. One patient presented with extensive scleroderma affecting almost all of his body surface area. After the completion of a 28-month course of treatment with ECP, he achieved complete resolution of skin scleroderma and immunosuppression was discontinued. Another one patient with extensive sclerodermatous changes had a partial skin response, but not allowing for any decrease in immunosuppression. Three patients had progressive GVHD while on treatment with ECP, while no response was observed in two patients.

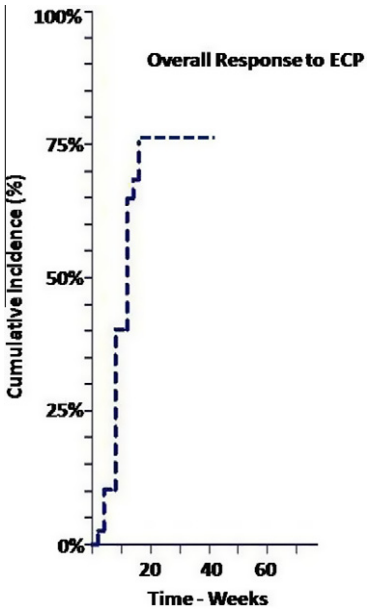

Fig. 1. Cumulative incidence of overall response to ECP (Cohort 1).

3.2. Influence of ECP on CD4+, CD8+, CD20+, and CD56+ lymphocyte subsets (Cohort 1)

Patients in Cohort 1 were divided in those who responded to ECP (25 patients) and in non-responders (14 patients). The absolute numbers of lymphocyte subsets (CD4+, CD8+, CD20+, and CD56+) at the onset of treatment and after 1 month of treatment with ECP were not different between responders and non-responders to ECP (data not shown). Three and 6 months after the onset of ECP responders had higher absolute numbers of CD4+ and CD8+ cells in comparison with non-responders to ECP (Fig. 2). No difference in the absolute numbers of CD20+, and CD56+ cells was observed between responders and non-responders to ECP (data not shown).

3.3. Influence of GVHD on T-cell subpopulations (Cohort 2)

The percentages of naive-CD8+, CM-CD8+ and EM-CD8+ cells (among total CD8+ cells) were not different between patients with GVHD and healthy donors ($p = 0.12$, 0.88, and 0.11, respectively). Similarly the percentages of naive-CD4+, and CM-CD4+ (calculated among total CD4+ cells) were not different between patients with GVHD and healthy donors ($p = 0.12$, 0.10, respectively). Patients with GVHD had a higher percentage of EM-CD4+ cells in comparison with healthy donors ($p = 0.046$). The percentages of Tregs cells were not different between patients with GVHD and volunteer donors

