13. A. A. Khusinov, Ya. K. Kurambaev and D. Kh. Ziyaeva, Disturbance of Mechanisms of Regulation and Their Correction [in Russian], Vol. 1, Moscow (1989), p. 217.
14. J. Ahlqvist, Psychoneuroimmunology, New York (1981), pp. 355-403.
15. E. Dupont, J. Wybran, and C. Toussaint, Transplantation, **37**, No. 4, 331 (1984).
16. W. J. Irvine, Immunology-80, London (1980), pp. 204-206.
17. C. Lafarge-Frayssint and E. Decloitre, J. Immunopharmacology, **4**, No. 2, 43 (1982).
18. E. Okaushi, J. Physiol. Soc. Jpn., **38**, 325 (1976).

ANTIBODY LEVELS TO DIFFERENT DETERMINANTS OF GROUP A STREPTOCOCCAL POLYSACCHARIDE AND AUTOANTIBODIES TO THE BASAL LAYER OF THE SKIN EPITHELIUM IN RHEUMATIC FEVER

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In various autoimmune processes in man and in New Zealand mice autoantibodies reacting with the basal layer of the skin epithelium (BLSE) and also with the epithelium of the cortical and medullary zones of the thymus [6], which is a type of endocrine epithelium [13], have been found It was shown previously that the streptococcal group A polysaccharide (A-PSC) contains a cross-reacting (CR) determinant, to which autoantibodies directed toward the above-mentioned epithelial cells of the skin and thymus mentioned above are directed [14]. During isolation of the antibodies by affinity chromatography it was shown that these reactions are linked with high-affinity antibodies to a group-specific (GS) determinant of A-PSC [5]. Similar reactions with epithelium of the thymus and skin have been found with the aid of monoclonal antibodies (MCA), obtained to one of the rhamnose determinants of A-PSC MCA evidently arising against another rhamnose determinant of A-PSC, react with all layers of epithelium of the skin and thymus [7]. It has been suggested that injury to the endocrine epithelium of the thymus by autoantibodies leads to immunoregulatory disturbances, namely to a deficiency of suppressor T cells, a characteristic feature of autoimmune processes [6].

A high level of antibodies to the GS determinant of A-PSC, containing β-N-acetylglucosamine, is found as a rule in primary active rheumatic fever (PAR), in a smaller percentage of cases in recurrent active rheumatic fever (RAR), in individual cases In an inactive phase of rheumatic fever, and in not more than 10% of control sera. Conversely, in PAR a high level of antibodies to rhamnose determinants of A-PSC is found in individual cases, and rather more often in healthy blood donors and patients with RAR [2, 3, 4]. Autoantibodies to BLSE and to epithelium of the cortical and medullary zones of the thymus have been found in a high percentage of cases in PAR, less frequently in RAR, and in individual cases in healthy controls [10]. These autoantibodies did not inhibit A-PSC in all cases, and it is not clear to which CR determinant of A-PSC they are directed. It likewise has not been established whether any connection exists between the presence of autoantibodies to BLSE and the level of antibodies to GS, and also to the rhamnose determinants of A-PSC in rheumatic fever and in healthy control sera Since a high level of antibodies to the GS determinant of A-PSC [2] and autoantibodies to BLSE [10] are observed very rarely in control sera, it is rational to undertake a special analysis of sera of the control group with a high level of antibodies to A-PSC.

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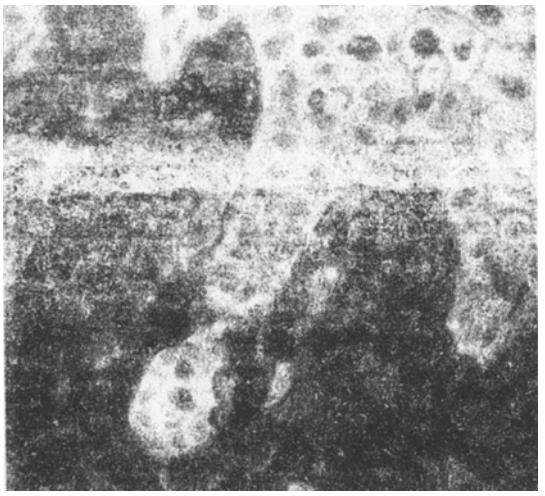

Fig 1. Reaction of serum of rheumatic fever patient with BLSE. (Reaction with DLSE can be observed simultaneously).

