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Dendritic Cells and Regulatory T Cells Changes During ECP for Chronic GvHD in Pediatric Patients

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Abbreviations: mDC: Myeloid Dendritic Cells; pDC: Plasmocytoid Dendritic Cells; Treg: Regulatory T Cells; GvHD: Graft-versus-host disease; APCs: Antigen-Presenting Cells; ECP: Extracorporeal Photopheresis; HSCT: Hematopoietic Stem Cell Transplantation; DCs: Dendritic Cells; PB: Peripheral Blood; PA: Photoactivated Apheresis; WBC: White Blood Cell

Mini Review

Graft-versus-host disease (GvHD) is a leading cause of post HSCT morbidity and mortality. It is mediated by alloreactive mature donor T lymphocytes, resulting in a harmful inflammatory response and tissue injury [1]. The pathophysiology of GvHD is constituted by precise sequences of immunological events such as the activation of antigen-presenting cells (APCs), activation, differentiation and migration of T cells, and finally the development of their full effector functions [2-4]. Dendritic cells (DCs) constitute the most professional APCs, promoting alloreactivity or clonal antigen-specific T cell responses. Moreover, tolerogenic DCs may play a pivotal role in GvHD exerting an immunomodulatory or even immunosuppressive effect on T cells [5]. DCs can be divided into two major subsets, plasmacytoid DCs (pDCs) and myeloid DCs (mDCs) which have distinct functions. pDCs play a pivotal role in peripheral tolerance through the generation of regulatory T (Treg). In addition to pDCs, mDCs also promote Th2 and Th0/Tr1 responses, depending on the activation signal types [6,7].

Although corticosteroids, with potent immunosuppressive and anti-inflammatory effects, are the first-line treatment for GvHD, only 25-50% of patients respond [1,8]. Extracorporeal photopheresis (ECP) is an alternative therapeutic strategy in patients who are resistant/refractory to steroids. ECP appears to act in an immunomodulatory fashion, inducing immunotolerance in GvHD by regulatory T lymphocytes, dendritic cells, in concert with the normalization of a T lymphocyte subset.

This pilot study focuses on these two cell populations, as well as on the patients' whole immunological pattern [9-11]. Data from eleven patients affected by chronic GVHD were included and analyzed. The median age was 9 years (range 2-18), 6 out of 11 patients were females and suffered of myeloid malignancies (4 patients), acute lymphoblastic leukemia (2 patients), neuroblastoma (1 patient), Hemophagocytic Lymphohistiocytosis (1 patient), Ewing sarcoma (1 patient), Blackfan-Diamond Anemia (1 patient).

Seven patients received an unrelated Hematopoietic Stem Cell Transplantation HSCT (3 bone marrow, 3 peripheral blood, 1 cord blood), three patients had a sibling HSCT (1 patient with 1 Ag mismatch graft, all patients had bone marrow as the stem cell source) and one patient had an phenotypic identical-HSCT). All patients suffered moderate to severe cGvHD which was resistant/refractory to steroids. All patients received ECP from January 2016 to December 2016 in our center according to previously published techniques [12]. The aim of this study was to: i) monitor peripheral blood changes after 30 days of ECP and ii) describe the immunological changes in aphaeresis samples after UVA treatment.

A sample of peripheral blood (PB), a sample of apheresis pre-UVA photoactivation (pre-PA) and a sample of photoactivated apheresis (PA) were collected at the first day of ECP and every week for the first month of treatment. Informed consent was obtained from all patients. PB, pre-PA and PA samples were characterized at day 0, 8, 15, 21, 30 of ECP treatment. The percentage obtained by cytofluorimetric analysis was used to calculate the absolute number of cells/μl based on the white blood cell (WBC) number counted using a standard hemacytometer (DASIT).

Statistical analysis was performed using NCSS for Windows. Descriptive statistics are reported as medians, continuous variable differences between groups were calculated with the Student T test. The fold change was calculated by the ratio between the

difference (day 30 – day 0) /day 0. The P value below 0.05 was considered as statistically significant.