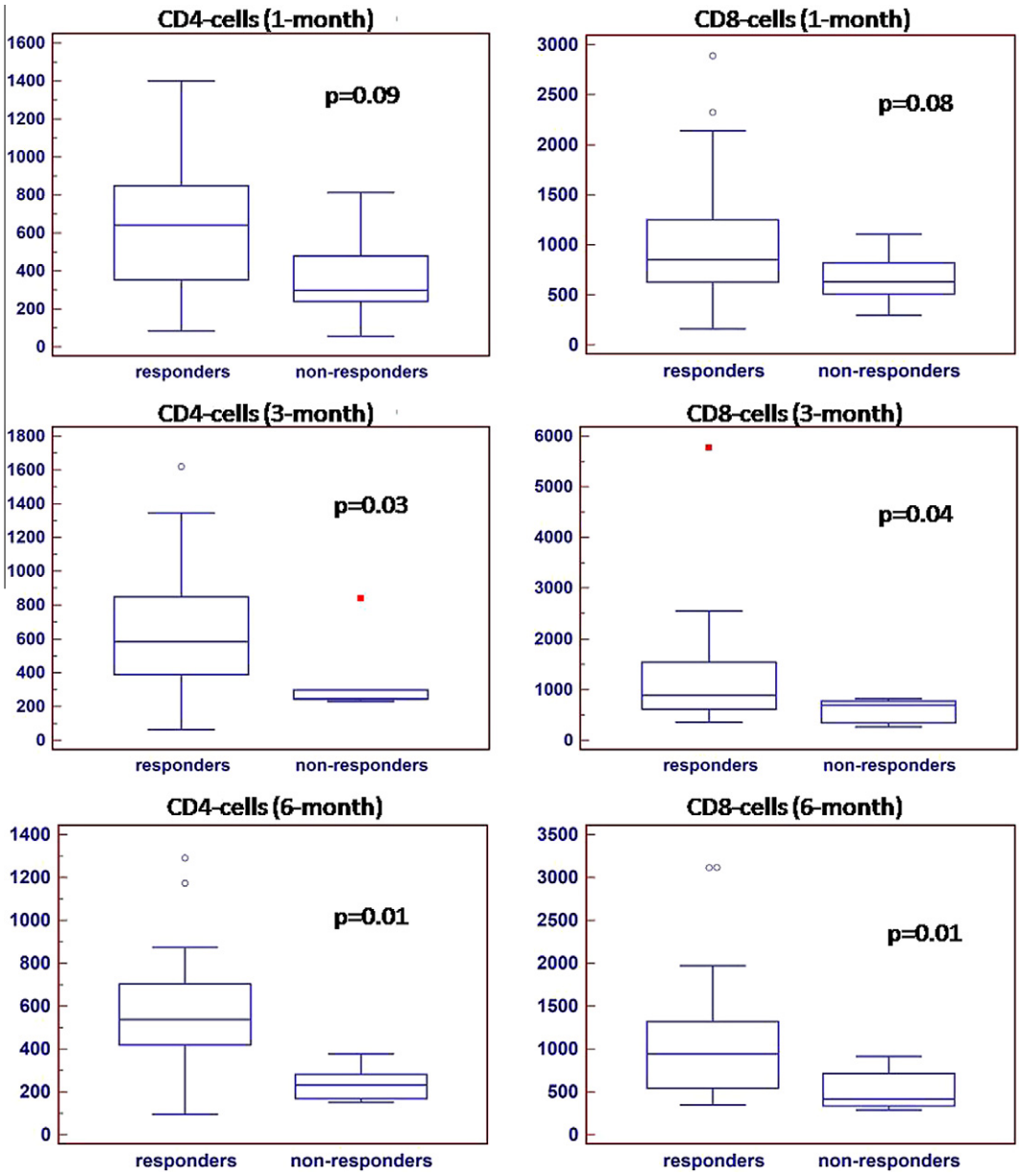

Fig. 2. Influence of ECP on CD4+, CD8+, CD20+, and CD56+ lymphocyte subsets (Cohort 1).

($p = 0.32$, and 0.49 for T-regs1 and T-regs2, respectively) (Figs. 3 and 4).

3.4. Influence of ECP on T-cell subpopulations (Cohort 2)

ECP did not have any effect on the following T-cell subsets: the percentages of naive-CD4+, CM-CD4+, and EM-CD4+ (among total CD4+ cells) were not significantly different before and after 3 months treatment with ECP ($p = 0.35$, 0.56, and 0.37, respectively). Similarly, there was no significant difference in the percentages of naive-CD8+, CM-CD8+, and EM-CD8+ cells before and after 3 months of treatment with ECP ($p = 0.43$, 0.81, 0.68) (Fig. 5).

3.5. Influence of ECP on Tregs (Cohort 2)

However, in the subset of Tregs a statistically significant increase was observed after 3 months of treatment with ECP ($p = 0.015$) (Fig. 6). The same result was obtained irrespectively of the method used for the estimation of Tregs (Tregs1 or Tregs2).

