

ЭКСПРЕССИЯ CD39 РЕГУЛЯТОРНЫМИ Т-ЛИМФОЦИТАМИ ПРИ ХРОНИЧЕСКОМ И ОСТРОМ САРКОИДОЗЕ

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Резюме. Саркоидоз – заболевание неустановленной этиологии, характеризующееся развитием эпителиоидно-клеточных гранул без некроза в различных органах. В настоящее время выделяют два основных клинических варианта дебюта саркоидоза: вариант острого/подострого течения саркоидоза (ОС, или синдром Лёфгрена) с более благоприятным прогнозом, а также хроническая форма течения саркоидоза (ХС, или «не Лёфгрена-синдром») с высоким (около 20%) риском развития фиброза легких. Целью данного исследования было изучение фенотипических характеристик регуляторных Т-лимфоцитов (Treg) периферической крови больных с острым (n = 11) и хроническим (n = 46) дебютом саркоидоза, контролем служили образцы периферической крови, полученные от 26 условно здоровых добровольцев. С использованием многоцветной проточной цитометрии было показано, что при ОС уровень Т-клеток относительного группы контроля, а при ХС уменьшается уровень CD3⁺CD4⁺ клеток при сравнении с контролем. Более того, при ХС как относительное, так абсолютное содержание Treg в периферической крови достоверно ниже значений контрольной группы (2,83% (2,47; 3,36) против 3,33% (2,79; 3,84) при p = 0,021 и 37 кл/мкл (29; 52) против 50 кл/мкл (42; 65) при p = 0,004 соответственно). Относительное содержание CD39-позитивных клеток в рамках общей популяции Treg возрастает с 39,52% (11,55; 46,34) как при хроническом (до 51,02% (38,20; 61,62), p < 0,001), так и при остром (до 48,64% (41,46; 63,72), p = 0,007) дебюте саркоидоза. Анализ экспрессии CD39 и CD73 основными субпопуляциям Treg не выявил достоверных различий по относительному содержанию позитивных клеток между всеми группами в рамках «наивных» CD45R0⁻CD62L⁺ клеток. Вместе с тем уровень CD39⁺ клеток среди CD45R0⁺CD62L⁺Treg у больных как ОС, так и ХС достоверно (p < 0,001 и p = 0,004 соответственно) превышал контрольные значения (69,66% (61,92; 79,34) и 67,62% (61,92; 79,34), соответственно, против 47,55% (15,74; 65,32)). В случае CD45R0⁺CD62L⁻Treg, способных покидать кровотоки и мигрировать в очаги воспаления, увеличение относительного содержания CD39-позитивных Treg было отмечено лишь при сравнении ХС и группы контроля (61,79% (55,12; 73,09) и 57,27% (16,03; 66,98) соответственно, при p = 0,006). Получен-

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ные нами результаты указывают, что при саркоидозе в циркуляции увеличивается уровень CD39⁺Treg, способных эффективно подавлять иммунные реакции за счет утилизации провоспалительного АТФ и инициации каскада реакций, приводящих к формированию провоспалительного аденозина.

Ключевые слова: саркоидоз, регуляторные T-лимфоциты, экспрессия CD39, проточная цитометрия, многоцветный анализ

CD39⁺ EXPRESSION BY REGULATORY T CELLS IN PULMONARY SARCOIDOSIS AND LOFGREN'S SYNDROME

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Abstract. Sarcoidosis is a disorder of unknown etiology characterized by development of necrosis-free epithelioid cell granulomas in various tissues. There are two main phenotypes of pulmonary sarcoidosis (PS): Lofgren's syndrome (LS) is an acute form with favorable outcome, while non-Lofgren's syndrome (nLS) is a chronic type of disease that can lead to pulmonary fibrosis in 20% of cases.

Our study was aimed at investigating changes in the main cell-surface differentiation antigens on peripheral blood regulatory T cells (Tregs) from the patients with first diagnosed PS without treatment (LS, n = 11) and nLS (n = 46) compared to healthy volunteers (HC, n = 26).

These indexes might be used as immunological markers for predicting severity of this disorder. Flow cytometry analysis of peripheral blood cell samples demonstrated that the nLS patients had decreased relative numbers of CD3⁺ cells vs healthy controls, as well as diminished CD3⁺CD4⁺ cells vs HC and LS patients. Furthermore, the relative and absolute Treg numbers were also decreased in nLS group vs HC (2.83% (2.47; 3.36) vs 3.33% (2.79; 3.84), p = 0.021), and 37 (29; 52) cells vs 50 (42; 65), p = 0.004, respectively) per one microliter of peripheral blood. Relative number of CD39-positive Tregs in chronic vs acute sarcoidosis patients was associated with 51.02% (38.20; 61.62) vs 48.64% (41.46; 63.72) that was significantly (p < 0.001 and p = 0.007, respectively) higher than in HC (39.52% (11.55; 46.34)). We have found that "naïve" (CD45R0⁻CD62L⁺) Tregs did not significantly differ in percentage of CD39- and CD73-positive cells in all the groups tested. Moreover, CD45R0⁺CD62L⁺ Tregs in LS and nLS patients contained significantly more CD39-positive cells (69.66% (61.92; 79.34) and 67.62% (61.92; 79.34), respectively, compared to 47.55% (15.74; 65.32) in HC (p < 0.001 and p = 0.004, respectively). In case of CD45R0⁺CD62L⁻ Tregs able to exit from the circulation and migrate to the site of inflammation, an increased percentage of CD39-positive subset was noted only in patients with chronic sarcoidosis and HC (61.79% (55.12; 73.09) and 57.27% (16.03; 66.98), p = 0.006). Enhanced CD39 expression on Tregs seems to be related to chronic immune response, so that antigen elimination becomes impossible due to Treg overactivation, as shown in patients with sarcoidosis and some other chronic autoimmune and infectious disorders.

Keywords: sarcoidosis, regulatory T cells, CD39 expression, flow cytometry, multi-color immunophenotyping

Introduction

Sarcoidosis is a disorder of unknown etiology characterized by development of necrosis-free epithelioid cell granulomas in various tissues [14; 32]. Numerous studies point at cell-mediated immune reactions mediated by type 1 (Th1) and type 17 (Th17) T helper cells playing an important role in formation of sarcoid granuloma (for more detail see [16] and [22]). Th1 cells producing interferon-gamma

(IFN γ) display CCR4⁻CCR6⁻CXCR3⁺ phenotype, whereas Th17 cells mainly produce IL-17A and express CCR6⁺CCR4⁺CXCR3⁻ markers on the surface [26]. More recently, "non-classical" Th17 cell subset called "Th1/Th17" or "Th17.1" exhibiting features of both Th1 and Th17 cells was described in human, which also expresses nuclear transcription factors TBX21 and RORC able to produce IFN γ and IL-17A [30]. Perhaps, a major role in pathogenesis of sarcoidosis is played solely by these cells able to

activate simultaneously tissue macrophages as well as polymorphonuclear leukocytes migrating from peripheral blood to the site of inflammation [34].

In addition, still it remains unclear what T cell subset migrating to target organs as well as genetic predisposition factors are particularly responsible for beneficial or unfavorable course of sarcoidosis. Some investigators noted that development of spontaneous remission in sarcoidosis occurs in 7-20% (or even more) cases [1, 2, 7]. Moreover, a more favorable prognosis was associated with Lofgren's syndrome or acute or subacute onset of sarcoidosis [7, 13]. Perhaps, various forms of sarcoidosis may develop due to distinct immunoregulatory mechanisms either contributing to regression or resulting in chronic course and fibrogenesis.

