INDICATORS OF IMMUNOGRAM IN PATIENTS WITH ACUTE AND CHRONIC BRUCELLOSIS

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Abstract

Background

This article considers indicators of immunogram in patients sick with acute and chronic brucellosis. Characteristic features of the immunity indicators have been analyzed in 36 patients sick with acute and in 22 patients sick with chronic brucellosis.

Aims: The goal of the present research was to evaluate immunological parameters in the patients with acute and chronic brucellosis.

Methods: Immunological parameters were studied in 58 patients with acute and chronic brucellosis: 36 patients with acute brucellosis (20 men and 16 women) in the age of 18 to 51 years old (average age is 31.1±7.76 years) and 22 patients sick with chronic brucellosis (10 men and 12 women) in the age of 14 to 53 years (average age is 34.3±11.56 years).

Results: Based on the carried out research there was observed a significant increase in lymphocytes in both groups, while in the group of patients with chronic brucellosis such increase is more pronounced (significantly higher than in the group of acute brucellosis). At the same time there are no observed significant changes in CD95+ cells in patients and in both groups there were not observed significant changes in the average content of B-lymphocytes. Based on an analysis of indicators of immunity it has been determined a marked inhibition of phagocytic activity of neutrophils, which is indirectly confirmed by the pronounced decrease of LPS induced neutrophil phagocytic activity in the patients, in both acute and chronic stage of the disease. It has been determined on the basis of analysis a high level of B-lymphocytes in patients with hypergammaglobulinemia and high titers of Wright's reaction in this study.

Conclusion: It was determined a marked reduction of spontaneous capturing capability of neutrophils in response to
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pyrogenal; increase at the oxygen-dependent bactericidal activity in chronic brucellosis in spontaneous and stimulated antigen brucellosis liquid options of NBT test, and in acute brucellosis it has been observed a marked reduction of the induced activity.

Key Words

Introduction
Incidence of a disease of brucellosis remains nowadays high. Galińska et al. confirm that this disease appears all over the world but it is mostly spread in the countries with low level of medical care and with bad control over the animal health.¹ It is known that countries of Mediterranean basin (Portugal, Spain, South of France, Italy, Greece, Turkey, North Africa) shall be referred to the regions with high level of infection with brucellosis disease as well as countries of South and Central America, Asia, Africa, Caribbean basin and Middle East as well as Peru, Kuwait and some regions of Saudi Arabia.²³

Regulatory T cells (Tregs) have become a popular subject for immune researches in the recent years. Numerous studies have identified in people basic biological aspects of Treg, with the characteristic of various subpopulations of T cells, including naturally occurring of CD4+CD25+Tregs, of induced Tregs [IL-10 production, such as CD4+IL regulatory T cells (Tr1), and T helper type 3 (Th3) cells] and CD4+CD25+T cells, which are developed in the periphery by transforming CD4+CD25 of T cells. All of these different populations of T cells with regulatory function performs and promotes immune suppression.⁴⁶

The high degree of chronic brucellosis is a consequence of characteristic features of the course of infection process, as well as developing at that insufficiency of immunological protection of the body. At present, the preservation of the persistence of the pathogen in the body of the patient is more associated with the formation of secondary immune deficiency in patients with brucellosis.⁷ Against the suppressed immunoreactivity clinical effect of antimicrobial agents application has been reduced.⁸ Adequate assessment of immunological changes during brucellosis infectious process allows to justify approaches to improve the effectiveness of the treatment of acute/sub-acute brucellosis and prevention of chronicity.⁹
Magomedov\textsuperscript{10} in his work informs that patients with brucellosis have been marked by a general reduction in the functional activity of the mononuclear phagocyte system, that manifests in a decrease of coefficient of the functional activity of monocytes, amount of phagocytic monocytes and coefficient of phagocytic activity in monocytes, in spite of the noted increase in the amount of functionally active cells.\textsuperscript{11} Think, that Brucella has developed a stealth strategy by reducing the expression of PAMP-receptors, which provides a low level of stimulation of the immune system cells and reduces the activity of phagocytosis. The author believes that this feature makes it is possible for pathogen to achieve replication before antimicrobial mechanisms of adaptive immunity would be switched on. Furthermore, at brucellosis infection there is imbalance of cellular immunity with suppression of major T cell subsets of the immune system, increasing number of cytotoxic T lymphocytes and receptor expression of D16+ CD23+, CD25+.\textsuperscript{12-16} It is necessary to underline that given indicators point at functional inferiority of anti-infective immune response in patients with brucellosis.\textsuperscript{15,16} According to the data by Atahodzhayeva,\textsuperscript{12} received in the survey of 45 patients with sub-acute brucellosis, we can judge the significant decrease of the population of CD4+ (T-helpers/inducers) and CD3+cells (T lymphocytes), in 1.3 times and in 1.1 times, respectively, and increase of the relative number of CD8+cells in 1.1 times as compared with control group. The author also points out that the study observed an increase in CD16+cells in the population in 1.1 times compared to the control, that could possibly be determined by the increased concentration of immature Natural Killers cells in peripheral blood.\textsuperscript{12} It is shown that there is a direct impact of imbalance in cellular link of immunity on its cytokine link.\textsuperscript{12,17-20}