4. Discussion

ECP was introduced in the clinical practice in 1987 as an effective treatment modality for a subset of patients with CTCL, such as erythrodermic mycosis fungoides and Sezary syndrome [8]. The therapeutic potential of ECP was tested

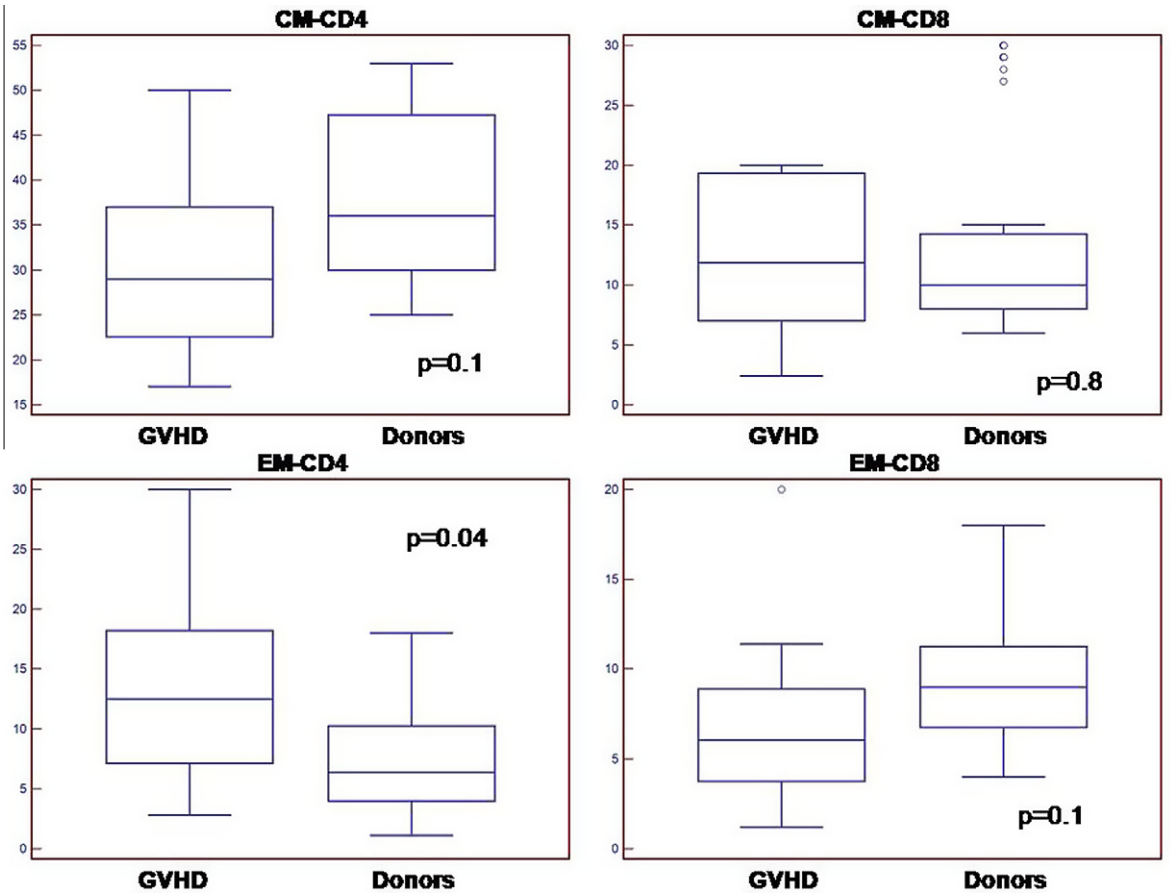

Fig. 3. Influence of GVHD on EM-CD4+, CM-CD4+, EM-CD8+, and CM-CD8+ cells. Comparison between patients with chronic GVHD and healthy donors.

in many diseases, and ECP proved to be effective in various autoimmune and alloimmune disorders, such as, solid organ graft rejection, GVHD, systemic sclerosis, etc. ECP procedure consists of four steps: (1) collection of peripheral blood mononuclear cells (buffy coat fraction), (2) ex-vivo incubation of mononuclear cells with 8-methoxypsoralen (8-MOP), (3) irradiation of cells with UVA, (4) reinfusion of treated cells to the patient [9]. The effects of ECP, on ex-vivo treated mononuclear cells have been extensively studied in the past. Photoactivation of 8-MOP after UVA irradiation results in DNA damage due to cross-linking between the DNA strands, followed by the activation of apoptotic machinery. T-lymphocytes are sensitive to ECP-effects and laboratory evidence of apoptosis has been observed in more than 80% of the treated cells, within 24 h after ECP. Monocytes are resistant to ECP apoptotic stimuli, and probably due to adherence to plastic surface differentiate to immature dendritic cells with enhanced phagocytic ability [9].

