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Blood myeloid and lymphoid dendritic cells reflect Th1/Th2 balance in sarcoidosis and Extrinsic Allergic Alveolitis

Sarcoidosis is a multisystem disease of unknown etiology characterized by the expansion of activated oligoclonal $CD4^+$ T cells and macrophages at sites of disease. The lungs are the most frequently affected organ, and, if untreated, progressive pulmonary inflammation and fibrosis can result in significant respiratory impairment and death. The formation of sarcoid granulomas is associated with characteristic Th1 profile. T cells isolated from the lungs of patients with sarcoidosis show a predominantly Th1 cytokine expression, with elevated mRNA and protein levels of IFN-γ and IL-2. Furthermore, sarcoid alveolar macrophages (AMs) produce high amounts of IL-12, a cytokine which is known to stimulate IFN-γ production and the proliferation of activated T-cells, and is involved in the differentiation of Th0 cells into Th1 cells (2,3,11,12,16).

Extrinsic Allergic Alveolitis (EAA) is a syndrome caused by repeated inhalation and sensitization to an organic antigen. It presents as a clinical syndrome of cough and dyspnoea in some individuals who are exposed to organic dusts, chemicals or drugs and is an immunological, inflammatory response involving alveoli, bronchioles and interstitium. The inciting antigens reach alveoli by inhalation, e.g. organic dusts, but may also do so by bloodstream, e.g. drugs. EAA is an important disease since it may result in acute or chronic illness. In patients with chronic EAA, unless the diagnosis is established and exposure to the antigen terminated, progressive irreversible lung disease may occur with fibrosis and pulmonary insufficiency. Although there is a widespread exposure to antigens, it has been estimated that only between 5 and 15% of exposed population will develop EAA (5,6,8,9). Little is known about the Th1/Th2 regulatory network in EAA. Animal models suggest that EAA is facilitated by overproduction of IFN-γ, and that IL-10 ameliorates severity of the disease indicating Th1 response. Few clinical studies seem to confirm the assumption (6,19).

Dendritic cells (DCs) are now recognized as essential regulators of both innate and acquired arms of the immune system. DCs, in addition to their distinctive morphology, have a number of phenotypic characteristics that make them the best of all Antigen Presenting Cells (APC). DCs bear sole responsibility for the stimulation of naive T lymphocytes, a property that distinguishes them from all other APC. They may also be important for the regulation of the Th1 or Th2 type of T cell mediated immune responses. Two distinct lineages of DCs have been described in humans. Blood myeloid dendritic cells (BDCA-1+), when stimulated, produce high levels of Il-12 (just as AMs) and drive T cell differentiation into Th1 lymphocytes. Blood lymphoid dendritic cells (BDCA-2+) have been recently described in human peripheral blood

and lymphoid tissue. BDCA-2+ cells after appropriate activation induce T cell differentiation into Th2 cells (7,10,13,17).

MATERIAL AND METHODS

Patients. The stud population consisted of five patients with I stage sarcoidosis of mean age 41 ± 11 years, six patients with EAA of mean age 43 ± 16 years. The diagnosis of pulmonary sarcoidosis was based on clinical presentation, X-ray abnormalities and histopathological analysis. The diagnosis of EAA was based on exposure history, clinical assessment, radiographic and physiological findings, removal of the patient from the suspected etiologic exposure and inhalation challenge to the suspected antigen in a hospital setting.

Material. The material consisted of bronchoalveolar lavage fluid (BALF) and peripheral blood. Bronchoalveolar lavage (BAL). The patients were pre-medicated intramuscularly with atropine (0.5 mg). After local anaesthesia with 4% lignocaine a flexible fibreoptic bronchoscope (Olympus BF-20) was wedged into a middle lobe bronchus and 140 ml sterile physiological saline solution at body temperature was instilled in seven aliquots of 20 ml. The first aliquot recovered was used for bacteriological analysis and the remaining were pooled. The fluid was mixed and strained through double layer of nets. The cells were centrifuged at 500 x g for 5 min. at 4 degrees C and the pellet cells were resuspended in phosphate buffered saline (PBS) without Ca^{2+} and Mg^{2+}, with 0.5% bovine serum albumin (BSA, Sigma, USA) and 2mM EDTA (Sigma, USA) for future staining. The cells were counted in Bürker chamber.

