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DYNAMICS OF CHANGES IN THE IMMUNE STATUS OF PATIENTS WITH ABDOMINAL TUBERCULOSIS DURING THE KOCH IMMUNOPROVOCATION TEST

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Despite the high level of development of modern diagnostics, there is still a problem of diagnosing extrapulmonary and latent forms of tuberculosis. The purpose of the study was to determine the features of the immune status during the Koch immunoprovocation test. Studies were conducted in the venous blood of 50 patients with abdominal tuberculosis before and 72 hours after the Koch skin test. Indicators of cellular immunity were determined by the indirect immunofluorescence method and the content of cytokines by the immunoenzymatic method. The existence of statistically significant differences in the immunogram and in the cytokine profile of the examined patients before and after the Koch test was proved. The Koch immunoprovocation test exacerbates the pathogenetic manifestations of the chronic inflammatory process, which is manifested by a change in the cytokine spectrum of the blood and makes it possible to clarify the diagnosis of abdominal tuberculosis. Determination of the level of TNF-α in the blood serum of patients after the Koch test can be a marker of the latent form of the course of abdominal tuberculosis.

Key words: immune system, cytokines, extrapulmonary tuberculosis, Koch test.

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ДИНАМІКА ЗМІН ІМУННОГО СТАТУСУ ХВОРИХ НА ТУБЕРКУЛЬОЗ ОРГАНІВ ЧЕРЕВНОЇ ПОРОЖНИНИ ПРИ ПРОВЕДЕННІ ІМУНОПРОВОКАЦІЙНОЇ ПРОБИ КОХА

Незважаючи на високий рівень розвитку сучасної діагностики, все ще існує проблема діагностики позалегеневих та латентних форм туберкульозу. Метою дослідження було визначення особливостей імунного статусу при проведенні імунопровокаційної проби Коха. Дослідження проводили у венозній крові 50 хворих на туберкульоз органів черевної порожнини до та через 72 години після проведення шкірної проби Коха. Показники клітинного імунітету визначали методом непрямої імунофлюоресценції та вміст цитокінів імуноферментним методом. Доведено наявність статистично значущих відмінностей в імунограмі та цитокіновому профілі обстежених пацієнтів до і після проведення проби Коха. Імунопровокаційний тест Коха загострює патогенетичні прояви хронічного запального процесу, що проявляється зміною цитокінового спектру крові та дає можливість уточнити діагноз туберкульозу органів черевної порожнини. Визначення рівня фактора некрозу пухлин-α в сироватці крові хворих після проведення проби Коха може бути маркером латентної форми перебігу абдомінального туберкульозу.

Ключові слова: імунна система, цитокіни, позалегеневий туберкульоз, проба Коха.

The study is a fragment of the research project "To study the potential of tuberculosis infection in order to develop modern methods aimed at stabilisation of the epidemic situation", state registration No. 0121U000227.

One of the reasons for the difficulties in diagnosing abdominal tuberculosis (AT) is the fact that tuberculosis is traditionally associated by physicians primarily with lung damage. There are also objective reasons for the difficulty of diagnosis, as there are no specific clinical signs of tuberculosis lesions of the abdominal cavity [1, 2, 14]. The immune response to any foreign agent depends on the nature, dose and duration of its exposure, as well as on the ability of the immune system and its immunocompetent cells to respond fully to the antigenic stimulus [1, 2, 3, 12]. The immune response to Mycobacterium tuberculosis (MBT) differs significantly from immunity in other infectious diseases. Infection with MBT is not always accompanied by the development of the disease due to their natural resistance. In response to the penetration of MBT, specific immunological changes develop in the body, which determine the acquired anti-TB immunity [1, 2]. Acquired immunity is also not absolute; it can be overcome by massive exposure to MBT, especially in various types of immunodeficiency [1, 3, 5].

Mantoux and Koch tests are used to diagnose tuberculosis. In recent years, the Koch subcutaneous test has been widely used in combination with various laboratory tests (biochemical, immunological, bacteriological). The subcutaneous Koch test is more sensitive than the Mantoux test. The Koch test is an immunoprovocative auxiliary method for assessing the general reaction of the body to subcutaneous injection of tuberculin. This test is important in determining the latent activity of a specific process, as well as in the differential diagnosis of a specific and nonspecific process [4, 7, 8, 13].

The purpose of the study was to investigate the immune and cytokine profile before and after the Koch test to detect changes in the immune status.