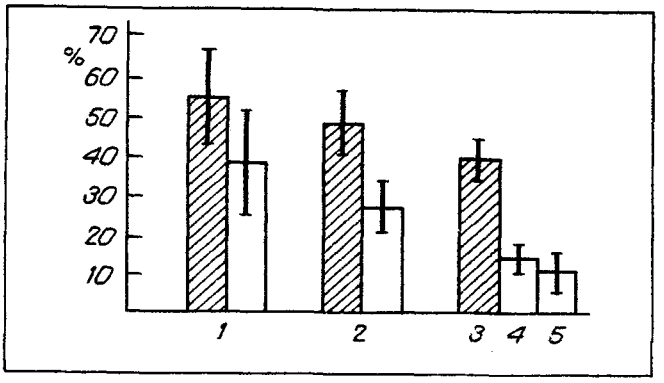

Fig. 2. Autoantibodies to BLSE in rheumatic fever and in control sera with different levels of antibodies to A-PSC. Percentage detection of reactions with BLSE: shaded columns – sera with high antibody titer to A-PSC (≥12,800); unshaded columns – titer of antibodies to A-PSC in sera ≤6400, or antibodies not present. 1) PAR, 2) RAR, 3) control No. 1, 4) control No. 2, 5) control No. 3.

The aim of this investigation was: to compare the presence of autoantibodies reacting with the basal layer of epithelium of the skin in primary and recurrent active rheumatic fever, and also in control sera, with the level of antibodies to the GS determinant and to the rhamnose region of group A streptococcal polysaccharide.

EXPERIMENTAL METHOD

Antibodies to A-PSC were determined by enzyme immunoassay (EIA) [3] and in the immunodiffusion (ID) test with different doses of A-PSC. The level of antibodies to rhamnose determinants of A-PSC was determined by EIA, by calculating the percentage inhibition of reactions of sera with A-PSC on the addition of streptococcal A-variant polysaccharide (V-PSC) [3].

Altogether 257 sera were tested. Of that number 41 were from patients with PAR (age 10-20 years), 81 from patients with RAR (18-60 years), and 135 sera were from clinically healthy individuals (control). Thirty sera (control No. 1 – adults) with high titers of antibodies to A-PSC in ID and EIA (≥12,000), which was evidently associated with previous streptococcal infections, were specially selected.

An additional 73 adult sera with a lower level of antibodies to A-PSC or with negative reactions (control No. 2), and 32 sera from clinically healthy persons (aged 10-20 years), without subdivision by level of antibodies to A-PSC (control No. 3) also were tested. Preparations of A-PSC and V-PSC (V-PSC is a homopolymer of rhamnose, identical with the rhamnose region of A-PSC) were obtained [12] from group A streptococci (No. 6/49, Prague collection) and A-variant (No. 32/18, from M. McCarty, USA).

Autoantibodies to BLSE were tested by the indirect immunofluorescence method (IIFM) in sections of human group 0 embryonic skin [14] with antibodies to human IKG, labeled with fluorescein isothiocyanate (the antibodies were obtained from Professor K. L. Shakhinina, Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR). The LYUMAM I-1 fluorescence microscope with ×40 objective was used. For photography, a Homal ×3 objective was used. The reaction was considered positive if marked fluorescence appeared on testing sera diluted 1/8-1/32 with BLSE. In experiments to inhibit the reaction with A-PSC in the ID test or the reaction with BLSE, A-PSC or V-PSC was added in doses of 2 mg/ml serum [3].