Previous studies examined the effect of treatment with ECP on T-cell subsets in small number of patients (10–11 patients) with chronic GVHD. Interpretation of data showed that the absolute number of various T-cell subsets (CD4+, and CD8+ cells) increased towards to normal values in patients with chronic GVHD who responded to treatment with ECP, while T-cell subpopulations remained low in non-responders [10,11]. We examined the effect of ECP in a large number of patients (no = 39), and similar to previous studies we observed statistically significantly higher absolute numbers of CD4+ and CD8+ cells in responders soon after the initiation of treatment with ECP (3rd month). The higher CD4+, CD8+ counts in responders could not be attributed to withdrawal of immunosuppression since no discontinuation or tapering of immunosuppression occurred during the first 3 months of treatment. Therefore it is reasonable to assume that slowing down the allo-reactive process results in augmentation of immune reconstitution. These data further support the concept that ECP does not cause immunesuppression, but should be better considered as an immune-modulating treatment.

The pathophysiology of chronic GVHD remains poorly understood however, it is accepted that donor derived, anti-host reactive, T cells play a significant role. Human T-cells can be divided into three distinct populations: (1) naïve T-cells (CD45RA+, CCR7+, CD62L^{high}), (2) central memory T-cells (CD45RA-, CCR7+, CD62L^{high}) and, (3) effector memory T-cells (CD45RA-, CCR7-, CD62L^{low}). Chemokine receptor CCR7 and CD62L (L-selectin) are expressed on the surface of cells destined to travel from

