Analysis of the level of B-cell populations in patients with pulmonary sarcoidosis*

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Sarcoidosis is a granulomatous disease of unknown etiology. The role of B-cells in pathogenesis of sarcoidosis was shown in several studies. The importance of B-lymphocytes activation in sarcoidosis can be confirmed by the efficacy of anti-CD20 treatment. However, there is no data about alteration of certain B-cells subsets. In this study changes in subsets of B-lymphocytes in patients with sarcoidosis (n = 37) and a control group (n = 30) are analyzed. In patients with sarcoidosis misbalance of B-naïve and B-memory cells was found, the level of B-naïve cells proved to be more than 70.00 % and B memory cells less than 30.00 % (sensitivity 76 %, specificity 70 %). Among regulatory B cells there was detected an elevation of CD24+++CD38+++ B cells more than 6.5 % (sensitivity 91 %, specificity 88 %) and CD5+CD27-B cells than 12.45 % (sensitivity 76 %, specificity 80 %). These results show, that in sarcoidosis pathogenesis are involved B-naïve and B-memory cells, that is characteristic for granulomatous diseases, and also regulatory B cells, that produce anti-inflammatory cytokines.

Keywords: sarcoidosis, autoimmunity, subsets, B cells, humoral immune response.

Introduction

Nowadays the theory of the autoimmune origin of sarcoidosis is becoming increasingly popular. One of the most important evidence is the presence of an autoimmune component of the humoral immune response. In patients with sarcoidosis there were found an increase in immunoglobulins, a change in the ratio of B-cell subsets, an increase in B-cell growth factor, elevated levels of various autoantibodies [1–3]. The additional confirmation of the role of B cells in the pathogenesis of sarcoidosis is the effectiveness of anti-B cell therapy in patients with sarcoidosis [4].

Aim of the study

To characterize changes in subsets of CD19 + B cells in patients with pulmonary sarcoidosis.

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Materials and methods

In 2016–2018 the whole peripheral blood samples of 37 patients with sarcoidosis stage 2 (men, n = 25 (67.6 %), women, n = 12 (32.4 %), the average age 32.4 (±6.7) years) and 35 age and sex-matched control healthy controls were studied at the St. Petersburg Research Institute of Phthisiopulmonology. The diagnosis of pulmonary sarcoidosis was performed according to the standard criteria of the American Thoracic Society (ATS), the European Respiratory Society (ERS) and the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG). All patients underwent a complex examination, including medical survey, multispiral chest computed tomography (MSCT), laboratory blood tests, the level of angiotensin converting enzyme (ACE), standard tuberculosis tests (TB. T-SPOT), histological verification of the lung and intrathoracic lymph nodes lesions (using a transbronchial and videothoracoscopic biopsy).

Multicolor flow cytometry was made with Navios flow cytometer using specific fluorochrome-conjugated monoclonal antibodies to IgD, CD183 (CXCR3), CD19 (BioLegend, Inc. (USA)), CD38, CD27, CD24, CD5, CD45 (Beckman Coulter, Inc (USA)). Thus in each sample 5000 CD19+ B cells were determed. Obtained data were analyzed with Kaluza software (Beckman Coulter, Inc., USA). Using this monoclonal antibodies CD19+ B cells were classified as B-naïve (IgD+CD27-), B-memory (IgD-CD27+) and B-regulatory cells, that includes CD24+++CD38+++ B cells and CD5+CD27- B cells.

The statistical analysis was performed using the Mann–Whitney U test, ROC-analysis. The differences between the groups were considered significant when p values were < 0.05. All of the statistical analysis of data was carried out with GraphPad Prism Version 6.0.

The results

The level of B cells in sarcoidosis patients was significantly higher than in HC (14.48 % (9.86; 17.29) vs. 11.09 % (8.52; 13.28), $p = 0.008$).

According to the Mann–Whitney U analysis there was found a significant statistical difference in comparison with healthy controls in following subsets: B-naïve IgD+CD27– cells, B-memory IgD–CD27+cells, CD24+++CD38+++ B cells and CD5+CD27- B cells ($p < 0.0001$ for all).

With the ROC-analysis there were determined the possible cut-off meanings for sarcoidosis patients. The most significant difference with the healthy controls was found in CD24+++CD38+++ B cells subset with increase more than 6.5 % (sensitivity 91 %, specificity 88 %, AUC = 0.904). Also for patients with sarcoidosis was typical an increase of B naïve cells more than 70.00 % (sensitivity 76 %, specificity 70 %), and decrease of B memory cells less than 30.00 % (sensitivity 76 %, specificity 70 %), AUC = 0.819. Analysis of level CD5+CD27-B cells showed the possible reference meaning that is more than 12.45 % (sensitivity 76 %, specificity 80 %, AUC = 0.7951) for patients with sarcoidosis.

Conclusions

In this study there were demonstrated the possible characteristics of changes in B-cells subpopulations in sarcoidosis patients. It was shown that for sarcoidosis is typical misbalance of B-naïve and B-memory cells, where the level of B-naïve cells is more than 70.00 % and B memory cells less than 30.00 % (sensitivity 76 %, specificity 70 %). The same changes were found in patients with chronic form of sarcoidosis [5] and several autoimmune diseases [6].

Analysis of regulatory B cells showed the increase of CD24+++CD38+++ B cells more than 6.5 % (sensitivity 91 %, specificity 88 %) and CD5+CD27-B cells more than 12.45 % (sensitivity 76 %, specificity 80 %). These cells produce an anti-inflammatory Il-10 and the misbalance of it with an increase was shown in some autoimmune diseases such as Sjogren's syndrome and systemic lupus erythematosus [7]. Such alteration in regulatory B-cells subsets might be explained by temporal compensation during the inflammatory process [8].

These results demonstrate the role of B-naïve, B-memory and B-regulatory cells in pathogenesis of sarcoidosis. The obtained cut-off values of their content can be useful in comparative studies of different granulomatous diseases.

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