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CORRELATION OF SPONTANEOUS AND ANTIGEN-SPECIFIC PROLIFERATIVE ACTIVITY OF B-LYMPHOCYTES WITH CLINICAL AND LABORATORY SIGNS IN UROGENITAL REACTIVE ARTHRITIS

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КОРРЕЛЯЦИЯ ПОКАЗАТЕЛЕЙ СПОНТАННОЙ И АНТИГЕНСПЕЦИФИЧЕСКОЙ ПРОЛИФЕРАТИВНОЙ АКТИВНОСТИ В-ЛИМФОЦИТОВ С КЛИНИКО-ЛАБОРАТОРНЫМИ ПРИЗНАКАМИ ПРИ УРОГЕНИТАЛЬНОМ РЕАКТИВНОМ АРТРИТЕ

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Abstract. In this work, the subject of the study is the study of spontaneous (SPABL) and antigen-specific proliferative activity of B lymphocytes (AGPABL) with a clinical and laboratory sign of urogenital reactive arthritis (UReA). The purpose of this study was to evaluate the correlation of SPABL and AGPABL indicators in patients with UReA. The method of quantitative cytofluorometry (QCF) was used to determine SPABL and AGPABL. As a result of the work, a high correlation was noted between the indicators of SPABL and AGPABL with the generally accepted clinical and laboratory signs of UReA. A high correlation of SPABL and AGPABL indicators was established in patients with oligoarthritis, asymmetric arthritis, arthritis of the I-th metatarsophalangeal joint of the foot, urethritis, chlamydia from the urethra, HLA-B27 and serum antibodies to chlamydia, the severity of joint pain, with high levels of CRP and immunoglobulin G (IgG). Thus, studies of SPABL and AGPABL in combination with clinical and laboratory data contribute to the early diagnosis of UReA.

Аннотация. Предметом исследования является изучение спонтанной и антиген специфической пролиферативной активности В-лимфоцитов при клинико-лабораторных признаках урогенитального реактивного артрита. Целью настоящего исследования явилась оценка корреляции показателей спонтанной и антиген специфической пролиферативной активности В-лимфоцитов у больных урогенитальным реактивным артритом. Использовали метод количественной цитофлюорометрии для определения спонтанной и антиген специфической пролиферативной активности В-лимфоцитов. В результате работы отмечена высокая коррелятивная связь показателей спонтанной и антиген специфической пролиферативной активности В-лимфоцитов с общепринятыми клинико-лабораторными признаками урогенитального реактивного артрита. Установлена высокая корреляция показателей спонтанной и антиген специфической пролиферативной активности В-лимфоцитов у больных с олигоартритом, несимметричным артритом, артритом I-го

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плюснефалангового сустава стопы, уретритом, хламидий в соскобе из уретры, HLA-B27 и сывороточных антител к хламидиям, выраженностью болей в суставах, с высокими уровнями С реактивного белка и иммуноглобулином G (IgG). Таким образом, исследования спонтанной и антигенспецифической пролиферативной активности В-лимфоцитов в комплексе с клинико-лабораторными данными способствуют ранней диагностике реактивного артрита.

Keywords: urogenital reactive arthritis; spontaneous proliferative activity; antigen-specific activity; B-lymphocytes.

Ключевые слова: урогенитальный реактивный артрит; спонтанная пролиферативная активность; антиген специфическая активность; В- лимфоциты.

Reactive arthritis (ReA) is a non-purulent "sterile" inflammatory disease of the musculoskeletal system caused by an infection that is not detected by conventional microbiological methods [1].

In this case, the focus of infection is located outside the joint, primarily in the genitourinary tract or intestines. ReA belongs to the group of seronegative spondylarthritis and fully meets the criteria of the European Group for the Study of Spondylarthritis (ESSG) [2].

The role of infection in the development of ReA is evidenced by the dissemination of infection from foci of infection into joints or other organs, phagocytosis of microorganisms by macrophages and dendritic cells, persistence of trigger microorganisms and their antigens in the synovial membrane and articular fluid, as well as the identification of viable microorganisms capable of division [3].

At the same time, one of the triggers for the development of immunopathological reactions in ReA is the stimulation of the B-cell immune response by chlamydia infection, which is confirmed by the detection of antibodies to chlamydia in blood serum, the ability of lymphocytes to produce antibodies in the presence of chlamydia, as well as the presence of B cells sensitized to chlamydia [4-6].

