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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

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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

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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