Preparation of cells from peripheral blood. Blood samples from patients were taken just before BAL was performed. Ten milliliters of blood were taken by venipuncture from each patient and collected in sterile heparinized tubes. Mononuclear cells were isolated by density gradient centrifugation on Lymphoprep (Nycomed, Norway). Interphase cells were removed, washed twice in PBS without Ca^{2+} and Mg^{2+}, with 0.5% bovine serum albumin (BSA, Sigma, USA) and 2mM EDTA (Sigma, USA) and than resuspended for future immunostaining. The cells were counted in Bürker chamber.

Monoclonal antibodies. The cells from BALF and peripheral blood were labeled by direct staining with the following monoclonal antibodies: IgG1-FITC/IgG2a-PE (Becton Dickinson, USA), mouse anti-human BDCA-1-FITC (Miltenyi-Biotec, Germany), BDCA-2-FITC (Miltenyi-Biotec, Germany), CD123-PE (Becton Dickinson, USA), CD19-CyChrome (Pharmingen, USA). After washing, 20 µl of FcR Blocking Reagent (Miltenyi-Biotec, Germany) were added to every 10^7 cells resuspended in 80 µl of the buffer. Monoclonal antibodies were added according to the manufacturer's protocol and incubated with the cells for 10 min in the dark at 4°C. After the incubation the cells were washed in 10 volumes of the buffer and centrifuged for 10 min at 300g and 4°C.

Table 1. Percentages of DCs in peripheral blood and BALF

		Peripheral blood			BALF		
		BDCA-1	BDCA-2	BDCA-1/BDCA-2 ratio	BDCA-1	BDCA-2	BDCA-1/BDCA-2 ratio
EAA	mean	0.38	0.13	4.09	2.28	7.85	2.96
	SD	0.15	0.11	2.86	1.66	14.33	4.57
I stage sarcoidosis	mean	0.64	0.42	1.45	0.96	2.41	0.59
	SD	0.58	0.29	0.55	0.68	2.49	0.47

Flow cytometric analysis. All samples were measured on a FACSCalibur flow cytometer equipped with 488-nm argon laser (Becton Dickinson, USA) and analyzed with CellQuest Software. We collected 300 000 of events in total.

Statistical analysis. We analyzed the percentages of BDCA-1+ and BDCA-2+ cells and the BDCA-1+/BDCA2+ ratio in BALF and peripheral blood in the two groups of patients. The results were presented as mean with SD. Mann-Whitney U test was applied for the statistical comparison, the $P<0.05$ was considered significant.

RESULTS

It was observed that the percentage of lymphoid dendritic cells in peripheral blood from patients with sarcoidosis was significantly higher ($p<0.05$) compared to the EAA. Furthermore, in sarcoidosis the BDCA-1/BDCA-2 ratio in peripheral blood was significantly lower ($p<0.05$) than in EAA. We did not find any statistical differences of myeloid DCs percentages in peripheral blood and both myeloid and lymphoid DCs percentages from BALF in sarcoidosis vs EAA, although the BDCA-1/BDCA-2 ratio was higher in EAA. In case of sarcoidosis we found that the BDCA-1/BDCA-2 ratio was significantly higher in peripheral blood than in BALF ($p<0.05$). In EAA the ratio is also higher, but without statistical significance.

DISCUSSION

To our knowledge, this is the first study trying to assess the role of dendritic cells subpopulations in sarcoidosis and Extrinsic Allergic Alveolitis.

T helper CD 4^+ lymphocytes are divided into three subsets, according to the type of produced cytokines. T helper 1 (Th1) lymphocytes produce interleukin-2 and IFN-γ and induce cell-mediated immunity. T helper 2 (Th2) lymphocytes produce Il-4, Il-5, Il-10 and Il-13 and enhance B lymphocytes proliferation and differentiation (in general humoral-mediated immunity). These subpopulations of T helper lymphocytes originate from the third subset of T helper lymphocytes – T helper 0 (Th0) lymphocytes, which produce both types of cytokines (1,14,15).