Materials and methods

53 patients with reactive arthritis aged 17 to 45 years (17 women and 36 men) were examined. The first degree of activity of the pathological process was noted in 18 (33.9%) patients, the second — in 22 (41.5%) and the third degree — in 13 (24.5%) patients. The acute course of the disease was observed in 28 (52.8%) patients, prolonged in 15 (28.3%) and chronic in 10 (18.8%) patients.

The peripheral lymphocytes were isolated to determine the spontaneous proliferative activity of B-lymphocytes. The lymphocytes were isolated from peripheral venous blood stabilized with an anticoagulant at a density gradient of 1,007 g/cm3 verografin-ficoll. The gradient was prepared as follows: one part of a 76% verografin solution was mixed with four parts of a ficoll solution. After careful mixing, the mixture was ready for use (for long-term storage, the verografin-ficoll mixture is placed in the refrigerator at 4^0C). 2.5 ml of verografin-ficoll mixture was poured into a bacteriological tube (the height of the column of the mixture was 2-2.5 cm).

The tube was left on the table until the mixture reached room temperature. 5 ml of blood was taken from the ulnar vein. To prevent clotting, anticoagulants were added to the blood during sampling: heparin (20 units per 1.0 ml of blood) or 0.1 ml of 5% sodium ethylenediaminetetraacetate (EDTA) solution per 1.0 ml of blood. Using a pipette, whole 4 ml anticoagulant-stabilized blood was carefully layered onto the gradient, avoiding mixing of the gradient and blood. Then centrifuged at 1500 rpm for 30 minutes. In this case, red blood cells and

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granulocytes settle to the bottom of the tube, and mononuclear cells are located at the interface between the gradient and the blood. A layer of mononuclears (a dense cloud above the mixture) was sucked off with a Pasteur pipette over the entire cross-sectional area of the tube at the interface of the phases. The cells stuck to the tube wall were collected with the tip of a pipette. The lymphocytes were transferred to a clean centrifuge tube. The isolated cells were washed twice from plasma with medium 199 by centrifugation at 1000 rpm for 5 minutes. The appendages were discarded, and the lymphocytes were resuspended with a nutrient solution.

The isolated lymphocytes were washed 1 more time with medium 199, by centrifugation at 1000 rpm for 5 minutes, then resuspended in 3-4 drops of medium 199, bringing the cell concentration to 4-5x106 /ml.

The study of the proliferative function of lymphocytes was carried out according to the method described by E. G. Tsai [7].

This study was carried out in monolayer cultures created on slides [8].

A drop of thick, freshly isolated suspension of lymphocytes was applied to 2 clean, fat-free slides (control and experiment), incubated in a moist chamber at room temperature for 3-5 minutes. After that, the non-adhering cells were washed off with medium 199. As a result, a well-formed spot of a cellular monolayer of viable cells remained on the glass. Then, glasses with a formed monolayer of lymphocytes, preventing drying, were immediately placed in culture chambers with a complete nutrient medium consisting of medium 199 with the addition of 10% bovine serum, L-glutamine (300 mg/ml) and gentamicin (0.08 mg/ml). Then, B-cell mitogen, LPS 5 mpd/ml, was added to both lymphocyte cultures (control and experiment). After that, the chamber with the control lymphocyte culture was immediately placed in a refrigerator at 4 °C, and the chamber with the experimental culture was placed in a thermostat at 37 °C with a wet chamber. The chambers were incubated for 2 hours. Then the glasses were removed, rinsed in medium 199, and fixed for 15 minutes with a 70% ethanol solution. Then the smears were stained with 0.001% acridine orange (AO) according to R. Rigler, excluding the stage of protein acetylation [9].

