PRECIPITATING MONOCLONAL ANTIBODIES TO AN ANTIGENIC DETERMINANT OF GROUP A STREPTOCOCCAL POLYSACCHARIDE

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The specific antigenic determinant of group A streptococcal polysaccharide (A-PS) is known to be the terminal region of the molecule, containing N-acetylglucosamine with a β-bond. A-PS also contains rhamnose oligosaccharides, a structure that is common to polysaccharides of different groups of streptococci-including the A-variant streptococcus (V-PS) [13]. Cross-reactions also have been found between A-PS and the polysaccharide of group L streptococcus (L-PS) [1, 8]. According to some data, this depends on the presence of N-acetylglucosamine in the L-PS molecule [9]. The partial identity of A-PS and L-PS also has been established during immunodiffusion tests with antibodies taken from an immunosorbent containing β-N-acetylglucosamine [2].

Hybridomas synthesizing monoclonal antibodies (MCA) against A-PS have now been obtained [7]. On testing by the precipitation method in a liquid medium it was shown that some MCA react simultaneously with streptococcal polysaccharides of groups A, L, and E (E-PS), and in one case, with A-PS and E-PS. Inhibition of the reaction of some MCA with A-PS by monosaccharides in the composition of polysaccharides of other groups of streptococcus also has been found [7].

Antigenic determinants of A-PS, common with polysaccharides of other groups of streptococcus, have thus not been completely studied.

The aim of this investigation was to obtain MCA against group A streptococcal polysaccharide and to test these antibodies with polysaccharides from different groups of streptococci by immunoenzyme assay and by immunodiffusion.

EXPERIMENTAL METHOD

Streptococcal cultures of 17 groups: A, B, C, D, E, G, H, K, L, M, O, P, Q, R, S, T (the Prague Collection was obtained from Dr. J. Rotta) and A-variant of streptococcus (obtained from M. McCarty, USA) were used. HCl extract from whole microbial cells were prepared by Lancefield's method. Preparations of polysaccharides were obtained by extraction with formamide from whole microbial cells or cell walls [6]. Peptidoglycan and teichoic acid also were isolated from A-variant streptococcus [13].

Fractions containing nontype-specific (NTS) cell wall protein antigens of group A streptococcus were obtained from HCl extracts by preparative electrophoresis [11] or by treating the cultures with KCNS (KCNS fraction) [3].

BALB/c mice weighing 17-18 g were immunized with a culture of group A streptococcus (strain J-17A^4) treated with pepsin [5]. Sera from rabbits immunized with the same culture, treated with pepsin, were used in control experiments [14].

To obtain hybridomas, the following mouse plasmacytoma lines were used: NPS, P-2/0. Cells were fused with polyethylene-glycol with mol. wt. of 4000 daltons, (from Serva and Merck, West Germany). Hybridization was carried out by the usual method [10]. Peritoneal

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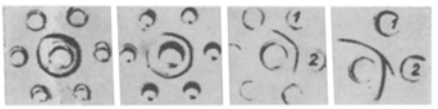

Fig. 1. Reaction of MCA to A-polysaccharide (clone AIII/2) with A- and L-polysaccharides on testing by the immunodiffusion method. Central wells contained: A, B, C — MCA to A-PS, D — control (serum of a rabbit immunized with group A streptococcus, treated with pepsin). Peripheral wells contained: A) A-PS (500-15 μg/ml); B) L-PS (50-15 g/ml); C, D: in well 1) A-PS, in well 2) L-PS.

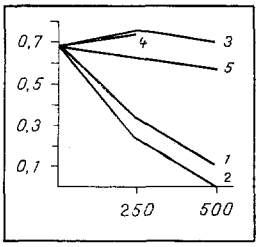

Fig. 2. Testing MCA to A-PS (clone AIII/2) by enzyme immunoassay on culture of group A streptococcus treated with pepsin. Abscissa, concentration of antibodies (in μg/ml); ordinate, optical at 492 nm. Determination of inhibitory action after addition of various preparations: 1) A-PS, 2) L-PS, 3) V-PS, 4) C-PS, 5) peptidoglycan.

macrophages of syngeneic and allogeneic mice were used feeder cells. For cloning, the method of limiting dilutions (0.5-1 cell/well) was used.