A large number of works by foreign and domestic authors are devoted to the common factors of change in the number of cytokines circulating in blood with pro-and anti-inflammatory effects as in the active and in inactive form of the disease.\textsuperscript{12,17-23} It is known that Brucella is able to allocate endotoxin, in response to which macrophages and lymphocytes release pro-inflammatory cytokines, as evidenced by increased levels of IL-1\textbeta and IL-6 in the blood of patients with brucellosis. Moreover, at brucellosis infection there is an increase in anti-inflammatory cytokines product: IL-4, IL-10.\textsuperscript{12,24-25}

Bahadoret al.\textsuperscript{26} in conclusion of his study, noted that there is a significant increase in circulating of CD4+CD25+, CD4+FoxP3+and CD4+CD25+FoxP3+Tregs and their CD25high or FoxP3high subpopulation of patients with acute and chronic brucellosis. Tregs play a negative role not only in the modulation of effector immune response by inhibiting secretion of gamma-interferon and cell proliferation by Brucella antigen stimulation, but also in maintenance of the impact of chronic brucellosis disease.
These results show that modulation of CD4+CD25+regulatory T cells may be a potential therapeutic strategy in the treatment of chronic form of this infection. Furthermore, this study noted a significant increase in the proportions of CD4+FoxP3+cells and their FoxP3high subpopulations and CD4+CD25+FoxP3+cells and their CD25high or FoxP3high subsets in chronic brucellosis compared with patients with acute brucellosis and healthy people. In addition, CD4+Treg CD25high cells in acute brucellosis group showed a significant increase in frequency as compared with the control group. The goal of the present research was to evaluate immunological parameters in the patients with acute and chronic brucellosis.

**Method**

Immunological parameters were studied in 58 patients with acute and chronic brucellosis: 36 patients with acute brucellosis (20 men and 16 women) in the age of 18 to 51 years old (average age is 31.1±7.76 years) and 22 patients sick with chronic brucellosis (10 men and 12 women) in the age of 14 to 53 years (average age is 34.3±11.56 years). The control group was consisted of 30 mostly healthy people (average age is 26.4±4.1 years), in whom chronic infectious diseases were excluded by method of EIA.

The diagnosis of brucellosis was verified by exuding of blood culture of Brucella melitensis and/or by detection of specific antibodies in the reactions of Wright Haddlsona in diagnostically significant titers, lymphocyte antigen detection of brucellosis specificity.

Bacteriological examination of the blood of patients sick with brucellosis was carried out in the first days of hospitalization. Bacteriological examination was conducted in the laboratory of especially dangerous infections of Health Inspection Services in the city of Almaty.

Delivery of material into the bacteriological laboratory was carried out by using transportation containers, which are sealed sterile vials with 20 ml of liquid nutrient medium, which is storage medium. Melitensis B blood cultures were determined in 66 (41.8%) patients with acute and in 11 (38%) with chronic brucellosis.

**Method to detect antigen-binding lymphocyte of brucellosis specificity**

Determination of antigen-binding lymphocytes of brucellosis specificity: blood in an amount of 3 ml with anticoagulant (heparin) was taken in the morning on an empty stomach (the patients were recommended to refrain from abundant and fatty foods on the day before the collection of blood). Lymphocytes were marked on Ficoll density gradient of verografin ρ=1.077 g / ml. The content of the antigen-binding specificity of brucellosis lymphocytes was determined by indirect rosette formation on the difference between the experimental and control
Immunological methods

In order to perform immunogram a heparinized venous blood was used. Lymphocytes were allocated on a gradient ficoll-verografin (p=1.077). Research of lymphocyte subpopulation composition was performed by flowing cytometry «FacsCalibur» (Becton Dickenson/USA) by method of indirect membrane immunofluorescence with the use of mAb panel to surface receptors on lymphocytes: CD3+(T lymphocytes), CD4+(T-helper cells), CD8+(T-cytotoxic lymphocytes), CD16 +, CD56+(NK-cells - natural killer cells); CD20+(B cells), CD95+(signaling receptors for apoptosis induction), CD25+receptor for IL-2), HLA-DR+ Phagocytic activity of peripheral blood leukocytes was studied by using of a method which is based on registration of phagocytosis of latex in spontaneous and stimulated with lipopolysaccharide versions (LPS of E.coli), as well as the specific stimulation of brucellosis allergen (brucellosis liquid antigen). To evaluate the oxygen-dependent activity of phagocytes by using of NBT-test spontaneous and stimulated by lipopolysaccharide by E.coli and brucellosis liquid antigen.