The working solution of AO was prepared on the day of the experiment from the mother solution of AO at a concentration of 1:1000, diluting it with citrate buffer to a concentration of 1:100000. Then the preparation was washed for 10 minutes in pure citrate buffer, dried and fluorimetrated by the CCF method. CTF was performed using an original method based on a LUMAM-IZ microscope using an FMEL-1 photometric prefix. The excitation source was a DRK-120 lamp, which gave a stable discharge, the source was installed according to the lighting option from above, the exciting filter CC-15-4, the locking filter LC-9. The light-emitting system was installed according to the dark-field version with a dark-field OPAC lens with a low magnification of 9x0.20. To ensure maximum intensity recording, luminescence was carried out on a FEU-39A with a base voltage of the amplification complex of 1000-1500 volts with the output of the results to a digital voltmeter in a register of 2-20 volts. Cytofluorometry of lymphoid cells stained with AO was performed as follows. A photometer lens was focused on an arbitrary section of the preparation under non-exciting lighting, in which one of the microprobes was previously removed in order to provide measurement from the entire lens area. After focusing the lens, the position corresponding to the removed microprobe was set, the light filter was replaced with an exciting one, and the fluorescence intensity in the 640 nm region was measured, highlighting this area with an interference light filter built into the photometer. After recording the result, the interference filter was replaced with another one by turning the disk and fluorescence was measured in the 530 nm region. The entire procedure of direct measurements takes 20-30 seconds, which practically eliminates the effect of AO photo destruction.

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The results obtained were expressed by the ratio of the fluorescence of the AO complex with RNA (640 nm) to the AO complex with DNA (530 nm). This ratio (F640/F530) is known as parameter A, reflecting the degree of activity of nuclear chromatin cells. Thus, the ratio of RNA/DNA of nuclear chromatin was determined, which naturally changes during the activation of lymphocytes.

Comparing the level of parameter, A in the control and the experiment, the indicator of spontaneous proliferative activity of B lymphocytes (SPABL) was derived according to the formula: SPABL = (A experiment: A control) x 100 conl. units.

At the same time, as indicated in the works of the developers of this method [9], the value of SPABL equal to 125 conl. units or more was accepted as a positive result of this study.

Determining the antigen-specific proliferative activity of B-lymphocytes (AGPABL), peripheral lymphocytes were isolated, as described above.

Lymphocytes were collected from the interphase, which were washed once with medium 199, by centrifugation at 1000 rpm for 5 minutes. The additives were discarded, and the mononuclear were resuspended with 1.0 ml of medium 199. 0.5 ml of lymphocyte suspension was added to 2 (two) centrifuge tubes (control and experiment) with a complete nutrient medium, the composition of which is described above. 1 drop of saline solution was added to the control, and 1 drop of diluted chlamydia antigen was added to the experiment. Then, the experimental and control samples were placed in a thermostat at 37°C with a wet chamber. The samples were incubated for 18 hours in hermetically sealed centrifuge tubes.

Then, as described above, parameter A. was determined.

Comparing the levels of parameter, A in the experiment and the control, the indicator of antigen-specific proliferative activity of B-lymphocytes (AGPABL) was derived using the formula: AGPABL = (And experience: And control) ×100 conl. units.

Statistical processing of the obtained results was carried out on a personal computer using special programs with the calculation of the arithmetic mean (M), standard deviation (δ), arithmetic mean error (m), confidence coefficient (t), probability index (P), correlation coefficient (r).

In ReA patients, before treatment, the correlation of SPABL and AGPABL indicators with the 18 following generally accepted clinical and laboratory indicators of the disease was performed. The results are presented in (Table).

Table

CORRELATION OF SPABL AND AGPABL INDICATORS WITH SOME GENERALLY ACCEPTED CLINICAL AND LABORATORY INDICATORS OF UREA BEFORE TREATMENT.

Signs of ReA	*Values of the correlation index (r)*	
	SPABL	*AGPABL*
Oligoarthritis	0,78	0,81
Asymmetrical arthritis	0,82	0,84
Arthritis of the first metatarsophalangeal joint of the foot	0,65	0,69
Arthritis of the II, III, IV and V metatarsophalangeal joints of the foot	0,44	0,47
Unilateral sacroiliitis	0,47	0,51
Urethritis	0,68	0,76
Conjunctivitis	0,38	0,42
Keratoderma of soles and/or palms	0,27	0,28
Severity of joint pain (VAS in mm)	0,74	0,62
Number of painful joints	0,62	0,56
Number of swollen joints	0,56	0,51
Severity of enthesopathies (VAS in mm)	0,61	0,57