Materials and methods. The blood of 50 patients with AT was examined before and after the Koch immunoprovocation test. Koch's test was performed by subcutaneous injection of 20 tuberculin units (TU) of purified tuberculin (PPD-L) in the projection of the left scapula. In active tuberculosis, a general, local and focal reaction occurred 72 h after subcutaneous injection of 20 TO of PPD-L. When assessing the subcutaneous Koch test, changes in the general blood count, immune and cytokine systems were additionally taken into account. The average age of patients undergoing Koch immunoprovocation test was 36.5±2.5 years. Patients were treated in the surgical department of the Lviv Regional Phthisiopulmonological Centre, which hosts the Department of Phthisiology and Pulmonology, and in the Lviv Regional Hospital for Extrapulmonary Tuberculosis. The control group consisted of 20 healthy individuals aged 25–45 years.

All the subjects underwent venous blood sampling into vacuum tubes with an anticoagulant – K_3EDTA for hematological studies. To assess the morphological and functional parameters of leukocytes, blood smears were examined and the leukocyte formula was calculated. The number and morphological and functional parameters of peripheral blood cells were assessed using a light microscope.

In the group of patients with hypertension who underwent Koch's test, venous blood was taken before and 72 hours after the test.

The content of lymphocyte populations and subpopulations (LC) using monoclonal antibodies to CD3 (T-lymphocytes), CD4 (T-helper), CD8 (T-cytotoxic/suppressor), CD19 (B-lymphocytes), CD56 (NK cells), CD23 (activated B-lymphocytes) in an indirect immunofluorescence reaction with fluorescein isothiocyanate (FITC)-labelled antibodies. Phenotyping of peripheral blood lymphocytes was performed by indirect immunofluorescence detection using monoclonal antibodies produced by the R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of the National Academy of Sciences of Ukraine. Lymphocyte populations and subpopulations were counted using a luminescence microscope with a phase-contrast attachment (Lumam-8). Cytokine levels of interleukin 1β (IL-1β), interleukin 6 (IL-6), interleukin 8 (IL-8), and tumour necrosis factor-α (TNF-α) were also measured. The concentration of interleukins in the blood serum was determined using a set of reagents for enzyme-linked immunosorbent assay (Diaclone, France). The results of the study were analyzed by the method of variation statistics using STATISTICA 6 software (Statsoft, USA). Values are presented as arithmetic means (M), standard errors of the mean (m), n – sample size. Each index was tested for normal distribution using the Shapiro-Wilk test.

Results of the study and their discussion. Comparative analysis of immunograms in the examined patients with hypertension compared with practically healthy volunteers did not reveal a significant difference in the total number of blood lymphocytes. However, a significantly higher level of B-lymphocytes, $CD23^+$ B-lymphocytes, natural killer cells, and a significantly lower level of T-helper cells were detected (Table 1).

Table 1

Population composition of peripheral blood lymphocytes of patients with abdominal tuberculosis before and after the Koch test

Indices of cellular immunity, 10^9/L	Groups of respondents		
	Control group, n=20	Patients with abdominal tuberculosis before the Koch test, n=50	Patients with abdominal tuberculosis after the Koch test, n=50
Lymphocytes	2.0±0.08	2.05±0.03 p>0.05	2.44±0.01 p<0.05, p_1<0.05
$CD3^+$lymph	1.07±0.08	1.06±0.09 p>0.05	1.30±0.9 p<0.05, p_1<0.05
$CD4^+$lymph	0.93±0.05	0.67±0.05 p<0.05	0.80±0.06 p<0.05, p_1<0.05
$CD8^+$lymph	0.33±0.03	0.37±0.03 p>0.05	0.50±0.04 p<0.05, p_1<0.05
$CD19^+$lymph	0.36±0.02	0.50±0.04 p<0.05	0.62±0.04 p<0.05, p_1<0.05
$CD23^+$lymph	0.14±0.01	0.37±0.02 p<0.05	0.38±0.03 p<0.05, p_1>0.05
$CD56^+$lymph	0.15±0.01	0.44±0.04 p<0.05	0.51±0.04 p<0.05, p_1<0.05

Notes: p – significance of differences compared to the control group; p_1 – significance of differences compared to the group of patients before the Koch test.

In 72 h after the Koch test, a significant increase in the number of T lymphocytes, an increase in the level of T helper cells and T cytotoxic suppressors, an increase in the number of NK cells and activated B lymphocytes were observed in the blood of patients with hypertension

As we can see, there is a pronounced lymphocytic reaction to the Koch test in patients with abdominal tuberculosis, indicating the presence of sensitisation in the chronic inflammatory process. There is a pronounced type IV hypersensitivity, as indicated by a significant increase in T-cytotoxic lymphocytes after the Koch test.

The degree of changes in the main immunoregulatory subpopulations, which determine the strength and direction of the immune response, is quantified by the immunoregulatory index (IRI). An IRI in the range of 1.52.5 corresponds to a normoergic state of the body. There was no significant difference in the value of the IRI before and after the Koch test compared to the control group. Thus, in patients with hypertension before the Koch test, the IRI was 1.81±0.23, which is within the normal range. After the Koch test, there was a tendency to decrease the IRI, which was 1.61±0.18, but was within the normal range.