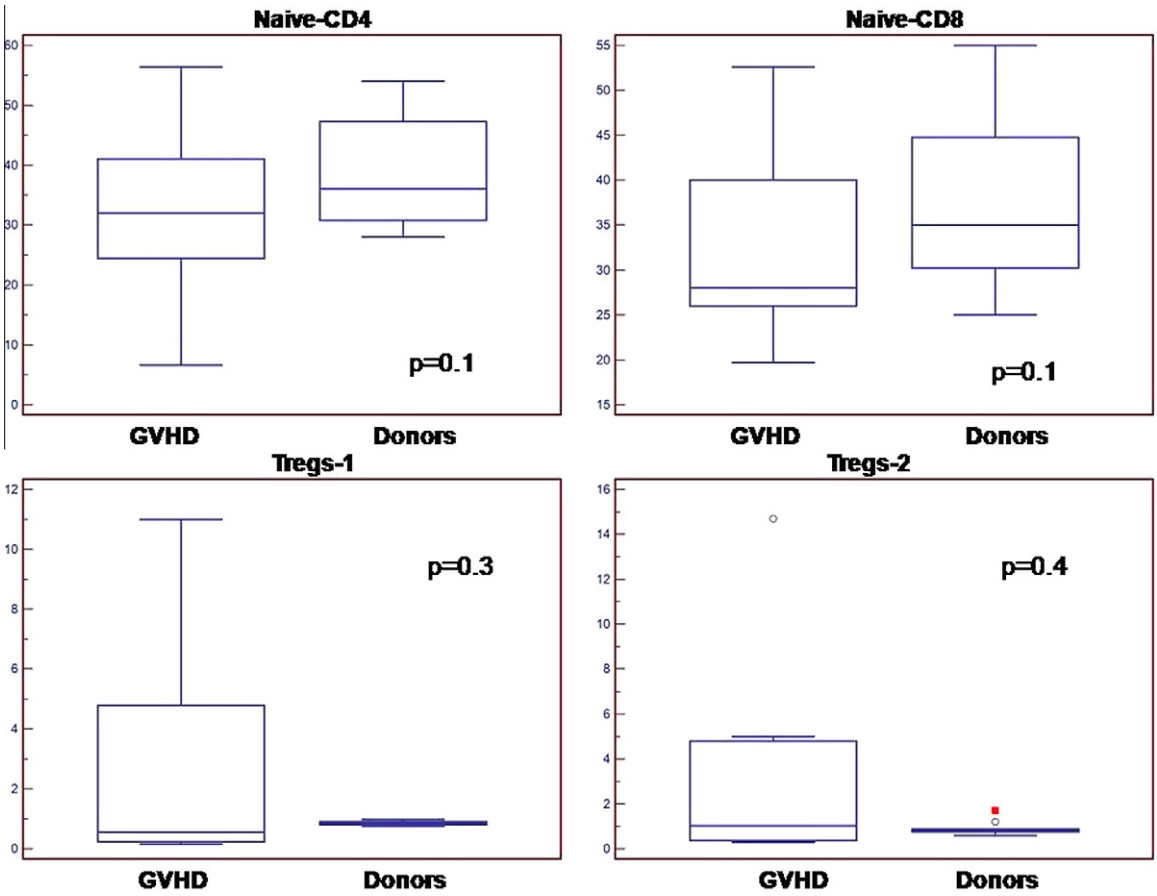

Fig. 4. Influence of GVHD on naïve T-cells and T-regulatory cells. Comparison between patients with chronic GVHD and healthy donors.

peripheral blood to secondary lymphoid organs. Central memory T-cells are antigen-experienced, migrate from peripheral blood to secondary lymphoid organs, and retain the capacity to differentiate into effector memory cells after antigen re-challenge. Conversely, effector memory cells migrate from peripheral blood to tissues and are actively involved in inflammation and cytotoxicity. In healthy individuals, central memory CD4+, T-cells are predominant in peripheral blood [12].

In a previous study, Yamashita et al. showed that severe chronic GVHD is characterized by an increased percentage of peripheral blood EM-CD4+, in relation to CM-CD4+ cells. Patients with cGVHD had a higher percentage of EM-CD4+ cells (35%), than healthy donors (13.8%), or patients without cGVHD (21.7%) and these differences were statistically significant [13].

Authors postulated that increased in vivo T cell stimulation observed in recipients with cGVHD promotes the transition of central memory to effector memory cells, resulting in the decrease of the central memory CD4+ T-cell pool. In a similar trial, authors showed that patients with cGVHD had a higher percentage of CM-CD8+, and a lower percentage of CM-CD4+ cells in comparison with healthy donors or patients without GVHD. Treatment with ECP resulted in restoration of T-cell subsets in responding patients [14].

In accordance with published data, we also observed that patients with GVHD had a higher percentage of EM-CD4+ cells in comparison with healthy donors. However, we did not observe any influence of treatment with ECP, on the various T-cell subpopulations. The percentages of these T-cell subsets were not significantly different in patients with GVHD before and after 3 months of treatment with ECP. The association, if any, of the different peripheral blood T-cell subsets with the clinical course of cGVHD should be further explored in a larger number of patients.

Tregs cells are an important component of peripheral immune tolerance and reduced levels have been observed in various autoimmune disorders as well as in cGvHD. In a murine model, the infusion of high levels of Tregs has been demonstrated to reduce acute GvHD mortality [15], while high levels of donor Tregs have been associated with a low risk of acute GvHD [16]. There are conflicting data regarding the percentage of Tregs in cases with chronic GVHD, as both increased and decreased levels have been reported [17,18]. In the present study, we did not observe any difference in the percentages of Tregs between patients and healthy donors.