EXPERIMENTAL RESULTS

Sera obtained from patients with PAR or RAR were divided into two groups depending on the titer of antibodies to A-PSC Sera with an antibody titer in the EIA test of ≥12,800 and with high levels of antibodies in the ID test were placed in group 1. Those with an antibody titer in EIA of 6400 or higher, and also those with a lower level of antibodies in the ID test, were placed in group 2. A number of sera in which no antibodies to A-PSC were found also were included in this group. During determination of antibodies to A-PSC a high level of antibodies to rhamnose determinants (inhibition of reactions in EIA with A-PSC by more than 40% under the influence of V-PSC), was found in only 6.7-6.6% in PAR, in 50 ± 10.6% in patients with RAR, and in 21 ± 9.3% in the control sera.

As a result of tests of sera by the IIFM, in the presence of PAR, reactions were found with BLSE in group 1 in 55 ± 11.8%, but in group 2, with a lower level of antibodies to A-PSC, in only 38.4 ± 14%. With RAR reactions of BLSE were found in 48.6 ± 8.4% in group 1 and 28.2 ± 6.7% in group 2 (Figs. 1 and 2). In the control sera with a high level of antibodies to A-PSC (control No. 1) antibodies to BLSE were determined also in a high percentage of cases (40 ± 5.4%). In control No. 2 with a lower level of antibodies to A-PSC a reaction with BLSE was found in 15 ± 4.1%, and in control No. 3, in 12 ± 5.7%. To identify to which CR determinants of A-PSC the autoantibodies reacting with BLSE were directed, inhibition tests were carried out with A-PSC and V-PSC. The selected sera, containing a high level of antibodies to A-PSC and reacting intensively in IIFM with BLSE, were chosen. The results showed that the reaction of all five sera obtained from patients with PAR with BLSE is inhibited intensively by A-PSC, but in three cases by V-PSC also; conversely, in RAR (nine sera) and in the control group (seven sera) the reaction with BLSE was not inhibited by A-PSC but was strongly inhibited by V-PSC.

Thus dependence of reactions with BLSE on the level of antibodies to A-PSC was established. Autoantibodies of patients with PAR and RAR are probably directed toward different CR determinants of A-PSC Autoantibodies to BLS13 in PAR in certain cases evidently arise to the GS determinant of A-PSC. As a rule a high level of antibodies to the GS determinant of A-PSC is observed in these sera This is shown by data obtained by the ID method, in which antibodies are determined only to the GS determinant [2]. A high level of antibodies to the rhamnose determinants of A-PSC was found extremely rarely in these patients by EIA In addition, according to preliminary data, inhibition of reactions with BLSE during PAR is sometimes induced by A-PSC only At the same time, autoantibodies in RAR and in control sera evidently are CR to one or two different rhamnose determinants. This is confirmed by the results of inhibition of reactions with BLSE on the addition of V-PSC and the discovery of a high level of antibodies to rhamnose determinants, especially in RAR. The different results of inhibition of reactions of antibodies to rhamnose determinants with the aid of A- and V-PSC probably depend on the fact that rhamnose determinants or one of them is hidden on A-PSC by the terminal determinant, which contains β-N-acetylglucosamine and is more accessible in V-PSC [12]. Furthermore, as was pointed out above, data were obtained with the aid of MCA in relation to the presence of two CR determinants in the rhamnose region of A-PSC inducing production of autoantibodies to the epithelium and thymus and skin [7]. In some cases positive reactions with BLSE in the absence of antibodies to A-PSC may evidently be connected with antibodies to a rhamnose determinant which is hidden in A-PSC. However, autoantibodies of a different specificity may also be present If autoantibodies to BLSE are present for a long time, and are CR with A-PSC, production of autoantibodies to other tissue antigens may be possible [7]. These are problems for further study.

The possibility cannot be ruled out that autoantibodies arising to GS or to one other rhamnose determinant of A-PSC may differ in their action on the immune system, in connection with the reaction of different antigens of the thymus. It was shown that in a specially selected group of control sera with a high level of antibodies to A-PSC, reactions with DLSE were observed in a higher percentage of cases than when the antibody level was lower. According to other data, a reaction with BLSE is observed under normal conditions even less frequently (from 1.5 to 6% of cases) [6].