Activation of FoxP3⁺ regulatory T cells (Tregs) represents one of the key mechanisms restraining overactive immunological reactions. A timely Treg activation allows to prevent development of a whole set of immune-mediated pathological processes [8]. Moreover, it is currently believed that a sole balance between anti-inflammatory Tregs and pro-inflammatory Th17 cells plays a crucial role in restraining inflammatory reactions occurring within the site of inflammation [28]. Antagonistic interactions between these two helper T cell subsets in the circulation, secondary lymphoid organs and peripheral tissues mainly underlies an efficacy of immune response and the onset of regenerative processes under various pathological settings.

Currently, a whole body of evidence have been gained about origin, phenotypic features, intracellular signal transduction and suppression mechanisms related to Tregs. Owing to this, they are considered as targets for new therapeutic strategies in autoimmune and allergic disorders as well as in oncology diseases. Importantly, a number of various subsets may be distinguished within Treg population, by describing at least two major subsets differing by their origin [15]. Firstly, thymus-derived or natural Tregs are differentiated in the thymus during antigen-independent stage of T cell maturation, which express markers of naïve peripheral blood CD45R0-CD62L⁺T cells. On the other hand, peripheral or inducible Tregs are generated during antigen-dependent differentiation in various peripheral lymphoid tissues. In addition, some investigators [25] refer CD45R0⁺Tregs to resting or non-activated regulatory T cell subset, whereas activated Tregs are defined by expressing CD45R0 surface marker.

Innate (tissue macrophages, antigen-presenting cells, NK cells) and adaptive (effector cytotoxic T cells, helper T cells, B cells) immune cells are the main targets for Tregs. Various mechanisms are involved in mediating functions of Tregs including cell contact-dependent effects via interaction between

Treg receptors and surface proteins on target cells, as well as cell contact-free effects mediated by soluble molecules released by regulatory T cells [33]. Surface CTLA-4 (CD152), neuropilin-1 and LAG-3 (CD223) on Tregs are able to downregulate expression of co-stimulatory molecules and major histocompatibility complex class II molecules on antigen-presenting cells that result in controlling magnitude of cell clonal expansion [19].

It was shown that interaction between galectin-9 on the surface of Treg and TIM-3 (CD366) protein on effector lymphocyte subset results in induction of apoptosis in the latter cell type. In addition, apoptosis of effector T cells may also be mediated via cell contact-free mechanisms by perforins and granzymes secreted by Tregs acting on target cells [19]. Tregs produce anti-inflammatory cytokines such as IL-10, IL-35, and TGF- β , which mainly target effector immune cells subsequently resulting in decreased proliferative potential as well as their differentiation towards effector subsets, down-modulated production and secretion of various cytokines. While acting on antigen-presenting cells, anti-inflammatory cytokines decrease density of the expressed costimulatory proteins, efficacy of antigen presentation and cytokine production necessary for T cell maturation [19].

On the other hand, degradation of pro-inflammatory extracellular ATP to adenosine that acts on a broad range of innate and adaptive immune cells represents another important immunosuppressive mechanism mediated by Tregs both in lymphoid tissues and within the site of inflammation [19]. In particular, two major ectonucleosides CD39 (E-NTPDase1, or ectonucleoside triphosphate diphosphohydrolase 1) and CD73 (Ecto-5'NTase, or ecto-5'-nucleotidase) cleave ATP to ADP, pyrophosphate and AMP, as well as to adenosine and phosphate, respectively [4, 37].

Clinical and immunological comparisons performed in patients with various course of sarcoidosis prior to the onset of immunosuppressive therapy, analysis of chemokine receptor profile on peripheral blood lymphocyte subsets including regulatory T cells allow to obtain new data about a potential role of these cell types played in pathogenesis of sarcoidosis. Therefore, our study was aimed at investigating changes in the key surface differentiation markers on Tregs in peripheral blood from patients with sarcoidosis prior to immunosuppressive therapy, which might be used as immunological markers for predicting severity of this disorder.

Materials and methods

Peripheral blood samples were collected into vacuum test tubes added with K₃EDTA. All patients provided an informed consent. Manipulations and assays were performed in accordance with Helsinki Declaration of the World Medical Association on

the Ethical Principles of Scientific Medical Research with Human Participation (1964, with subsequent additions, including the 2000 version) as well as the Order No. 266 On Approval of Rules of Good Clinical Practice in the Russian Federation (dated of June 19, 2003, registered by the Ministry Health of the Russian Federation).

All laboratory experiments were conducted at the day collecting blood samples. There were enrolled 11 and 46 patients with acute (Lofgren's syndrome) and chronic (non-Lofgren's syndrome) sarcoidosis, respectively. A comparison group consisted of 26 age- and sex-matched healthy volunteers. Peripheral blood samples were prepared for flow cytometry assay according to recommendations published before [12]. A panel of monoclonal antibodies conjugated to various fluorochromes was used to identify main T cell subsets and to examine their CD39 and CD73 surface expression [3]: CD39-FITC (clone A1, cat. #328206, BioLegend, Inc., USA), CD25-PE (clone B1.49.9, cat. #A07774, Beckman Coulter, USA), CD62L-ECD (clone DREG56, cat. #IM2713U, Beckman Coulter, USA), CD45R0-PC5.5 (clone UCHL1, cat. #IM2712U, Beckman Coulter, USA), CD4-PC7 (clone SFC112T4D11 (T4), cat. #737660, Beckman Coulter, USA), CD8-APC (clone B9.11, cat. #IM2469, Beckman Coulter, USA), CD3-APC-Alexa Fluor 750 (clone UCHT1, cat. #A94680, Beckman Coulter, USA), CD73-Pacific Blue (clone AD2, cat. #344012, BioLegend, Inc., USA), and CD45-Krome Orange (clone J33, cat. #A96416, Beckman Coulter, USA). Flow-Count Fluorospheres (cat. #7547053, Beckman Coulter, USA) were used to measure absolute counts of the examined T cell subsets, by recalculating relative percentage into absolute numbers presented as cell count per 1 μ L peripheral blood. Distribution of antibodies in different fluorescence channels was performed in accordance with the principles for creating multi-color flow cytometry panels [11, 23], which were used to stain 100 μ L peripheral blood sample, according to the manufacturer's recommendations. Red blood cells were removed by adding *ex tempore* prepared 975 μ L lysis buffer VersaLyse (cat. #A09777) followed by adding 25 μ L IOTest 3 Fixative Solution (cat. #A07800). After red blood cells were lysed, samples were washed out once with excess saline at 330g for 7 minutes followed by decanting supernatant. Then, cell pellet was resuspended in saline (pH 7.2-7.4) containing 2% paraformaldehyde (Sigma-Aldrich, USA). Finally, blood samples were analyzed by using a flow cytometer Navios™ (Beckman Coulter, USA) equipped with 405, 488, and 638 nm diode lasers.