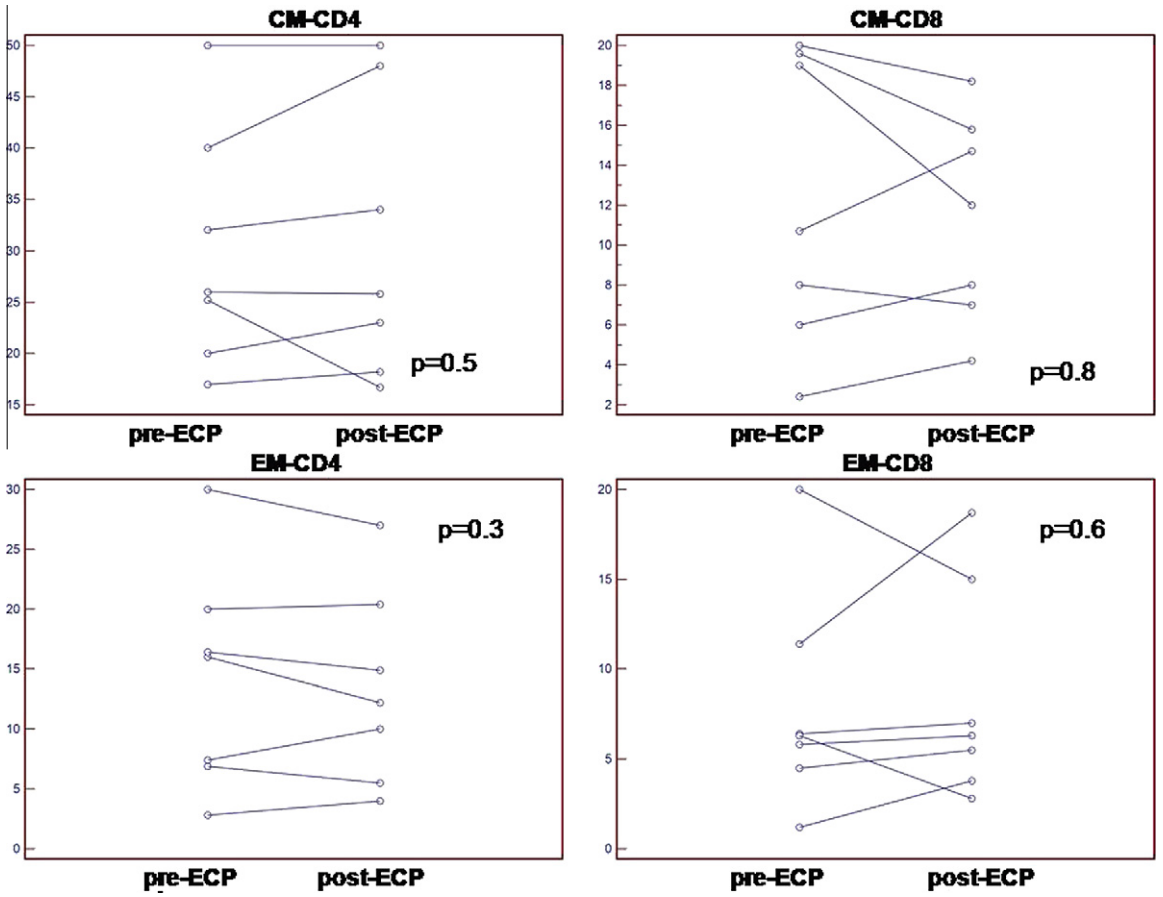

Fig. 5. Influence of ECP on EM-CD4+, CM-CD4+, EM-CD8+, and CM-CD8+ cells. Comparison between values before and after 3 months treatment with ECP.

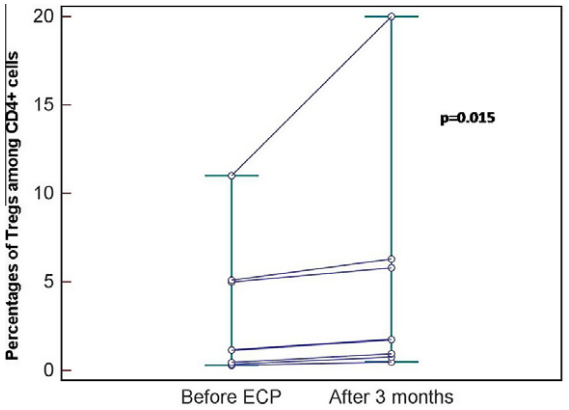

Fig. 6. Influence of ECP on Tregs. Comparison between values before and after 3 months treatment with ECP.