The hypothesis relating to the role of autoantibodies to BLSE, which usually react with the endocrine epithelium of the thymus [6], in various autoimmune processes is confirmed by certain new data. Preliminary results were obtained which indicate a decrease in the number of suppressor T cells in the presence of autoantibodies to BLSE in patients with glomerulonephritis. Positive correlation also has been found between a high level of autoantibodies to BLSE and a T-suppressor cell deficiency in hepatitis B [9]. The absence of rheumatic fever in the control group with a high level of antibodies to A-PSC and in the presence of autoantibodies to BLSE may depend on genetic factors, and also on infection by streptococci which do not contain antigens common with heart tissue antigens. Such CR antigens are known to be characteristic of rheumatogenic strains [7].

Autoantibodies to BLSE are evidently the first stage in the onset of the autoimmune process, in connection with a suppressor T-cell deficiency, which must facilitate the development of cellular cytotoxic reactions to streptococcal CR antigens [6]. It has been suggested on the basis of experimental data that antibodies to the rhamnose region of A-PSC, and reacting not only with BLSE but also with the cytoplasm of cells of differentiated epithelial layers, can prevent the development of an autoimmune process, in connection with the possible suppression of cellular cytotoxic reactions Conversely, antibodies to the GS determinant of A-PSC, directed only against BLSE, evidently prevent suppression of these reactions [8, 1]. These problems require further study under experimental conditions and in autoimmune diseases in man.

In conclusion, it must be pointed out that the discovery of autoantibodies to BLSE is evidently an indicator of activity of rheumatic fever. This method should also be used, evidently, to predict the development of autoimmune processes after acute streptococcal infections.

LITERATURE CITED

1. E. A. Bazanova, É. V. Gnezditskaya, I. M. Lyampert, et al., Byull. Éksp. Biol. Med., No. 8, 170 (1990).
2. N. A. Borodiyuk, I. M. Lyampert, I. N. Mikhailova, et al., Zh. Mikrobiol., No. 6, 77 (1985).
3. N. A. Borodiyuk, N. G. Puchkova, A. V. Nekrasov, et al., Immunologiya, No. 4, 60 (1990).
4. N. A. Borodiyuk, E. A. Bazanova, I. M. Lyampert, et al., Zh. Mikrobiol., No. 5, 41 (1991).
5. V. Yu. Kolesnikova, É. V Gnezditskaya, and I. M. Lyampert, Byull. Éksp. Biol. Med., No. 12, 708 (1980).
6. I. M. Lyampert, Immunologiya, No. 4, 5 (1988).
7. I. M. Lyampert, É. I. Dobryashevskaya, E. V. Ryzhikova, et al., Progress in Science and Technology Series: Immunology [in Russian], Vol. 22, Moscow (1988), pp 43-67.
8. I. M. Lyampert, E. A Bazanova, and N. A. Borodiyuk, Abstracts of Proceedings of the First All-Union Congress of Immunologists [in Russian], Vol. 2 (1989), p. 81.
9. N. R. Mursyaeva, L. V. Chistova, and I. M. Lyampert, Pediatriya, No. 1, 13 (1991).
10. E. V. Ryzhikova, G. V. Stepina, V. A. Cherkasov, et al., Vestn. Akad. Med. Nauk SSSR, No. 7, 45 (1986).
11. D. G. Braun, W. Schalen, L. S. Rodkey, and J. K. Wright, Pathogenic Streptococcus, ed. by M. J. Parker, Oxford (1979), pp. 84-85.
12. J. H. Coligan, J. J. Kindt, and R. M. Krause, Immunochemistry, **15**, 755 (1978).
13. B. F. Haynes, Advances in Immunology, **36**, 84 (1984).
14. I. M. Lyampert (J. M. Lyampert), L. V. Beletskaya, N. A. Borodiyuk (N. A. Borodijuk), et al., Immunology, **31**, 47 (1976).