IMMUNOGLOBULIN RECEPTORS OF GROUP A STREPTOCOCCUS

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UDC 576.851.214.097.3

The use of the gel precipitation test showed that adsorption of preparations of human IgG, the Fc fragments isolated from them, and of preparations of rabbit IgG with cultures of group A streptococcus inhibits the reaction of these preparations with antiglobulin sera. No inhibition of reactions of $F(ab')_2$ fragments took place as a result of their adsorption by the same cultures. The results confirm that certain strains of group A streptoccocus contain immunoglobulin receptors (Ig receptors), capable of reacting with Fc sites of human and rabbit IgG. The Ig receptors were shown to be destroyed by pepsin. No Ig receptors could be found by the use of this method in hydrochloric acid extracts obtained from streptococci containing these receptors. The method used in the investigation is suitable for detecting the presence of Ig receptors in streptococcal cultures.

KEY WORDS: group A streptococcus; immunoglobulin receptors.

Immunoglobulin receptors (Ig receptors) reacting with the four subclasses of human IgG and with rabbit IgG have been found in streptococci of groups A, C, and G. Reactions with Ig receptors do not take place on account of the combining site of the antibodies. However, reactions with the Fc fragment of the immunoglobulins could not be obtained [9]. According to other observations, the Ig receptor reacts with Fc regions of IgG [7]. It is not yet clear whether the Ig receptors are destroyed by proteolytic enzymes. On treatment of whole microbial cells with trypsin only a partial decrease in the intensity of reactions connected with Ig receptors could be obtained [6]. The problem regarding the presence of Ig receptors in HCl extracts prepared from streptococci likewise has not been completely solved. According to some data, Ig receptors are almost completely destroyed in the course of 10 min at pH 2.0 and at 96°C, i.e., under the conditions used for preparing HCl extracts [6]. According to other observations, Ig receptors can be found in large numbers in HCl extracts [8].

The objects of the present investigation were: 1) to detect Ig receptors by testing preparations of immunoglobulins and also F(ab')2 and Fc fragments before and after adsorption with cultures of group A streptococcus, by the immunodiffusion method with antiglobulin sera; 2) to study the sensitivity of Ig receptors to a proteolytic enzyme; 3) to search for Ig receptors in HCl extracts obtained from group A streptococcus.

EXPERIMENTAL METHODS

Cultures of group A streptococci of the following types were used: 1 (Nos. 2/55 and 2/49), 5 (No. 6/55), 29 (No. 15/55), and 17 (No. 10/55),* and also a culture of an A variant of streptococcus (No. 32/18).† A 24-h culture grown on broth with casein digest was washed off 3 times with 0.85% NaCl solution and killed by heating to 56-58°C for 60 min. On treatment with pepsin (Soviet preparation, from Moscow) 10 ml of 0.25% pepsin solution was added to $1 \cdot 10^{12}$ heat-killed microbial cells. The microbial mass, whether untreated with pepsin or treated and carefully washed, was freeze-dried and used for adsorption. HCl extracts were prepared from unheated cultures and cultures not treated with pepsin [10].

*Prague Collection. Obtained from J. Rott (Czechoslovakia).
†Obtained from McCarty (USA).

Laboratory of Streptococcal Infections, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR P. A. Vershilova.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 88, No. 9, pp. 319-322, September, 1979. Original article submitted December 5, 1978.

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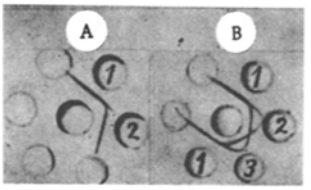

Fig. 1

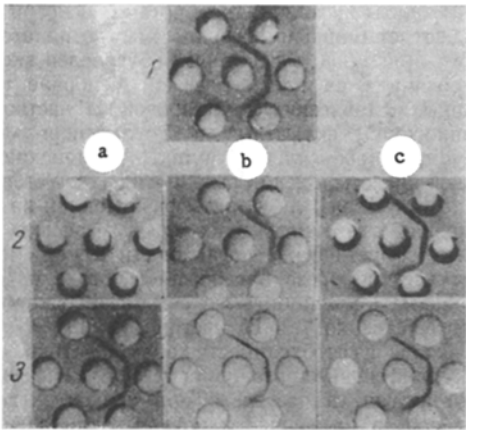

Fig. 2

Fig. 1. Testing of sera against human and rabbit serum globulins with preparations of IgG, $F(ab')_2$ fragments, and Fc fragments. Central wells contain: sera against rabbit (A) and human (B) serum globulins. Peripheral wells contain: 1) preparation of rabbit (A) and human (B) IgG in concentrations of 1 mg/ml; 2) preparations of $F(ab')_2$ fragments of rabbits (A) and human (B) IgG in concentrations of 500 μg/ml; 3) preparation of Fc fragments of human IgG (300 μg/ml).