Previous studies assessing the efficacy and mechanism of action of ECP in the treatment of GVHD, and prevention of solid organ rejection, showed a significant increase in the percentage of Tregs post treatment [19–23]. However the influence of ECP on Tregs has been explored in a limited number of patients with chronic GVHD, and requires further investigation.

In accordance to previous reports, in our study, we observed a significant increase in the percentage of Tregs after 3-months of treatment with ECP. Moreover, the greatest increase in Tregs was observed in the only patient who achieved complete skin response.

Generation of regulatory T-cells after treatment with ECP remains a topic of active research. Immature dendritic cells (DCs) upon exposure to microbial products, receive the so called "danger signals", differentiate into mature DCs, migrate in regional lymph nodes and finally activate antigen-specific T-cells. On the contrary, DCs acquire a tolerogenic phenotype after phagocytosis of apoptotic cells (ACs) [24]. Under normal conditions, apoptotic cells, resulting from the normal cellular turnover, are removed by DCs. Engulfment of apoptotic cells represents the basic mechanism of peripheral tolerance. ECP induces massive apoptosis of lymphocytes. Apoptotic bodies are removed by various cell types and among them, antigen professional cells (like immature DCs) are the more efficient. Recognition of ACs is mediated through specific receptors on DCs. These receptors recognize ligands such as phosphatidyl serine and oxidized lipids expressed on ACs. Previous studies showed that ingestion of ACs by DCs regulates immune responses. Immune regulation occurs primarily by a functional shift of DCs to a tolerogenic profile. Tolerogenic DCs do not stimulate T cells and may induce

tolerance using several different mechanisms, including generation of Tregs [25,26].

In conclusion, refractory chronic GVHD represents a major challenge for the treating physician. ECP is a promising therapeutic modality with an excellent safety profile. The mechanism of action of ECP, as well as parameters predictive of response need to be further explored in future studies. Understanding the mechanism of action of ECP might lead to innovative and more effective immune tolerance therapeutic modalities.

References

[1] Vogelsang GB. How I treat chronic graft-versus-host disease. Blood 2001;97:1196–201.

[2] Heald PW, Perez MI, Christensen I, Dobbs N, McKiernan G, Edelson R. Photopheresis therapy of cutaneous T-cell lymphoma: the Yale-new Haven hospital experience. The Yale J Biol Med 1989;62:629–38.

[3] Foss FM, Gorgun G, Miller KB. Extracorporeal photopheresis in chronic graft-versus-host disease. Bone Marrow Transplant 2002;29:719–25.

[4] Flowers ME, Apperley JF, van Besien K, Elmaagacli A, Grigg A, Reddy V, et al. A multicenter prospective phase 2 randomized study of extracorporeal photopheresis for treatment of chronic graft-versus-host disease. Blood 2008;112:2667–74.

[5] Filipovich AH, Weisdorf D, Pavletic S, Socie G, Wingard JR, Lee SJ, et al. National Institutes of Health Consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and Staging Working Group Report. Biol Blood Marrow Transplant 2005;11:945–55.

[6] Greinix HT, Volc-Platzer B, Rabitsch W, Gmeinhart B, Guevara-Pineda C, Kalhs P, et al. Successful use of extracorporeal photochemotherapy in the treatment of severe acute and chronic graft-versus-host disease. Blood 1998;92:3098–104.

[7] Pavletic SZ, Martin P, Lee SJ, Mitchell S, Jacobsohn D, Cowen EW, et al. Response Criteria Working Group. Measuring therapeutic response in chronic graft-versus-host disease: National Institutes of Health Consensus development project on criteria for clinical trials in chronic graft-versus-host disease: IV. Response Criteria Working Group Report. Biol Blood Marrow Transplant 2006;12:252–66.

[8] Edelson R, Berger C, Gasparro F, Jegasothy B, Heald P, Wintroub B, et al. Treatment of cutaneous T-cell lymphoma by extracorporeal photochemotherapy. Preliminary results. N Engl J Med 1987;316:297–303.

[9] Heshmati F. Mechanisms of action of extracorporeal photochemotherapy. Transf Apher Sci 2003;29:61–70.