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Signs of ReA	*Values of the correlation index (r)*	
	SPABL	*AGPABL*
ESR, mm/h	0,62	0,54
CRP, mg/ml	0,71	0,66
IgG, g/l	0,56	0,67
HLA-B_{27}	0,66	0,72
Chlamydia in urethral scraping	0,62	0,81
Serum antibodies to chlamydia	0,56	0,88

As can be seen from (Table 1), a high correlation of the SPABL index with the frequency of detection of oligoarthritis (r=0.78), asymmetric arthritis (r=0.82), arthritis of the ith metatarsophalangeal joint of the foot (r=0.65), urethritis (r=0.68), chlamydia in urethral scraping (r=0.62), HLA-B27 (r=0.66) and serum antibodies to chlamydia (r=0.56), the severity of joint pain (r=0.74) and enthesopathy (r=0.61), the number of painful joints (r=0.62), with high levels of ESR (r=0.62) and CRP (r=0.71).

There was an average correlation between the SPABL index and the frequency of detection of arthritis of the II, III, IV and V metatarsophalangeal joints of the foot (r=0.44) and unilateral sacroiliitis (r=0.47), the number of swollen joints (r=0.56) and high IgG levels (r=0.56).

The value of SPABL was weakly correlated with the frequency of detection of keratoderma of soles and/or palms (r=0.28).

As follows from (Table 1), a high correlation of the AGPABL index with the frequency of detection of oligoarthritis (r=0.81), asymmetric arthritis (r=0.84), arthritis of the ith metatarsophalangeal joint of the foot (r=0.69), urethritis (r=0.76), chlamydia in urethral scraping (r=0.81), HLA-B27 (r=0.72) and serum antibodies to chlamydia (r=0.88), severity of joint pain (r=0.62), with high levels of CRP (r=0.71) and IgG (r=0.67).

There was an average correlation between the AGPABL index and the frequency of detection of arthritis of the II, III, IV and V metatarsophalangeal joints of the foot (r=0.47), unilateral sacroiliitis (r=0.51) and conjunctivitis, the number of painful and swollen joints (r=0.56 and r=0.51), the severity of enthesopathies (r=0.57) and high the level of ESR (r=0.54).

The AGPABL value was weakly correlated with the frequency of detection of keratoderma of the soles and/or palms (r=0.27).

Thus, the following can be noted. A correlative relationship of the SPABL index with all 18 generally accepted clinical and laboratory signs of ReA was noted.

At the same time, in ReA patients, the SPABL index correlated with the presence of oligoarthritis, asymmetric arthritis, arthritis of the first metatarsophalangeal joint of the foot, urethritis, chlamydia in urethral scraping, HLA-B27 and serum antibodies to chlamydia, the severity of joint pain and enthesopathies, the number of painful joints, with high levels of ESR and CRP were high.

With ReA, the correlation between the value of SPABL, on the one hand, and the frequency of detection of arthritis of the II, III, IV and V metatarsophalangeal joints of the foot and unilateral sacroiliitis, the number of swollen joints and high IgG levels, on the other hand, was average.

A weak correlation was found between the SPABL index and the frequency of detection of conjunctivitis and keratoderma of the soles and/or palms in ReA.

The correlative relationship of the AGPABL index with all 18 generally accepted clinical and laboratory signs of ReA was noted.

Thus, in ReA patients, a high correlation was found between the AGPABL index and the presence of oligoarthritis, asymmetric arthritis, arthritis of the first metatarsophalangeal joint of the foot, urethritis, chlamydia in urethral scraping, HLA-B27 and serum antibodies to chlamydia,

severity of joint pain, high levels of CRP and IgG. At the same time, in ReA patients, the correlation between the value of AGPABL and such signs of the disease as the frequency of detection of arthritis of the II, III, IV and V metatarsophalangeal joints of the foot, unilateral sacroiliitis and conjunctivitis, the number of painful and swollen joints, the severity of enthesopathies and a high level of ESR was average. A weak correlation was found between the AGPABL index and the frequency of detection of keratoderma of the soles and/or palms during ReA.

Conclusions:

In patients with ReA, there was a high correlation between the indicators of SPABL and AGPABL with the generally accepted clinical and laboratory signs of ReA;

The index of SPABL and AGPABL weakly correlated with the frequency of detection of keratoderma of soles and palms in ReA;

Studies of SPABL and AGPABL in combination with clinical and laboratory data contribute to the early diagnosis of ReA.

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