To identify major subsets among peripheral blood T cells, an algorithm earlier described by [29] was applied. In brief, at least CD45-positive 40,000 singlet events were analyzed in each sample. After that,

regulatory T cells were identified as CD4⁺CD25^{bright} T cells. Our preliminary data and those published by others [18] allowed to conclude that a relative percentage of T cells expressing a transcription factor FoxP3 within this population was as high as 90-92%, therefore allowing to apply this approach for identifying Tregs without permeabilization and subsequent co-staining for nuclear FoxP3 transcription factor. In addition, Tregs were further evaluated for surface expression of CD45R0 and CD62L markers as published before [9, 10]. In particular, naïve Tregs were defined as CD45R0⁻CD62L⁺, central memory (CM) subset – as CD45R0⁺CD62L⁺, effector memory (EM) – as CD45R0⁺, whereas CD45RA-positive terminally differentiated effector T cells (TEMRA) – as CD45R0⁻CD62L⁻ cells. Finally, these Treg subsets were analyzed for surface expression of CD39 and CD73 markers [29].

Flow cytometry data were processed by using software Navios Software v.1.2 and Kaluza™ v. 1.2 (Beckman Coulter, USA). Statistical analysis was performed by applying software Statistica 8.0 (StatSoft, USA) as well as GraphPad Prism 4.00 for Windows (GraphPad Prism Software Inc., USA). A normality of a distribution was checked by using Pearson's Chi-square test. The data in the main text and in Figure notes on percentage of marker-positive cells out of T cell subset as well as absolute number per 1 μ L of peripheral blood, by showing results as median and inter-quartile range (25%; 75%). Paired quantitative comparisons were analyzed by applying a non-parametric Mann-Whitney U-test.

Results

Flow cytometry analysis of peripheral blood samples demonstrated that patients with primary chronic sarcoidosis vs control group had decreased both relative (70.83% (63.93; 77.26) vs (77.06% (75.33; 78.52), respectively, $p < 0.001$) and absolute numbers (931 (717; 1086) vs 1218 (964; 1758) cells per 1 μ L of peripheral blood, respectively, $p = 0.010$) of total CD3⁺T cells. No significant differences between these parameters were found in patients with acute sarcoidosis vs control group as well as comparing with chronic sarcoidosis ($p = 0.984$ and $p = 0.075$, as well as $p = 0.335$ and $p = 0.283$, respectively).

A percentage of total CD3⁺CD4⁺T (Th) cells was significantly lower in patients with chronic sarcoidosis (41.66% (34.05; 45.94) out of total lymphocyte population) compared to both control (48.40 (43.66; 50.77), $p < 0.001$) group and patients with acute-onset sarcoidosis (53.57% (43.30; 55.84), $p = 0.004$). Moreover, a number of Th cells in patients with chronic sarcoidosis was decreased from 737 (501; 1198) down to 529 (423; 702) cells per 1 μ L ($p = 0.006$), whereas in patients with acute sarcoidosis it was (656 (541;

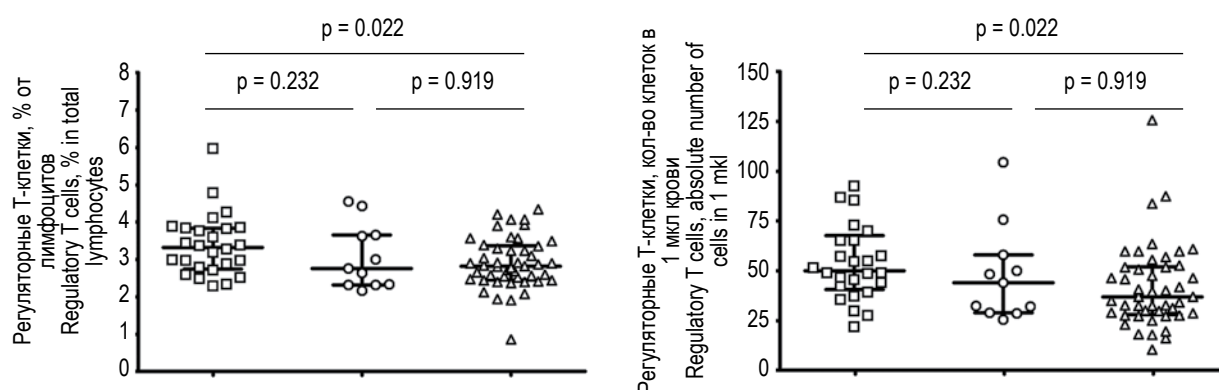


Рисунок 1. Относительное (слева) и абсолютное (справа) содержание регуляторных Т-клеток в периферической крови больных с острым и хроническим саркоидозом

Примечание. Здесь и далее на рисунках 2-4: квадраты – группа условно здоровых доноров (n = 26); круги – больные с острой формой саркоидоза (n = 11); треугольники – больные с хронической формой саркоидоза (n = 46). Результаты представлены в виде медианы и интерквартильного размаха ($Q_{0,25}$ - $Q_{0,75}$). Различия между сравниваемыми группами указаны согласно непараметрическому критерию Манна–Уитни.

Figure 1. Relative (left) and the absolute (right) number of regulatory T cells in peripheral blood from patients with acute (Lofgren's Syndromes) and chronic (non-Lofgren's Syndromes) sarcoidosis

Note. Hereinafter, symbols in Figure 1-4 denote the following groups: squares, healthy control group (n = 26); circles, patients with acute sarcoidosis (Lofgren's Syndromes, n = 11); triangles, patients with chronic sarcoidosis (non-Lofgren's Syndromes, n = 46). The data are presented as median with interquartile range ($Q_{0,25}$ - $Q_{0,75}$). Differences between the groups calculated by nonparametric Mann–Whitney U-tests are shown.

Left: % Tregs out of total lymphocyte count. Right: Abs. number Tregs.

864) cells/ μ L); however, no significant differences in both comparison groups were found.

Analysis of the relative and absolute $CD3^+CD4^+CD25^{bright}$ Treg cell counts in peripheral blood is shown in Figure 1. In particular, patients with chronic sarcoidosis were noted to have lowered Treg percentage in the total lymphocyte population from 3.33% (2.79; 3.84) to 2.83% (2.47; 3.36, $p = 0.021$), which was even more pronounced for absolute counts: 50 (42; 65) vs 37 (29; 52) cells per 1 μ L, respectively ($p = 0.004$).

Assessing expression of ectonucleotidases CD39 and CD73 playing a crucial role in cleaving pro-inflammatory ATP to anti-inflammatory adenosine [4] on the surface of circulatory Tregs revealed marked differences between the groups examined (Figure 2). A relative percentage of CD39-positive Treg subset was remarkably higher in patients with sarcoidosis vs control group. In particular, chronic vs acute sarcoidosis was associated with 51.02% (38.20; 61.62) vs 48.64% (41.46; 63.72) that was significantly ($p < 0.001$ and $p = 0.007$, respectively) higher than in control group (39.52% (11.55; 46.34). further examination demonstrated that relative percentage of CD73-positive Tregs was decreased only in patients with chronic sarcoidosis compared to control group (3.55% (2.11; 4.78) and 4.78% (3.67; 5.91), respectively, $p = 0.011$).