Hybrid cells were injected intraperitoneally into mice in a dose of 10^7. Ascites fluid was obtained on the 14th-30th day and frozen at −20°C. The clones were kept at −70°C or in liquid nitrogen. Enzyme immunoassay (EIA) [14] with rabbit antibodies against mouse immunoglobulins conjugated with peroxidase was used to screen the hybrid clones and to determine antibodies in the supernatant during culture of the monoclones, and also in the ascites fluid (the conjugate was obtained from the laboratory of Immunologic Diagnosis, N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR; director K. L. Shakhanina).

A-PS and polysaccharides of other groups of streptococci, namely C, L, and V (10 μg/ml) or a culture of group A streptococcus treated with pepsin were used. The substrate was 5-aminosalicyclic acid or o-phenylenediamine. The reaction was read on a photometer (titerteck).

To determine MCA in the supernatants and ascites fluids, the method of immunodiffusion in agar gel also was used. In the inhibition experiments ascites fluid was mixed with various antigens, incubated for 2 h at 37°C and for 20 h at 4°C, and then tested by EIA.

EXPERIMENTAL RESULTS

Altogether 9 stable monoclones, recloned by the limiting dilutions method, were obtained and 25 samples of ascites fluid were prepared in mice. The samples were found to react with A-PS in titers of 1:6,400-1:100,000, and in very high titers when tested on group A streptococci treated with pepsin (1:50,000-1:1,000,000).

Determination of antibodies by the immunodiffusion method with A-, C-, L-, and V-PS revealed reactions of the ascites fluid in 6 cases with A- and L-PS.

Ascites fluid obtained from 4 mice (clone A/III-2) gave an intensive reaction, but in two other cases (clones B2/I and 2-I) a weaker reaction with A-PS and L-PS. Complete identity of the precipitation lines with the two polysaccharides was found. In control experiments, on testing A-PS and L-PS with rabbit sera to group A streptococcus, partial identity was found (Fig. 1).

Intensively precipitating MCA were tested additionally with C-PS and V-PS, peptidoglycan, teichoic acid, the NTS fraction isolated from the HCl extract, and KCNS fraction, and HCL extracts obtained from streptococci of all groups listed above. A reaction was observed only with HCl extracts prepared from streptococci of groups A and L. Complete identity of the precipitation lines with the above HCl extracts and A polysaccharide was observed. Positive reactions were not found with the HCl extract obtained from group E streptococci.

To obtain more detailed characteristics of the precipitating MCA, inhibition experiments were carried out. In this case, A-, C-, L-, and V-PS or peptidoglycan were added to ascites fluids, diluted in the ratio of 1:1,600-1:3,200. When tested by EIA with a culture of group A streptococcus, treated with pepsin, inhibition of the reactions was observed only after addition of A-PS or L-PS to the ascites fluid (Fig. 2).

Nonprecipitating MCA in ascites fluids obtained on injection of six different clones into mice were tested by EIA with A-PS. In all cases positive reactions were found.

Thus three clones synthesizing precipitating MCA were obtained, which reacted with a determinant common for both A- and I-PS. By contrast with results obtained by other workers [7], in the present experiments no cross reactions were observed with E-PS when MCA reacting with A- and L-PS were tested by the immunodiffusion test in gel.

It has been suggested that A-PS contains two antigenic determinants, linked with β-N-acetylglucosamine, which differ in location and specificity: one of them is a repeating determinant, located on the inner segments of A-PS chains, whereas the second determinant is terminal, and precipitating antibodies evidently do not appear against it [4]. Production of MCA reacting in the immunodiffusion test with A- and L-PS is evidently connected with the fact that the common determinant for these polysaccharides is the determinant repeated on A-PS chains. We were unable to obtain precipitation in gel with other MCA reacting with A-PS. The reason is probably that nonprecipitating MCA are aimed at the terminal determinant of A-PS. These antibodies required further study.

Incidentally, during testing not only of whole sera, but also of specific antibodies to A-PS, obtained from an immunosorbent containing synthetic β-N-acetylglucosamine by the immunodiffusion method, precipitating antibodies could be obtained [2]. MCA reacting in the precipitation test in liquid medium only with A-PS also have been obtained [7]. In the future it will be necessary to study the possibility of obtaining MCA specific for A-PS and reacting in the immunodiffusion test. Such antibodies can be used to improve the previous method of identification of group A streptococci by the precipitation test in gel [12]. Production of MCA specific for A-PS and reacting in the immunodiffusion test will also shed light on the particular features of the corresponding antigenic determinants of A-PS.