A very interesting picture of the distribution of the studied levels of interleukins (IL-1β, IL-6, IL-8, TNF-α) was observed after the immunoprovocation Koch test (Table 2). After the test, the level of TNF-α increased significantly (2.5 times). It is known that TNF-α is produced by cells only in response to an inducer. Tuberculin immunoprovocation (Koch's test) is the inducer that caused a significant increase in TNF-α levels.

The concentration of IL-8 after immunoprovocation with tuberculin decreased by almost half, which, in our opinion, is due to its local use in the inflammatory focus, as this interleukin is able to activate cells in the inflammatory focus.

Table 2

Indices of cytokine profile in patients with abdominal tuberculosis before and after the Koch test

Groups of respondents	IL-1β, pg/ml	IL-6, pg/ml	IL-8, pg/ml	TNF-α, pg/ml
Control group, n=20	4.86±0.90	5.87±0.49	2.0±0.20	4.97±0.18
Patients with abdominal tuberculosis before the Koch test, n=50	27.42±1.80 p<0.05	7.69±0.65 p<0.05	20.39±2.30 p<0.05	17.57±1.05 p<0.05
Patients with abdominal tuberculosis after the Koch test, n=50	9.00±0.80 p<0.05 p1<0.05	1.97±0.20 p<0.05 p1<0.05	12.17±1.10 p<0.05 p1<0.05	49.85±1.20 p<0.05 p1<0.05

Notes: p — significance of differences compared to the control group; p1 – significance of differences compared to the group of patients before the Koch test

Patients with hypertension had significantly elevated levels of IL-1β (5.6-fold), IL-8 (10-fold) and TNF-α (3.5-fold) compared to the control group.

After the Koch test, the level of IL-1β, which was significantly higher than in healthy individuals, decreased threefold. As is known from the literature, the main biological role of this interleukin is mediator, ensuring the interaction of various protective inflammatory mechanisms at the level of the whole organism.

The concentration of IL-6 after the Koch test also significantly decreased (by 4 times), even compared to the control group. Since IL-6 exhibits the properties of a cytokine that can stimulate the development of an immune response, our data show that immunoprovocation with tuberculin suppressed the synthesis of this interleukin.

While there are many diagnostic tests available to detect active tuberculosis, the detection of latent tuberculous infection (LTBI) remains a challenge. Current diagnostic testing for LTBI is performed using tuberculin skin test (TST) and interferon (IFN)-γ release assays (IGRA) [10]. Tuberculin skin test is a diagnostic method performed by subcutaneous injection of old tuberculin (OT) or purified protein derivative (PPD) as an antigen [7, 9]. It has been used for screening, diagnosis, and epidemiological studies of primary M. tuberculosis infection for more than 100 years and is the primary diagnostic test for detecting LTBI. Tuberculin diagnostics is widely used for screening and detection of TB due to its advantages of affordability, ease of use and minimal laboratory equipment. The test has some drawbacks, such as the need for a second visit to the doctor after 48–72 hours, inability to distinguish between PTB and active TB, false-positive results with Bacillus Calmette-Guérin (BCG) vaccination, cross-reactivity with nontuberculous mycobacteria, and false-negative results in immunosuppression and deficiency [10].

Thus, studies have shown that 72 hours after the Koch test, patients with abdominal tuberculosis have a pronounced systemic immune response of the cell type that is characteristic of the body's sensibilization [1, 5]. It has also been confirmed that the key regulatory cytokines in AT are IL-1β and TNF-α, which is consistent with the findings of other researchers [6, 11]. These cytokines, according to our studies, are also key in regulating the immune response after the Koch immunoprovocation test.

Our study has shown that tuberculin diagnostics will continue to be clinically useful in low- and high-endemic regions until more predictive tests become available to identify those at highest risk of progression to active TB [7].

The interferon release assay (IGRA), which assesses the cell-mediated immune response in vitro, is now being proposed alongside the tuberculin skin test, and the results are based on the level of IFN-γ produced by circulating memory effector cells and the frequency of IFN-γ-producing effector T cells. It is also possible to assess changes in cellular immunity and regulatory cytokines by examining the immunogram in the dynamics, as shown in our work [8, 9].

Conclusion

Thus, there are statistically significant differences in the immunogram and cytokine profile of the examined patients before and after the Koch test. Immunoprovocation Koch test exacerbates pathogenetic manifestations of chronic inflammatory process, which is manifested by changes in the cytokine spectrum of blood and allows to clarify the diagnosis of abdominal tuberculosis Determination of TNF-α level in the blood serum of patients after Koch test can be a marker of latent form of abdominal tuberculosis.

Prospects for further research on the immune status of patients with AT is a promising area that will improve the differential diagnosis of abdominal pathologies and help identify the latent period of AT.

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