Next, experiments were performed by analyzing surface expression of CD45R0 and CD62L on Tregs

allowing to subdivide them as follows: naïve Tregs bear $CD45R0^+CD62L^+$, whereas central memory (CM) and effector memory (EM) Treg subsets had phenotype $CD45R0^+CD62L^+$ and $CD45R0^+CD62L^-$, respectively. Frequency and absolute numbers of these Treg subsets are shown in Figure 3. In particular, it was found that patients with chronic sarcoidosis vs control group were characterized by increased percentage of EM Tregs (13.48% (10.69; 17.97) vs 11.21% (8.07; 13.96), $p = 0.026$). Upon that, absolute number of naïve and CM Tregs was decreased in patients with chronic sarcoidosis vs controls (9 (6; 17) vs 15 (11; 24) cells/ μ L and 21 (17; 26) vs 29 (21; 37) cells/ μ L, $p = 0.007$ and $p = 0.002$, respectively). However, these parameters did not significantly differ in patients with acute sarcoidosis vs comparison groups.

Multi-color flow cytometry allowed to assess surface expression of membrane-bound ectonucleotidases CD39 and CD73 on various Treg subsets (Figure 4). It was found that naïve Tregs did not significantly differ in percentage of CD39- and CD73-positive cells in all examined groups. $CD45R0^+CD62L^+$ Tregs patrolling peripheral lymphoid organs in patients with acute and chronic sarcoidosis contained significantly more CD39-positive cells (69.66% (61.92; 79.34) and 67.62% (61.92; 79.34), respectively, compared to 47.55% (15.74; 65.32) in control group ($p < 0.001$ and $p = 0.004$, respectively). Moreover, the level of CD73 expression was markedly lower on CM Tregs in patients with chronic sarcoidosis (as low as 3.07%

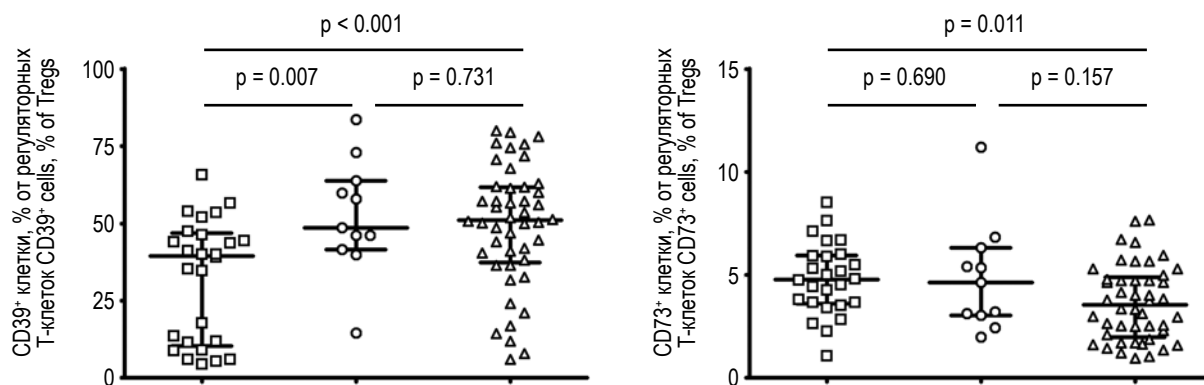


Рисунок 2. Экспрессия нуклеотидаз CD39 (слева) и CD73 (справа) регуляторными Т-клетками периферической крови больных с острым и хроническим саркоидозом

Figure 2. Expression of CD39 (left) and CD73 (right) on peripheral blood regulatory T cells from the patients with acute (Lofgren's Syndromes) and chronic (non-Lofgren's Syndromes) sarcoidosis. Left: %CD39⁺Treg cells. Right: %CD73⁺Treg cells

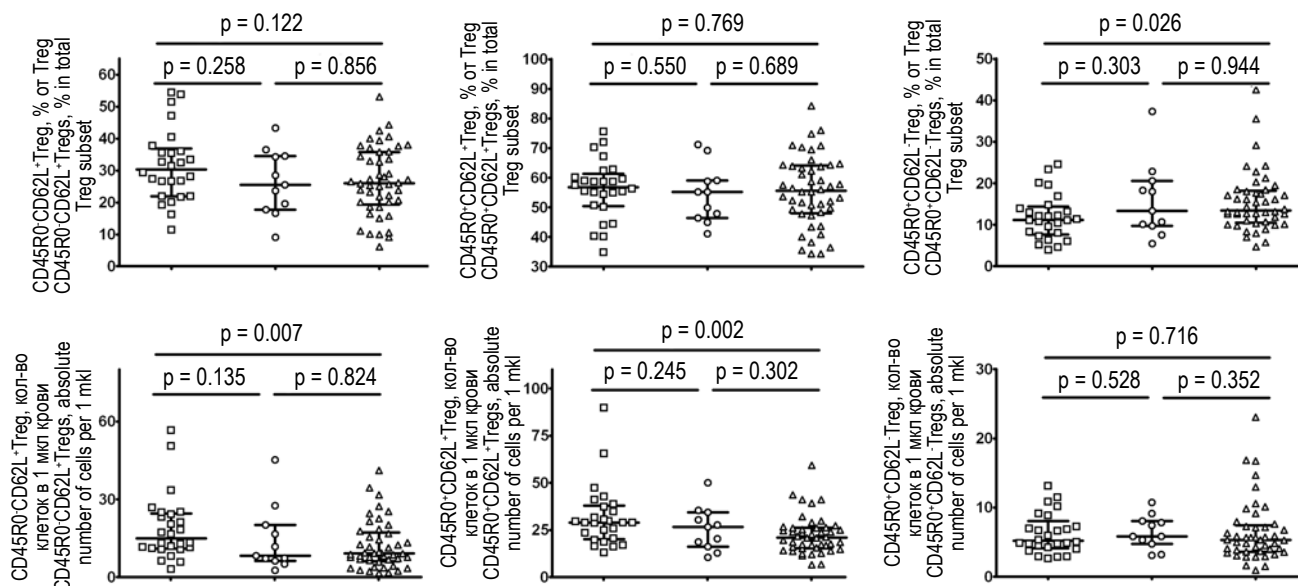


Рисунок 3. Относительное (сверху) и абсолютное (снизу) содержание регуляторных Т-клеток периферической крови больных с острым и хроническим саркоидозом, находящихся на различных стадиях созревания

Примечание. Верхний ряд гистограмм – относительное содержание регуляторных Т-клеток с фенотипами CD45RO⁺CD62L⁺, CD45RO⁺CD62L⁻ и CD45RO⁻CD62L⁺ соответственно, в рамках общей популяции Treg. Нижний ряд гистограмм – абсолютное (кол-во клеток в 1 мкл периферической крови) содержание Т-клеток с фенотипами CD45RO⁺CD62L⁺, CD45RO⁺CD62L⁻ и CD45RO⁻CD62L⁺ соответственно.

Figure 3. Relative and the absolute number of regulatory T cell subsets in peripheral blood from patients with acute (Lofgren's Syndromes) and chronic (non-Lofgren's Syndromes) sarcoidosis

Note. Upper panel: %CD45RO⁺CD62L⁺Treg cells, %CD45RO⁺CD62L⁻Treg cells and %CD45RO⁻CD62L⁺Treg cells, respectively. Lower panel: Abs. number CD45RO⁺CD62L⁺Treg cells, Abs. number CD45RO⁺CD62L⁻Treg cells and Abs. number CD45RO⁻CD62L⁺Treg cells, respectively.