Fig. 2. Adsorption of preparation of rabbit IgG by cultures of group A streptococcus. Central wells contain serum against rabbit globulins. Peripheral wells (1-6) contain: 1) preparation of rabbit IgG (500-15 μg/ml); 2) preparation of rabbit IgG adsorbed with cultures of group A streptococcus of types 1 (a), 5 (b), and 29 (c); 3) preparation of rabbit IgG adsorbed by cultures of group A streptococcus of types 1 (a), 5 (b), and 29 (c), treated with pepsin.

Preparations of IgG, and also $F(ab')_2$ fragments and Fc fragments isolated from human IgG were used.* Preparations of rabbit IgG (from Calbiochem and Serva) and a preparation of $F(ab')_2$ fragments obtained from rabbit IgG also were used.†

To carry out adsorption, 20 mg freeze-dried streptococcal culture, treated or untreated with pepsin, or 2 ml of freeze-dried HCl extracts (4 mg protein) was added to solutions of the above-mentioned preparations in a volume of 0.2 ml and in a concentration of 125-500 μg/ml. The mixture was kept for 2 h at 37°C and overnight at 4°C.

After centrifugation the supernatant was tested in the agar gel diffusion test (DT). Unadsorbed preparations of IgG, $F(ab')_2$ fragments and Fc fragments were used as the control. Tests were carried out with sera of rabbits immunized with the globulin fraction isolated from human serum and also with donkey serum against rabbit globulins.‡ Antibodies against rabbit IgG isolated from antiglobulin serum by means of an immunosorbent prepared by the method of Avrameas and Ternynck [5], also were used as described previously [2].

EXPERIMENTAL RESULTS

Antibodies against IgG and $F(ab')_2$ fragments were detected in the antiserum against rabbit globulins. Serum against human globulins contained antibodies against IgG and $F(ab')_2$ fragments and Fc fragments (Fig. 1).

*Prepared in the Laboratory of Luminescent Diagnostic Sera, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR.

†This preparation was supplied by Professor R. S. Nezlin (Institute of Molecular Biology, Academy of Sciences of the USSR).

‡The sera were prepared in the Laboratory of Luminescent Diagnostic Sera, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR.

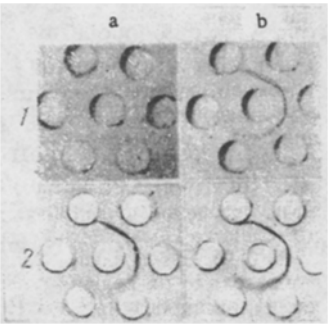

Fig. 3. Adsorption of preparations of $F(ab')_2$ and Fc fragments of a culture of A variant of streptococcus obtained from human IgG. Central wells contain serum against human globulins. Peripheral wells contain: 1) preparation of Fc fragments (300-9 μg/ml); 2) preparation of $F(ab')_2$ fragments (500-15 μg/ml). a) Unadsorbed preparations; b) adsorbed.

Adsorption of preparations of human or rabbit IgG by cultures of group A type 1 and A variants of streptococcus caused almost complete inhibition of reactions of the immunoglobulins with sera containing antibodies against these immunoglobulins. Adsorption with group A type 5 streptococcus reduced the intensity of the reaction of IgG preparations when tested with the same sera. Group A streptococci of types 17 and 29 had no such action. Treatment of cultures of group A type 1 streptococcus and A variant of streptococcus with pepsin made them unable to inhibit the reactions of preparations of human or rabbit IgG with the corresponding sera (Fig. 2).