Results

In patients with chronic brucellosis in comparison with the patients with acute brucellosis, there was a reduction of total peripheral blood leukocytes from 5.9±1.4h10⁹/l to 4.4±0.84h10⁹/l. At the same time in patients with chronic brucellosis was observed a more pronounced relative lymphocytosis of 43% to 59% (average 50.7±4.96%), as compared to leukocyte formula in patients with acute brucellosis (in average of 41.7±6.94%). Thus, the absolute lymphocyte contains in patients with chronic brucellosis was somewhat reduced as compared to patients with acute brucellosis and healthy individuals (Table 1).

Table 1: Contents of the main lymphocyte populations in acute and chronic brucellosis.

<table>
<thead>
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<th>control</th>
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<td>&gt;0.05</td>
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<tr>
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<td>±</td>
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Analysis of immunological parameters in patients with acute and chronic brucellosis showed both relative and absolute content of mature T lymphocytes and T-helpers with an equal degree of reliability.

In both groups, there were decreases of the relative and absolute number of total T lymphocytes that is a characteristic feature of the acute stage of infection due to redistribution phenomenon pool of T lymphocytes from circulation to the tissues. There was also observed a reduction of T-helper cells in the normal level of cytotoxic T-lymphocytes (Table 1).

In patients of both groups, there were not determined significant changes in the content of natural killer cells (CD56+). However, in the group with chronic brucellosis number of cells expressing CD56+and CD16+are equal and they were represented in one Gate that is almost all CD56+were CD16+. While the number of CD56+cells in acute brucellosis group exceeds the number of lymphocytes, expressing receptors to fragment of the Fc IgG (CD16+).

Expression of the receptor is an indication of the functional activity of the lymphocyte population and reflects the ability to bind immunoglobulins G, and therefore the ability to antibody-dependent cellular cytotoxicity (ADCCT). In patients with acute brucellosis, not all the cells have CD16, therefore not all the natural killer cells are capable to antibody-dependent cellular cytotoxicity (ADCCT). At the stage of chronic brucellosis an expression of CD16
increases significantly, nearly all NK cells express CD16 and therefore capable to antibody-dependent cellular cytotoxicity (ADCCT).

In both groups, it was observed a significant increase of lymphocytes expressing the receptor for IL-2 (CD25+). In the group of patients with chronic brucellosis such increase is more pronounced (significantly higher than in the group of acute brucellosis). At the same time in patients significant changes in CD95+cells (ready to apoptosis) were not observed. In acute brucellosis was noted a significant increase in other lymphocyte activation marker - HLA-DR. However, in chronic brucellosis HLA-DR+average values were not significantly different from the control due to the large variation indicators. That is, patients had HLA-DR+(21%) high values and low (6%).

In both groups there was not detected a significant change in the average grade of bursal lymphocytes. In earlier studies it was observed an increase in the content of bursal lymphocytes in patients with acute brucellosis, correlate with hypergammaglobulinemia. High levels of B-lymphocytes (20-22%) were also observed in patients with hypergammaglobulinemia and with high titers and Wright reaction (1: 400-1: 1200) and in this study. However, along with patients with high levels of bursal lymphocytes and IgG there were observed normal (5-9%) and even lower values of bursal lymphocytes (below 5%). In patients with acute brucellosis were observed significant changes in the capturing ability of neutrophil phagocytes, but there was a marked reduction of induced activity in response to a non-specific stimulus. In response to the antigen-specific stimulation of brucellosis in the acute phase of the capturing activity indicators have not been changed significantly (Figure 1). In chronic brucellosis there has been a decrease in spontaneous activity and reserves of the capturing ability of neutrophils have been significantly decreased. But in response to brucellosis antigen, as well as in acute brucellosis, latex phagocytosis has not been significantly changed.