(1.78; 4.88), whereas in acute sarcoidosis and control group it was 4.44% (3.13; 6.08) and 4.77% (3.26; 6.28) ($p = 0.026$ and $p = 0.011$, respectively). In case of CD45RO⁺CD62L⁻Tregs able to exit from the circulation and migrate to the site of inflammation,

an increased percentage of CD39-positive subset was noted only in patients with chronic sarcoidosis and control group (61.79% (55.12; 73.09) and 57.27% (16.03; 66.98), $p = 0.006$). Finally, a percentage of CD73-positive EM Tregs was decreased about by

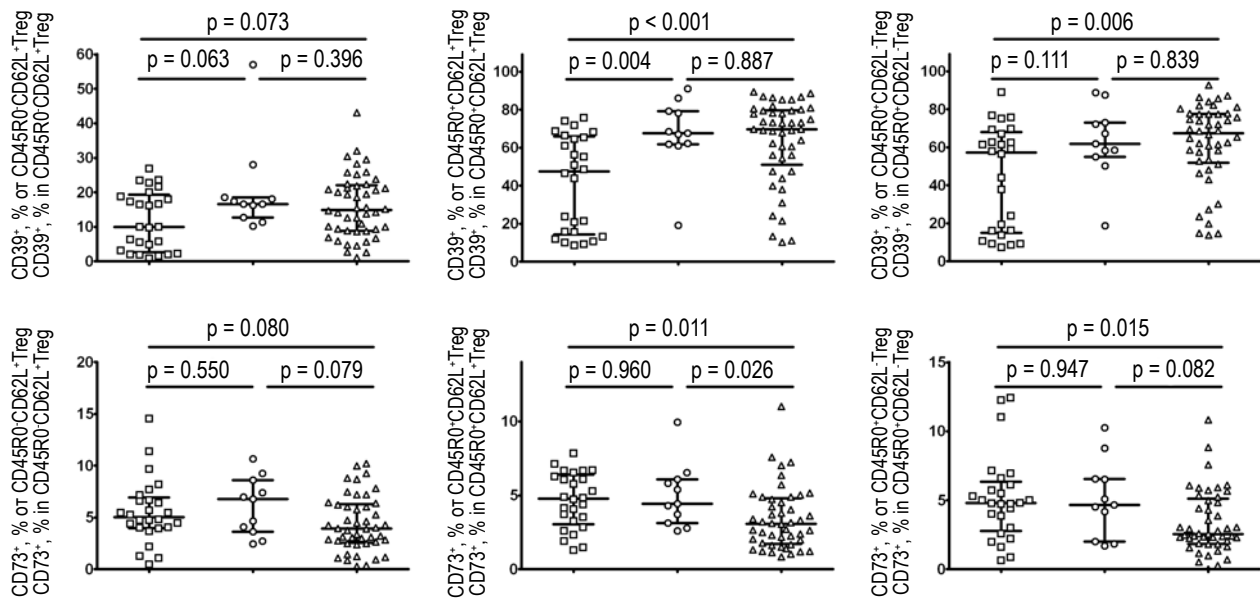


Рисунок 4. Экспрессия нуклеотидаз CD39 (сверху) и CD73 (снизу) основными субпопуляциями регуляторных Т-клеток периферической крови больных с острым и хроническим саркоидозом

Примечание. Верхний ряд гистограмм – относительное содержание CD39⁺ клеток среди Трег с фенотипами CD45RO⁺CD62L⁺, CD45RO⁺CD62L⁻ и CD45RO⁻CD62L⁻ соответственно. Нижний ряд гистограмм – относительное содержание CD73⁺ клеток среди Трег с фенотипами CD45RO⁺CD62L⁺, CD45RO⁺CD62L⁻ и CD45RO⁻CD62L⁻ соответственно.

Figure 4. Expression of CD39 and CD73 on various subsets of peripheral blood regulatory T cells from the patients with acute (Lofgren's Syndromes) and chronic (non-Lofgren's Syndromes) sarcoidosis

Note. Upper panel: %CD39⁺ out of CD45RO⁺CD62L⁺Treg cells, %CD39⁺ out of CD45RO⁺CD62L⁻Treg cells and %CD39⁺ out of CD45RO⁻CD62L⁻Treg cells, respectively. Lower panel: %CD73⁺ out of CD45RO⁺CD62L⁺Treg cells, %CD73⁺ out of CD45RO⁺CD62L⁻Treg cells and %CD73⁺ out of CD45RO⁻CD62L⁻Treg cells, respectively.

2-fold in patients with chronic sarcoidosis vs control group comprising 2.57% (1.86; 4.98) and 4.80% (3.01; 6.12, $p = 0.015$), respectively.

Discussion

Here, we present the data on investigating immunological parameters by profiling Tregs subsets in peripheral blood of patients with newly diagnosed sarcoidosis prior to applying immunosuppressive therapy. It allowed to examine signs of naturally developed immunopathogenesis of sarcoidosis upon manifestation of acute and primary chronic forms. Based on aligning immunological parameters with clinical signs of acute and primary chronic sarcoidosis, certain patterns were found, which contribute to better understanding a role of immune cells played in its pathogenesis. It was shown that the number of peripheral blood total T cells, helper T cells as well as Tregs was significantly decreased in patients with primary chronic sarcoidosis compared to control group, which is in agreement with the concept of compartmentalized immune responses and redistribution of effector immune cells exiting peripheral circulation and entering site of inflammation [16, 34].

However, such patterns were not detected upon acute onset sarcoidosis showing no significant differences in the number of total T cells, Th and Treg cell subsets as compared with control group. Importantly, the data published in various studies assessing number of Treg cells in peripheral blood of patients with sarcoidosis are highly controversial. For instance, Huang et al. observed that percentage of CD4⁺CD25^{high}FoxP3⁺T cells was decreased about by 3-fold in newly diagnosed sarcoidosis compared to healthy volunteers [20]. On the other hand, it was also found that the number of circulatory Tregs was increased in patients with relapsing sarcoidosis (clinical manifestations or chest X-ray verified relapse after corticosteroid withdrawal) compared to patients with stable remission after corticosteroid withdrawal [21]. On the contrary, percentage of CD4⁺CD25^{bright}Tregs was decreased by 5-fold in patients with overt sarcoidosis compared to control group and patients with clinically inactive form [24]. We can speculate that the more pronounced activation and migration of T cells from peripheral circulation to the lungs typical to primary chronic vs acute sarcoidosis most probably might determine severity of clinical manifestations. On the other hand, perhaps a more profound lung inflammation during primary

chronic sarcoidosis elicits a more augmented negative feedback reaction mediated by regulatory T cells. Indeed, the data obtained by us evidence that number of peripheral blood Treg cells was markedly decreased in patients with chronic sarcoidosis compared to control group that might point at a crucial role played by Tregs within the site of inflammation on restraining hypersensitivity immune reactions given that no such relationship was found in acute-onset sarcoidosis.