LITERATURE CITED

1. N. A. Borodiyuk, E. N. Semenova and I. M. Lyampert, Zh. Mikrobiol., No. 3, 22 (1980).
2. V. Yu. Kolesnikova, N. A. Borodiyuk, and I. M. Lyampert, Byull. Éksp. Biol. Med., No. 5, 433 (1979).
3. E. N. Semenova, V. B. Kolesnikova, and M. N. Smirnova, Byull. Eksp. Biol. Med., No. 3, 51 (1983).
4. D. G. Braun, Microbiol. Immunol., 27, 823 (1983).
5. D. G. Braun, B. Kindred, E. B. Jakobson, et al., Eur. J. Immunol., 2, 138 (1972).
6. J. E. Coligan, W. C. Schnute, and T. J. Kindt, J. Immunol., 114, 1154 (1975).
7. H. Herbst, D. Lavanchy, and D. S. Braun, Ann. Immunol. Inst. Pasteur, 134-D, 349 (1983).
8. J. Jelinkova, R. Bicova, and J. Rotta, J. Hyg. (Prague), 11, 323 (1967).
9. W. W. Karakawa, J. E. Wagner, and J. H. Pazur, J. Immunol., 107, 554 (1971).
10. G. Köhler and C. Milstein, Nature, 256, 495 (1975).
11. I. N. Lyanpert, S. G. Shuratova, V. V. Akimova, et al., Infect. Immun., 17, 21 (1977).
12. I. M. Lyanpert, N. A. Borodiyuk, T. K. Asoskova, et al., Infect. Immun., 19, 961 (1978).

13. C. K. Osterland, E. J. Miller, and W. Karakawa, J. Exp. Med., 123, 599 (1966).
14. A. Voller, in: Enzyme-Immunoassay, E. T. Magio, ed., Florida (1980).

INTERLEUKIN 2-INDUCED RECOVERY OF NATURAL KILLER ACTIVITY IN STRESS

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Interleukin 2 (IL-2), or T-cell growth factor, promotes the maintenance and proliferation of functionally active populations of T lymphocytes and natural killer cells (NKC) [5-7, 9]. This lymphokine is produced mainly by T helper/amplifier cells and it acts on cytotoxic lymphocytes [9]. Besides its distant action, which characterizes it as a hormone-like factor of the immune system, IL-2 can effect autocrine stimulation, as has been shown for some cytotoxic T lymphocytes and NKC [4, 10].

The writers previously showed inhibition of concanavalin A (con A) -induced IL-2 production by the spleen cells of mice exposed to stress [2]. On the basis of these findings it was postulated that disturbance of IL-2 production may lie at the basis of the pathogenesis of stress-induced disturbances of the immune system. A deficiency of this lymphokine is evidently one of the main links in the mechanism of stress-induced depression of NKC activity.

On the basis of the above remarks, and considering the inducing action of IL-2 on NKC, in the investigation described below IL-2 was studied as one of the pathogenetic agents in the experimental treatment of the immunodepressive state of the system of natural antitumor resistance, arising as a result of exposure to stress.

EXPERIMENTAL METHOD

Experiments were carried out on male CBA mice aged 12 weeks and exposed to immobilization stress for 6 h [2]. NKC activity was determined 24 and 48 h after the end of exposure to stress and injection of IL-2 in the microcytotoxicity test, with release of radioactive chromium from YAC-1 and K 562 target cells [1, 3]. IL-2 (titer (1:512), obtained from the supernatant of a culture of BALB/c mouse spleen cells (5×10^6 cells/ml), stimulated by con A (5 μg/ml), was used in experiments *in vivo* and *in vitro* [2]. The residual leukin in the IL-2 preparations was neutralized with α-methyl-D-mannoside (20 mg/kg) or was removed by adsorption on Sephadex G-150 [8].

EXPERIMENTAL RESULTS

There were two stages to the investigation. In the first stage the action of IL-2 was assessed *in vitro* on NKC of intact mice and of animals exposed to immobilization stress. IL-2 (10 μl) was added to the wells in culture panels containing effector and target cells (T lymphoma, YAC-1) in a total volume of 200 μl and cultured for 4 h at 37°C in a CO_2 incubator. During this time no changes were found in NKC activity in the presence of IL-2 in either of the test groups.

In the next experiments the effector cells were incubated with IL-2 for a longer period — 18 h. At the end of this the effector cells were washed to remove IL-2, after which ^{51}Cr-labeled YAC-1 target cells were added to it and incubation was continued for a further 4 h. Preliminary incubation of the spleen cells with IL-2 for 18 h led to an increase in NKC ac-

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