[10] Gomes da Silva M, Neto L, Guimarães A, Machado A, Parreira A, Abecasis M. Long-term follow-up of lymphocyte populations and cellular cytokine production in patients with chronic graft-versus-host disease treated with extracorporeal photopheresis. Haematologica 2005;90:565–7.

[11] González-Vicent M, Ramírez M, Pérez A, Lassaletta A, Sevilla J, Ángel Díaz M. Extracorporeal photochemotherapy for steroid-refractory graft-versus-host disease in low-weight pediatric patients. Immunomodulatory effects and clinical outcome. Haematologica 2008;93:278–1280.

[12] Sallusto F, Lenig D, Förster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. Nature 1999;401:708–12.

[13] Yamashita K, Choi U, Woltz PC, Foster SF, Sneller MC, Hakim FT, et al. Severe chronic graft-versus-host disease is characterized by a preponderance of CD4+ effector memory cells relative to central memory cells. Blood 2004;103:3986–8.

[14] Yamashita K, Horwitz ME, Kwatemaa A, Nomicos E, Castro K, Sokolic R, et al. Unique abnormalities of CD4+ and CD8+ central memory cells associated with chronic graft-versus-host disease improve after extracorporeal photopheresis. Biol Blood Marrow Transplant 2006;12:22–30.

[15] Taylor PA, Lees CJ, Blazar BR. The infusion of ex-vivo activated and expanded CD4+CD25+ immune regulatory cells inhibits graft-versus-host disease lethality. Blood 2002;99:3493–9.

[16] Rezvani K, Mielke S, Ahmadzadeh M, Kilical Y, Savani BN, Zeilah J, et al. High donor FOXP3-positive regulatory T-cell (Treg) content is associated with a low risk of GVHD following HLA-matched allogeneic SCT. Blood 2006;108:1291–7.

[17] Zorn E, Kim HT, Lee SJ, Floyd BH, Litsa D, Arumugarajah S, et al. Reduced frequency of FOXP3+ CD4+CD25+ regulatory T cells in patients with chronic graft versus-host-disease. Blood 2005;106:2903–11.

[18] Clark FJ, Gregg R, Piper K. Chronic graft-versus-host disease is associated with increased numbers of peripheral blood CD4+CD25 high regulatory T cells. Blood 2004;103:2410–6.

[19] Bladon J, Taylor P. Extracorporeal photopheresis normalizes some lymphocyte subsets (including T regulatory cells) in chronic graft-versus-host-disease. Ther Apher Dial 2008;12:311–8.

[20] Lamioni A, Carsetti R, Legato A, Landolfo A, Isacchi G, Emma F, et al. Induction of regulatory T cells after prophylactic treatment with photopheresis in renal transplant recipients. Transplantation 2007;83:1393–6.

[21] Di Biaso I, Di Maio L, Bugarin C, Gaipa G, Dander E, Balduzzi A, et al. Regulatory T cells and extracorporeal photochemotherapy: correlation with clinical response and decreased frequency of proinflammatory T cells. Transplantation 2009;87:1422–5.

[22] Schmitt S, Johnson TS, Karakhanova S, Naher H, Mahnke K, Enk AH. Extracorporeal photopheresis augments function of CD4+ CD25+ FoxP3+ regulatory T cells by triggering adenosine production. Transplantation 2009;88:411–6.

[23] Rubegni P, Sbano P, Cevenini G, Perari MG, Marotta G, Risulo M, et al. CD4+CD25+ lymphocyte subsets in chronic graft versus host disease patients undergoing extracorporeal photochemotherapy. Int J Immunopathol Pharmacol 2007;20:801–7.

[24] Mahnke K, Knop J, Enk AH. Induction of tolerogenic DCs: 'you are what you eat'. Trends Immunol 2003;24:646–51.

[25] Steinman RM, Turley S, Mellman I, Inaba K. The induction of tolerance by dendritic cells that have captured apoptotic cells. J Exp Med 2000;191:411–6.

[26] Peritt D. Potential mechanisms of photopheresis in hematopoietic stem cell transplantation. Biol Blood Marrow Transplant 2006;12:7–12.