Redistribution of naïve, central memory (CM) and effector memory (EM) regulatory T cells represents important immunosuppressive mechanism controlling hypersensitivity immune reactions. It is known that lung-resident EM regulatory T cells lose ability to express high amount of CD62L and display CD45R0⁺CD62L⁻ phenotype [36]. In turn, CM CCR7⁺CD62L⁺Tregs may migrate to the lungs followed by down-modulating expression of CCR7 and CD62L markers subsequently resulting in their transition into EM T cells. We found that number of naïve and CM Treg subsets was much lower in peripheral blood in patients with chronic sarcoidosis, whereas the number of EM Tregs was significantly higher as compared with control group. Moreover, no such changes were detected in patients with acute-onset sarcoidosis. Previously, it was found [36] that effector memory vs naïve and central memory T cells display lower antigen-specific activation threshold, do not depend on high expression of co-stimulation molecules on antigen-presenting cells, may be rapidly activated to a level higher than in naïve Tregs. Perhaps, a more extensive redistribution of Treg subsets from peripheral circulation to effector immune sites may be noted in primary chronic sarcoidosis, which, in turn, underlies lower severity of organismal pathological processes.

Adenosine exerting marked suppressive effects on a broad range of innate and adaptive immune cells is another important and poorly investigated soluble factor released by Tregs along with anti-inflammatory cytokines TGF- β and IL-10 [19]. Comparing CD39 expression level on peripheral blood regulatory T cells showed that percentage of CD39⁺Tregs was elevated both in patients with acute-onset and primary chronic sarcoidosis. Moreover, such changes were also found both in total and CD45R0⁺CD62L⁺Treg subset that in chronic course was further extended to CD45R0⁺CD62L⁻Tregs patrolling peripheral lymphoid tissues and migrating to the site of inflammation, respectively. High CD39 expression level seems to be related to transition to chronic immune response, so that antigen elimination becomes impossible due to Treg cell over activation, as shown in some chronic disorders.

Phenotypic analysis comparing CD39⁺ and CD39⁻Tregs in adult donors showed that CD39⁺Tregs expressed higher levels of intracellular FoxP3 and

CTLA4 than CD39⁻Tregs [31]. Analysis of chemokine receptor expression revealed that a higher percentage of CD39⁺Tregs expressed CCR4, CXCR3 and CCR6, which are involved in the migration of T cells to sites of inflammation, while CCR5 was solely expressed by CD39⁺Tregs. Upon *in vitro* activation, TGF- β anchoring proteins GARP and LAP were expressed at higher level on CD39⁺Tregs. These data indicate that CD39⁺Tregs exhibit superior suppressive potential compared to CD39⁻Tregs.

Furthermore, unregulated CD39⁺ expression on circulatory Tregs was noted in chronic hepatitis B virus infection [35], but the increased viral load inversely correlated with the level of serum alanine aminotransferase. Another clinical study comparing HIV-infected patients vs control group demonstrated that both percentage of CD39⁺Tregs as well as density of CD39 expression were substantially higher [27]. Furthermore, an increase of these parameters paralleled elevated viral load and decreased percentage of peripheral blood CD4⁺T cells. A marked increase of circulatory CD39⁺Tregs was also noted in developing anti-cancer immune response [5]. In particular, colorectal cancer patients were shown to have elevated number of peripheral blood CD39⁺Tregs and showed a direct relationship between CD39 expressed on Tregs and increased mRNA level for adenosine receptor A2AR in peripheral blood leukocytes isolated from patients.

It is also worth noting that no significant differences in the number of thymus-derived CD39⁺CD45R0⁻CD62L⁺Tregs in peripheral blood were found. An impaired differentiation of antigen-specific Tregs in peripheral lymphoid tissues seems to occur in sarcoidosis. Moreover, both upregulated CD39 expression on CD45R0⁺CD62L⁻Tregs able to exit circulation and enter sarcoid granuloma as well as significantly decreased percentage of CD73⁺Tregs able to produce immunosuppressive adenosine were found in chronic sarcoidosis. Apparently, it may point at impaired mechanisms underlying emergence of effector functions in this Treg cell subset. In addition, it is remarkable that percentage of CD45R0⁺CD62L⁻Tregs in chronic sarcoidosis vs control group (together with decreased total Treg count and significantly decreased CD45R0⁻CD62L⁺ and CD45R0⁺CD62L⁺Treg subsets) were elevated, and their absolute numbers were increased up to normal level. Published data point that CD39⁺Tregs are able to infiltrate sarcoid granulomas diffusely located throughout pathological tissues [17]. Production of anti-inflammatory adenosine involved in suppression of hypersensitivity immune reactions seems to be among the key most common immunosuppressive mechanisms involved both in acute and chronic sarcoidosis.

Our data also confirm the previous results [37] evidencing about increased number of CD45R0⁺CD62L⁻Tregs able to exit peripheral circulation and migrate to the site of inflammation as well as an increased percentage of CD39⁺T cells in patients with chronic sarcoidosis compared to control group.

Thus, the our data contribute to better understanding a whole set of immunoregulation mechanisms during sarcoidosis. Further dynamic clinical and immunological examinations as well as a comparative analysis of the number and functional properties of the major T cell subsets in peripheral blood and bronchoalveolar fluid and/or lung biopsy is necessary to unveil intimate and universal immunopathogenetic mechanisms in sarcoidosis. Thus, it may be concluded that along with potential factors of genetic predisposition, or, probably, owing to genetic factors determining magnitude of immune response, based on a thorough investigation of regulatory T cell types and subsets differences in mechanisms of immune regulation were found depending on a form of sarcoidosis.

Conclusions

Based upon a concept about compartmentalized immune response and redistribution of immune cells

from peripheral circulation to the site of inflammation (here, lung tissues), the following conclusions may be drawn:

1. An important role played by Tregs in the processes regulating immune response to yet unidentified antigen(s) in sarcoidosis was uncovered.

2. The majority of peripheral blood Treg markers of patients with chronic (non-Lofgren's Syndromes) sarcoidosis vs control subjects was down-modulated that may be associated with their redistribution to the site of inflammation, thereby contributing to restraining immune-mediated processes in the lungs and subsequent development of chronic-onset sarcoidosis.

3. Mechanisms of immunosuppression mediated by Tregs via cleaving pro-inflammatory extracellular ATP to anti-inflammatory adenosine acting on a broad range of innate and adaptive immune cells represent high importance adaptation mechanisms in chronic sarcoidosis.

4. Acute-onset sarcoidosis might not be coupled to a marked activation of the mechanisms restricting immune-mediated inflammatory responses in the lung tissue, as the majority of surface markers on peripheral blood Treg cells did not significantly differ from those in control subjects.