**Figure 1: Indicators of phagocytosis of latex in Patients sick with acute and chronic brucellosis.**

The ability of neutrophils to produce reactive oxygen metabolites in acute brucellosis did not differ from the benchmarks in spontaneous and induced variants of brucellosis liquid antigen and in NBT test. But the induced activity in response to a non-specific stimulus was markedly reduced (Figure 2). In chronic brucellosis oxygen-
dependent activity of neutrophils tended to increase in spontaneous and induced antigen-specific options, and did not change significantly in response to a non-specific stimulus. It is well known that brucellosis infection is characterized by dysfunction of the phagocytic system. At that Brucella actively avoid bactericidal factors of phagocytic cells by inhibiting the process of liberation of the enzymes involved in the destruction of the pathogen. However, it was known that Brucella had virtually no effect on the capturing capability of neutrophils without inhibiting or stimulating it.30 When a human body has been infected with brucella a phagocyte system appears on the first line of defence. In patients activation has been observed as of microphages and macrophages. At that phagocytosis of Brucella can result in the lysis of the pathogen, or as a result of the blockade of the enzyme systems of phagocytic cells, Brucella is able not only survive but also to multiply inside the phagocyte, which creates conditions for the generalization of the process.31 The study of neutrophil phagocytic function in patients with brucellosis is remained by a simple and available method of assessment of immune reactivity of the body.32 At the surveyed patients with both an acute and chronic brucellosis a marked suppression of neutrophil phagocytic activity was observed. This fact may not be associated with specific features of the infection process, and relates to the premorbid state of examined patients. This assumption was indirectly confirmed by the pronounced decrease of LPS-induced neutrophil phagocytic activity in patients, both in acute and chronic stage of the disease. In the studies by Jurina it has been shown that in patients with acute brucellosis in light and medium-severe course of the disease stimulation by the specific antigen induced a more pronounced effect compared with stimulation by pyrogenal, whereas in donors, on the contrary, stimulation of non-specific antigen prevailed over stimulation by brucellosis allergen.30 In severe acute brucellosis, by contrast, antigen-specific stimulation could lead not to activation but to the suppression of NBT test indicators. As among studies patients there were predominant patients with severe acute brucellosis, the lack of stimulation of the NBT in response to the Brucellosis liquid antigen and the same indicators NBT induced in response to pirogenal and brucellosis antigen match the data by E.V. Jurina.

Figure 2: Indicators of NBT-test in the patients sick with acute and chronic brucellosis.
However, the results of a survey of patients with chronic brucellosis have controversial character. In earlier studies, they have not been marked by an increase in the spontaneous and antigenstimulated NBT-test in chronic brucellosis relatively to the similar indicators in acute brucellosis, as it has been found in the surveyed patients. At the same time, a higher oxygen-dependent stimulation of the activity of neutrophils in patients with chronic brucellosis compared with patients with acute brucellosis seems logical, since in terms of higher levels of specific immunoglobulins classes of G and A, metabolic and phagocytic ability of macrophages should respectively increase. The interaction of neutrophils receptor to Fc fragment of IgG is an important and independent mechanism of stimulation of neutrophils with the activation of the secretory function, oxygen-dependent bactericidal and capturing abilities.

Advanced study of population profile of peripheral blood lymphocytes in patients with acute and chronic brucellosis, showed no significant dynamics of population profile of lymphocytes in the transition from of acute to chronic brucellosis.

All differences in the analysis of groups with acute and chronic brucellosis have been limited to changes in the expression of the early activation markers - CD25 and HLA-DR. In chronic course of disease content of CD25+ was 2 times higher than in acute. In acute brucellosis the relative and absolute content of HLA-DR+ cells were also significantly increased. An interesting fact is that in chronic brucellosis number of CD25+ cells was in 2 times higher than the number in acute brucellosis, while the number of HLA-DR+ changed equidirectionally: both downwards and upwards.

In the course of brucellosis infection development, substantial and varied changes in the immune system of the body happen, reflecting the mobilization of the various links of immunity. The use of phenotyping of peripheral blood lymphocytes with a broad panel of monoclonal antibodies to the CD antigens has enabled to confirm the characteristics of acute brucellosis infection and reveal some new aspects of its immunopathogenesis.

**Discussion**

On the representative groups of patients we were able to establish that in acute and chronic brucellosis it was observed the same type of change in the profile of the population of lymphocytes. In patients with both an acute and chronic brucellosis it was found a significant and marked reduction in total number of mature T lymphocytes and T helper cells. At the same time in patients there were no significant changes in the content of T-cytotoxic lymphocytes, and the level of bursal lymphocytes remained normal.
Such dynamics in the level of the population, on the one hand, reflects the changes that are typical for the acute period of any infectious process: the decline in the share of T-helper cells, while maintaining the level of T-cytotoxic. It is associated with primary involvement in Fas-dependent activation of apoptosis and less Tx - Tc, with more intense proliferation of Tc in response to a specific stimulus (Perry M.B., Bundle D.R.)\textsuperscript{31}. It is believed that the initiation of a response to an antigen (which corresponds to clinical incubation period), the main functional load fall on Tx, whereas at the height of the response (the period of the manifestation of clinical manifestations) leading role proceeds to CD8+ effector cells.

On the other hand, it is not characteristic for an acute infectious process a significant reduction in the absolute content of T-lymphocytes and T-helper cells - normally there are changes only in the proportion of these cells among the remaining sub / lymphocyte populations. A significant reduction in CD3+ and CD4+ may be an indicator of suppressor of Brucella action and be a consequence of severity of infection course.

References


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