Dendritic cells (DCs) are the only antigen-presenting cells that can prime naive T cell to a new antigen. Two distinct lineages of DCs have been described in humans. Blood myeloid dendritic cells (BDCA-1+) express myeloid antigens CD11c, CD13 and CD 33, they originate from myeloid bone marrow precursors and require the presence of GM-CSF for their survival. In humans peripheral blood BDCA-1+ cells are identified as negative for lymphoid and myeloid cell-specific markers (lin^-) and $HLA\text{-}DR^+/CD11c^+$. BDCA-1+ cells produce high levels of Il-12 when stimulated with tumor necrosis factor-α (TNF-α) or CD40L and drive T cell differentiation into Th1 lymphocytes. Blood lymphoid dendritic cells (BDCA-2+) have been recently described in human peripheral blood and lymphoid tissue as $HLA\text{-}DR^+/lin^-/CD11c^-/CD4^+$Il-$3R\alpha^+$ plasmacytoid cells. BDCA-2+ cells after appropriate activation induce T cell differentiation into Th2 cells (7,10,13).

In order to assess the significance of dendritic cells in sarcoidosis and EAA we made an effort to estimate the populations of myeloid and lymphoid DCs in peripheral blood and BALF.

In our study we showed the prevalence of myeloid DCs in peripheral blood in both of the diseases, and in this way predominance of Th1-type immunity. This is consistent with the previous reports. Moller et al found elevated mRNA and protein levels of IFN-γ but not IL-4, in

sarcoid lung cells and fluid compared with those in normal subjects (11). Yamasaki et al. revealed increased production of IFN-γ in EAA, but not IL-4 (19). It is known that Il-12 is produced by antigen presenting cells and stimulates differentiation of T helper 0 cells into Th1 lymphocytes (6, 19). One source are alveolar macrophages, yet it seems possible that another of the sources are myeloid DCs. Moller et. al hypothesize that sarcoidosis is a Th1-mediated disease driven by chronic, dysregulated production of IL-12 at sites of disease (11). But the results from BALF indicate the dominance of myeloid DCs only in EAA. Although there is no statistical significance in this case, it may result from eosinophilic inflammation connected with Th2 immunity, which is quite often associated with EAA (4, 18). Explanation of higher BDCA-1+/BDCA-2+ ratio in peripheral blood than in BALF in sarcoidosis is difficult, however, the wide recognized lung compartmentalization in sarcoidosis may be considered. This may also suggest decreased antigen stimulation or impaired antigen presentation in lungs.

In conclusion, our results not only seem to confirm reports that sarcoidosis and EAA are Th1-type immunity diseases, but they also suggest involvement of dendritic cells in the immune regulation in the diseases.

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SUMMARY

Dendritic cells play a specific regulatory role in the immune system. In this paper, the significance of myeloid and lymphoid dendritic cells in sarcoidosis and extrinsic allergic alveolitis (EAA) was evaluated. Myeloid dendritic cells are connected with Th1 type of immunological response, whereas lymphoid ones – with Th2 type. The latest findings indicate that both diseases are characterized by serious disturbances of Th1/Th2 response to Th1 dominance. Our studies seem to confirm these suggestions. In the peripheral blood of patients with sarcoidosis as well as with EAA, myeloid dendritic cells outnumbered lymphoid ones.

Występowanie mieloidalnych i limfoidalnych komórek dendrytycznych we krwi obwodowej u chorych na sarkoidozę i zewnątrzpochodne zapalenie pęcherzyków płucnych

Komórki dendrytyczne pełnią szczególną funkcję regulacyjną w układzie immunologicznym. W niniejszej pracy oceniono znaczenie mieloidalnych i limfoidalnych komórek dendrytycznych w sarkoidozie i w alergicznym zapaleniu pęcherzyków płucnych (AZPP). Mieloidalne komórki dendrytyczne wiązane są odpowiedzią typu Th1, podczas gdy limfoidalne z Th2. Najnowsze dane wskazują na istotne zaburzenia równowagi odpowiedzi immunologicznej Th1/Th2 w obydwu tych chorobach z przesunięciem w kierunku Th1. Nasze badania wydają się potwierdzać te sugestie. We krwi obwodowej pacjentów z sarkoidozą jak i AZPP stwierdziliśmy przewagę mieloidalnych komórek dendrytycznych nad limfoidalnymi.