Список литературы / References

1. Визель И.Ю. Sarcoidosis: возможность спонтанной ремиссии // Вестник современной клинической медицины, 2012. Т. 5, № 2. С. 54-60. [Vizel I.Yu. Sarcoidosis: the possibility of spontaneous remission. *Vestnik sovremennoy klinicheskoy meditsiny = Bulletin of Contemporary Clinical Medicine*, 2012, Vol. 5, no. 2, pp. 54-60. (In Russ.)]
2. Визель И.Ю., Шмелев Е.И., Баранова О.П., Барламов П.Н., Бородин Г.Л., Денисова О.А., Добин В.Л., Кулбаисов А.М., Купаев В.И., Листопадова М.В., Овсянников Н.В., Оськин Д.Н., Петров Д.В., Соловьев К.И., Шульженко Л.В., Визель А.А. Состояние больных sarcoidosisом исходно и 10 лет спустя при различной тактике их ведения (мультицентровой анализ) // Пульмонология, 2012. № 4. С. 29-33. [Vizel I.Yu., Shmelev E.I., Baranova O.P., Barlamov P.N., Borodina G.L., Denisova O.A., Dobin V.L., Kulbaisov A.M., Kupaev V.I., Listopadova M.V., Ovsyannikov N.V., Oskin D.N., Petrov D.V., Solovyov K.I., Shulzhenko L.V., Vizel A.A. Health status of sarcoidosis patients in baseline and in 10 years under different treatment strategies (multicenter analysis). *Pulmonologiya = Russian Pulmonology*, 2012, Vol. 4, pp. 29-33. (In Russ.)]
3. Головкин А.С., Серебрякова М.К., Жидулева Е.В., Муртазалиева П.М., Титов В.А., Иртыга О.Б., Моисеева О.М., Кробинец И.И., Кудрявцев И.В. Экспрессия рецепторов пуринергического сигналинга на Т-лимфоцитах периферической крови здоровых доноров // Трансляционная медицина, 2017. Т. 4, № 5. С. 46-60. [Golovkin A.S., Serebryakova M.K., Zhiduleva E.V., Murtazaliev P.M., Titov V.A., Irtyuga O.B., Moiseeva O.M., Krobinets I.I., Kudryavtsev I.V. Purinergic signaling receptors expression on peripheral T-lymphocytes of healthy donors. *Translyatsionnaya meditsina = Translational Medicine*, 2017, Vol. 4, no. 5, pp. 46-60. (In Russ.)]
4. Головкин А.С., Асадуллина И.А., Кудрявцев И.В. Пуринергическая регуляция основных физиологических и патологических процессов // Медицинская иммунология, 2018. Т. 20, № 4. С. 463-476. [Golovkin A.S., Asadullina I.A., Kudryavtsev I.V. Purinergic regulation of basic physiological and pathological processes. *Meditsinskaya immunologiya = Medical Immunology (Russia)*, 2018, Vol. 20, no. 4, pp. 463-476. (In Russ.)] doi: 10.15789/1563-0625-2018-4-463-476.
5. Жулай Г.А., Олейник Е.К., Чуров А.В., Романов А.А., Кравченко (Семакова) П.Н., Олейник В.М. Роль Трег-клеток в аденозин-опосредованной иммунной супрессии при колоректальном раке // Медицинская иммунология, 2017. Т. 19, № 1. С. 89-94. [Zhulai G.A., Oleinik E.K., Churov A.V., Romanov A.A., Kravchenko (Semakova) P.N., Oleinik V.M. Significance of Treg cells for adenosine-mediated immune suppression in colorectal cancer. *Meditsinskaya immunologiya = Medical Immunology (Russia)*, 2017, Vol. 19, no. 1, pp. 89-94. (In Russ.)] doi: 10.15789/1563-0625-2017-1-89-94.

6. Илькович М.М. Диссеминированные заболевания легких. М.: ГЭОТАР-Медиа, 2011. 480 с. [Ilkovich M.M. Disseminated lung diseases]. Moscow: GEOTAR-Media, 2011. 480 p.
7. Илькович М.М., Кокосова А.Н. Интерстициальные заболевания легких: руководство для врачей. СПб.: Нордмедиздат, 2005. 560 с. [Ilkovich M.M., Kokosova A.N. Interstitial lung diseases: a guide for physicians]. St. Petersburg: Nordmedizdat, 2005. 560 p.
8. Козлов В.А. Клетки-супрессоры – основа иммунопатогенеза аутоиммунных заболеваний // Медицинская иммунология, 2016. Т. 18, № 1. С. 7-14. [Kozlov V.A. Suppressor cells – the basis of immunopathogenesis autoimmune diseases. *Meditsinskaya immunologiya = Medical Immunology (Russia)*, 2016, Vol. 18, no. 1, pp. 7-14. (In Russ.)] doi:10.15789/1563-0625-2016-1-7-14.
9. Кудрявцев И.В., Елезов Д.С. Анализ основных популяций цитотоксических Т-лимфоцитов периферической крови на основании уровня экспрессии CD27, CD28, CD45R0 и CD62L // Российский иммунологический журнал, 2013. Т. 7 (16), № 2-3 (1). С. 57-61. [Kudryavtsev I.V., Elezov D.S. Analysis of the main populations of cytotoxic T lymphocytes of peripheral blood on the basis level or the expression of CD27, CD28, CD45R0 and CD62L. *Rossiyskiy immunologicheskij zhurnal = Russian Journal of Immunology*, 2013, Vol. 7 (16), no. 2-3 (1), pp. 57-61. (In Russ.)]
10. Кудрявцев И.В., Савицкий В.П. Многоцветный анализ основных субпопуляций Т-хелперов и цитотоксических Т-клеток методом проточной цитофлуориметрии // Российский иммунологический журнал, 2012. Т. 6, № 3 (1) (14). С. 94-97. [Kudryavtsev I.V., Savitsky V.P. Multicolor flow cytometric analysis of the main subpopulations of T-helpers and cytotoxic T cells from peripheral blood. *Rossiyskiy immunologicheskij zhurnal = Russian Journal of Immunology*, 2012, Vol. 6, no. 3 (1) (14), pp. 94-99. (In Russ.)]
11. Кудрявцев И.В., Субботовская А.И. Опыт измерения параметров иммунного статуса с использованием шести-цветного цитофлуориметрического анализа // Медицинская иммунология, 2015. Т. 17, № 1. С. 19-26. [Kudryavtsev I.V., Subbotovskaya A.I. Application of six-color flow cytometric analysis for immune profile monitoring. *Meditsinskaya immunologiya = Medical Immunology (Russia)*, 2015, Vol. 17, no. 1, pp. 19-26. (In Russ.)] doi: 10.15789/1563-0625-2015-1-19-26.
12. Хайдуков С.В., Байдун Л.А., Зурочка А.В., Тотолян Арег А. Стандартизованная технология «Исследование субпопуляционного состава лимфоцитов периферической крови с применением проточных цитофлуориметров-анализаторов» (Проект) // Медицинская иммунология, 2012. Т. 14, № 3. С. 255-268. [Khaydukov S.V., Baydun L.A., Zurochka A.V., Totolian Areg A. Standardized technology “Research of lymphocytes subpopulation composition in peripheral blood using flow cytometry analyzers” (Draft). *Meditsinskaya immunologiya = Medical Immunology (Russia)*, 2012, Vol. 14, no. 3, pp. 255-268. (In Russ.)] doi: 10.15789/1563-0625-2012-3-255-268.
13. Чучалин А.Г., Визель А.А., Илькович М.М., Авдеев С.Н., Амиров Н.Б., Баранова О.П., Борисов С.Е., Визель И.Ю., Ловачева О.В., Овсянников Н.В., Петров Д.В., Романов В.В., Самсонова М.В., Соловьева И.П., Степанян И.Э., Тюрин И.Е., Черняев А.Л., Шмелев Е.И., Шмелева Н.М. Диагностика и лечение саркоидоза: резюме федеральных согласительных клинических рекомендаций (Часть II. Диагностика, лечение, прогноз) // Вестник современной клинической медицины, 2014. Т. 7, вып. 5. С. 73-81. [Chuchalin A.G., Vizel A.A., Ilkovich M.M., Avdeev S.N., Amirov N.B., Baranova O.P., Borisov S.E., Vizel I.Yu., Lovacheva O.V., Ovsyannikov N.V., Petrov D.V., Romanov V.V., Samsonova M.V., Solovyova I.P., Stepanian I.E., Tyurin I.E., Cherniayev A.L., Shmelev E.I., Shmeleva N.M. Diagnosis and treatment of sarcoidosis: Summary of federal conciliative clinical recommendations (Part II. diagnosis, treatment, prognosis). *Vestnik sovremennoy klinicheskoy meditsiny = Bulletin of Contemporary Clinical Medicine*, 2014, Vol. 7, no. 5, pp. 73-81. (In Russ.)]
14. Broos C.E., van Nimwegen M., Hoogsteden H.C., Hendriks R.W., Kool M., van den Blink B. Granuloma formation in pulmonary sarcoidosis. *Front. Immunol.*, 2013, Vol. 10, no. 4, p. 437.
15. Caramalho I., Nunes-Cabaço H., Foxall R.B., Sousa A.E. Regulatory T-cell development in the human thymus. *Front. Immunol.*, 2015, Vol. 6, p. 395.
16. Facco M., Cabrelle A., Teramo A., Olivieri V., Gnoato M., Teolato S., Ave E., Gattazzo C., Fadini G.P., Calabrese F., Semenzato G., Agostini C. Sarcoidosis is a Th1/Th17 multisystem disorder. *Thorax*, 2011, Vol. 66, no. 2, pp. 144-150.
17. Fujimura T., Kambayashi Y., Aiba S. Expression of CD39/Entpd1 on granuloma-composing cells and induction of Foxp3-positive regulatory T cells in sarcoidosis. *Clin. Exp. Dermatol.*, 2013, Vol. 38, no. 8, pp. 883-889.
18. Garcia Santana C.A., Tung J.W., Gulnik S. Human Treg cells are characterized by low/negative CD6 expression. *Cytometry A*, 2014, Vol. 85, no. 10, pp. 901-908.
19. Grant C.R., Liberal R., Mieli-Vergani G., Vergani D., Longhi M.S. Regulatory T-cells in autoimmune diseases: challenges, controversies and yet unanswered questions. *Autoimmun. Rev.*, 2015, Vol. 14, no. 2, pp. 105-116.
20. Huang H., Lu Z., Jiang C., Liu J., Wang Y., Xu Z. Imbalance between Th17 and regulatory T-cells in sarcoidosis. *Int. J. Mol. Sci.*, 2013, Vol. 14, no. 11, pp. 21463-21473.
21. Liu Y., Qiu L., Wang Y., Aimurolo H., Zhao Y., Li S., Xu Z. The circulating Treg/Th17 cell ratio is correlated with relapse and treatment response in pulmonary sarcoidosis patients after corticosteroid withdrawal. *PLoS ONE*, 2016, Vol. 11, no. 2, e0148207. doi: 10.1371/journal.pone.0148207.
22. Loke W.S., Herbert C., Thomas P.S. Sarcoidosis: immunopathogenesis and immunological markers. *Int. J. Chronic Dis.*, 2013, Vol. 2013, 928601. doi: 10.1155/2013/928601.