As shown in Table 1, at day 30 we observed a 0.48 change of CD3+. After 30 days of treatment there was an improvement of the CD4+/CD8+ ratio from 0.49 to 0.86 (0.75 times), that resulted in a change of CD3+CD4+ (from 234 to 384 x10^9/L) compared to CD3+CD8+ (from 475 to 448 x10^9/L). Moreover, in the same period of study, we had a change of 0.75 times of CD3-CD56+ from 272 to 478 x10^9/L 62 and a smaller rise of CD19+ from 214 to 256 x10^9/L. The naïve CD4+ and CD8+ lymphocytes rose from 4 to 39x10^9/L (8.75-fold change, P=0.02) and from 27 to 58x10^9/L (1.14-fold change) over a month while their memory counterpart fell by 0.62 and 0.55-fold. When we calculated the CD4+ and CD8+ naïve/memory ratio we found a 35- and 4-fold change (P=0.01 and 0.02). Comparing the day 0 and day 30 peripheral blood samples, we observed a high rise of 0.66 and 1.14 times for mDCs (mDC from 9 to 15 x10^9/L [P=0.03] and pDC from 7 to 15 x10^9/L [P=0.05]) respectively together with a change of Tregs (from 2.9 to 9.1 x10^9/L, 3.5-fold change [P=0.04]). Finally, when we analyzed the changes between pre- and post-UVA photoactivation a significant change of Treg was observed (0.35-fold change, P=0.05), while a decrease in cell number was observed for CD8+ (P=0.05) (Table 2).

Table 1: Immunological changes in peripheral blood following before and after one month of ECP treatment. CD4RA: naïve CD4+ cells, CD4RO: memory CD4+ cells, CD8RA: naïve CD8+ cells, CD8RO: memory CD8+ cells, Treg: regulatory T cells, mDC: myeloid dendritic cells, pDC: plasmocytoid dendritic cells.

	Day 0	Day 30	Fold Change	P
CD3+ x10^9/L	668	990	0,33	0.94
CD3+CD4+ x10^9/L	234	384	0,39	0.55
CD3+CD8+ x10^9/L	475	448	-0,06	0.63
CD4\CD8 RATIO	0,49	0,86	0,43	0.45
CD19 x10^9/L	214	256	0,16	0.53
CD3-CD56+ x10^9/L	272	478	0,43	0.45
CD4+RA+ x10^9/L	4	39	0,90	0.12
CD4+RO+ x10^9/L	282	107	-1,64	0.36
CD4RA+\CD4RO+ RATIO	0,01	0,36	0,96	0.05
CD8+RA+ x10^9/L	27	58	0,53	0.67
CD8+RO+ x10^9/L	290	129	-1,25	0.43
CD8RA+\CD8RO+ RATIO	0,09	0,45	0,79	0.9
Treg x10^9/L	2	9	0,78	0.04
mDC x10^9/L	9	15	0,40	0.03
pDC x10^9/L	7	15	0,53	0.05

Table 2: Details of immunological changes during ECP. Medians are reported. mDC: myeloid dendritic cells, pDC: plasmocytoid dendritic cells, Treg: regulatory T cells.

		Day 0	Day 8	Day 15	Day 21	Day 30	Fold Change	P
CD3+ x10^9/L	Pre UVA	3027	4112	3027	4331	3721	0,02	NS
	Post UVA	3931	4302	3339	4061	3090		
CD4+ x10^9/L	Pre UVA	971	1124	864	2247	1786	-0,13	NS
	Post UVA	1194	1063	701	1688	1482		
CD8+ x10^9/L	Pre UVA	2699	2864	2455	2705	2272	-0,14	0.05
	Post UVA	2218	2633	2169	2250	2130		
CD19+ x10^9/L	Pre UVA	1291	2489	927	2083	1456	0,08	NS
	Post UVA	1355	2583	1225	1930	1798		
CD3-CD56+ x10^9/L	Pre UVA	822	779	776	856	1854	0,16	NS

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	Post UVA	1002	940	962	1239	1821		
CD4+RA+ x10^9/L	Pre UVA	33	48	74	112	56	0,24	NS
	Post UVA	36	118	59	180	97		
CD4+RO+ x10^9/L	Pre UVA	1073	1582	801	1419	769	-0,22	NS
	Post UVA	1016	1256	974	1586	363		
CD8+RA+ x10^9/L	Pre UVA	340	347	901	464	1099	-0,37	NS
	Post UVA	635	237	564	497	470		
CD8+RO+ x10^9/L	Pre UVA	994	1328	1552	675	912	0,15	NS
	Post UVA	1466	1563	1466	1017	953		
mDC x10^9/L	Pre UVA	24	46	37	28	64	0,11	NS
	Post UVA	21	46	37	50	88		
pDC x10^9/L	Pre UVA	32	62	39	38	62	0,33	NS
	Post UVA	41	71	72	53	15		
Treg x10^9/L	Pre UVA	49	38	49	38	50	0,35	0.05
	Post UVA	88	53	88	53	75		

Considering the low number of patients enrolled in this study no firm conclusions can be drawn from a clinical point of view, while a biological effect was certainly highlighted. Our findings are in line with previous publications, however a larger cohort of patients is needed to establish a direct correlation between biological changes and clinical responses.

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