As a result of adsorption of normal rabbit sera with cultures of group A type 1 streptococcus and of A variant of streptococcus a sharp decrease took place in the intensity of the reaction of these sera when tested in DT in gel with antibodies against rabbit IgG. Adsorption of the above-mentioned sera with type 29 streptococcus had no such action.

Adsorption of $F(ab')_2$ fragments isolated from human or rabbit IgG with cultures of group A streptococcus of different types and of A variant of streptococcus did not lead to inhibition of the reaction of these preparations with antiglobulins sera. Meanwhile a culture of group A streptococcus type 1 or A variant caused inhibition of reactions of the Fc fragments with antiglobulin serum (Fig. 3).

The HCl extract prepared from group A streptococci of types 1, 5, and 29 did not cause inhibition of the reaction of IgG preparations with antiglobulin serum when tested by the gel DT.

On adsorption of preparations containing rabbit or human IgG, some of the cultures of group A streptococcus tested inhibited the reaction of these preparations with antiglobulin sera in the gel DT. The same phenomenon was observed after adsorption of a preparation containing Fc fragments isolated from human IgG by the above-mentioned cultures. Meanwhile adsorption of $F(ab')_2$ fragments obtained from human or rabbit IgG by the same cultures had no effect on the reaction of these preparations with antiglobulin sera. These results fully support those obtained by other workers [7] and are evidence that certain strains of A streptococcus and A variant possess receptors which can react with Fc regions of human and rabbit IgG. Experiment with pure antibodies against rabbit IgG confirm that streptococcal Ig receptors can in fact react with IgG. HCl extracts obtained from cultures possessing Ig receptors had no inhibitory action on the reaction of IgG with sera containing antibodies against immunoglobulins. These observations, and also the absence, as a rule, of positive reactions of HCl extracts with normal rabbit sera are evidence that Ig receptors cannot be found by the use of the gel DT in HCl extracts. The presence of Ig receptors in some strains of streptococcus must undoubtedly be taken into account in experiments involving adsorption of antibodies in order to obtain group and type sera.

Treatment of the streptococcus with pepsin was shown to render it unable to react with the Fc region of immunoglobulins. Accordingly, the use of the culture of the A variant treated with pepsin for the adsorption of sera intended for identification of group A streptococci was fully justified [1, 4].

The presence of Ig receptors in the group A streptococcus may be the reason why incorrect results have been obtained in studies of cross-reacting antigens common to the streptococcus and mammalian tissues [7]. It must be pointed out that Ig receptors of group A streptococcus can react with IgG of all subclasses [6]. Accordingly the use of so-called cross-adsorption of sera containing antibodies against cross-reacting antigens is a perfectly reliable control. Each culture under these circumstances should absorb only antibodies reacting in the immunofluorescence test with a definite tissue structure, against which they are produced during immunization with the present culture. Subsequent testing of the sera in the indirect immunofluorescence test with pure antibodies against IgG is a perfectly reliable criterion for confirming the validity of the results [3].

The test used in the present investigation for detecting Ig receptors in streptococcal cultures by the immunodiffusion method is simple and clear, and for that reason it can be used when screening streptococcal cultures during the study of the distribution of cross-reacting antigens in these cultures.

LITERATURE CITED

1. N. A. Borodiyuk, O. P. Galach'yants, I. I. Rassokhina, et al., Zh. Mikrobiol., No. 8, 63 (1975).
2. T. A. Danilova, N. A. Borodin, and G. A. Ugryumova, Byull. Éksp. Biol. Med., No. 4, 71 (1971).
3. I. M. Lyampert, N. A. Borodiyuk, and T. K. Asoskova, Infect. Immun., 19, 961 (1978).
4. S. Avrameas and T. Ternynck, Immunochemistry, 6, 53 (1969).
5. P. Christensen and V. -A. Oxelius, Acta Path. Microbiol. Scand., Sect. B., 82, 475 (1974).
6. P. Christensen, B. G. Johnsson, and G. Kronvall, Acta Path. Microbiol. Scand. Sect. C., 84, 73 (1976).
7. J. Havlicek, Exp. Cell. Biol., 46, 146 (1978).
8. G. Kronvall, J. Immunol., 111, 1401 (1973).
9. R. C. Lancefield, J. Exp. Med., 57, 571 (1933).
10. O. Ouchterlony, Prog. Allergy, 6, 30 (1962).