23. Mahnke Y.D., Roederer M. Optimizing a multicolor immunophenotyping assay. *Clin. Lab. Med.*, 2007, Vol. 27, no. 2, pp. 469-485.
24. Miyara M., Amoura Z., Parizot C., Badoual C., Dorgham K., Trad S., Kambouchner M., Valeyre D., Chapelon-Abric C., Debré P., Piette J.C., Gorochov G. The immune paradox of sarcoidosis and regulatory T cells. *J. Exp. Med.*, 2006, Vol. 203, no. 2, pp. 359-370.
25. Miyara M., Yoshioka Y., Kitoh A., Shima T., Wing K., Niwa A., Parizot C., Taflin C., Heike T., Valeyre D., Mathian A., Nakahata T., Yamaguchi T., Nomura T., Ono M., Amoura Z., Gorochov G., Sakaguchi S. Functional delineation and differentiation dynamics of human CD4⁺ T-cells expressing the FoxP3 transcription factor. *Immunity*, 2009, Vol. 30, pp. 899-911.
26. Mortaz E., Rezayat F., Amani D., Kiani A., Garssen J., Adcock I.M., Velayati A. The roles of T helper 1, T helper 17 and regulatory T cells in the pathogenesis of sarcoidosis. *Iran J. Allergy Asthma Immunol.*, 2016, Vol. 15, no. 4, pp. 334-339.
27. Nikolova M., Carriere M., Jenabian M.A., Limou S., Younas M., Kök A., Huë S., Seddiki N., Hulin A., Delaneau O., Schuitemaker H., Herbeck J.T., Mullins J.I., Muhtarova M., Bensussan A., Zagury J.F., Lelievre J.D., Lévy Y. CD39/adenosine pathway is involved in AIDS progression. *PLoS Pathog.*, 2011, Vol. 7, no. 7, e1002110. doi: 10.1371/journal.ppat.1002110.
28. Noack M., Miossec P. Th17 and regulatory T cell balance in autoimmune and inflammatory diseases. *Autoimmun. Rev.*, 2014, Vol. 13, no. 6, pp. 668-677.
29. Nurkhametova D., Kudryavtsev I., Khayrutdinova O., Serebryakova M., Altunbaev R., Malm T., Giniatullin R. Purinergic profiling of regulatory T-cells in patients with episodic migraine. *Front. Cell. Neurosci.*, 2018, Vol. 12, p. 326.
30. Paulissen S.M., van Hamburg J.P., Dankers W., Lubberts E. The role and modulation of CCR6⁺ Th17 cell populations in rheumatoid arthritis. *Cytokine*, 2015, Vol. 74, no. 1, pp. 43-53.
31. Rissiek A., Baumann I., Cuapio A., Mautner A., Kolster M., Arck P.C., Dodge-Khatami A., Mittrucker H.W., Koch-Nolte F., Haag F., Tolosa E. The expression of CD39 on regulatory T cells is genetically driven and further upregulated at sites of inflammation. *J. Autoimmun.*, 2015, Vol. 58, pp. 12-20.
32. Saidha S., Sotirchos E.S., Eckstein C. Etiology of sarcoidosis: does infection play a role? *Yale J. Biol. Med.*, 2012, Vol. 85, no. 1, pp. 133-141.
33. Sakaguchi S., Miyara M., Costantino C.M., Hafler D.A. FoxP3⁺ regulatory T-cells in the human immune system. *Nat. Rev. Immunol.*, 2010, Vol. 10, pp. 490-500.
34. Sakthivel P., Bruder D. Mechanism of granuloma formation in sarcoidosis. *Curr. Opin. Hematol.*, 2017, Vol. 24, no. 1, pp. 59-65.
35. Tang Y., Jiang L., Zheng Y., Ni B., Wu Y. Expression of CD39 on FoxP3⁺ T regulatory cells correlates with progression of HBV infection. *BMC Immunol.*, 2012, Vol. 13, p. 17.
36. Williams A.E. Immunology mucosal and body surface defences. *A.E. Williams. – A. John Willey and Sons, Ltd., Publication*, 2015. 380 p.
37. Yegutkin G.G. Enzymes involved in metabolism of extracellular nucleotides and nucleosides: functional implications and measurement of activities. *Crit. Rev. Biochem. Mol. Biol.*, 2014, Vol. 49, no. 6, pp. 473-497.

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