COMMON ANTIGEN TO HUMAN HEART TISSUE AND THE STREPTOCOCCUS

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The existence of common antigens in bacteria and man has been reported in the literature [1, 5]. It has been shown, in particular, that prolonged immunization of animals with group A streptococci causes the formation of antibodies against heart tissue [3, 7]. On the basis of this, the existence of a crossed antigen in human heart tissue and the streptococcus has been postulated. However, it has not yet been settled whether this antigen is a constant component of heart tissue, or whether it arises in response to prolonged action of streptococci.

In the present investigation the possibility that substances antigenically common to streptococci are constantly present in human heart tissue was studied.

EXPERIMENTAL METHOD

Two groups of rabbits, with 10 animals in each, were immunized with heart tissue homogenate prepared from the cadavers of healthy persons dying from accident and the cadavers of patients with rheumatic fever. To exclude the action of streptococci, the homogenate was injected with penicillin. Repeated seeding of the immunizing material on agar with gentian violet did not yield growth of streptococci. The same scheme of immunization was used with all the animals: each rabbit received a cycle of 6 injections of a saline extract of the heart on alternate days. The first injection was intravenous, the second subcutaneous, and the 3rd-6th intraperitoneal. In the intraperitoneal injections, besides the saline extract, minced heart tissue also was injected. Altogether three cycles of immunization were given at intervals of 2 months. The amount of antigen injected was determined exactly as protein. During the whole course of immunization each animal received about 250 mg protein.

The heart antisera thus obtained were investigated by four methods: the complement fixation reaction (CFR) in the cold [2], the precipitation reaction in agar as described by Ouchterlony [8], latex agglutination [9], and the passive anaphylaxis reaction [6]. For the detection of antibodies against heart tissue, a saline extract, the globulin and two nuclear fractions, and desoxyribonucleoprotein (DNP) were used as tissue test antigens. The globutin fraction was isolated with ammonium sulfate at 50% saturation, and the nuclear fractions by differential centrifugation of the heart tissue homogenate in sucrose solution with the addition of calcium chloride. DNP was obtained by extraction of the tissue in a solution of sodium versenate [10]. The raw material for preparation of the test antigens was the heart tissue from cadavers of healthy persons killed in accidents and from the cadavers of patients with rheumatic fever. Polysaccharide C and fractions of the membranes and intracellular antigens were used as streptococcal test antigens [4].

EXPERIMENTAL RESULTS

The blood of the experimental animals before immunization contained practically no heart or streptococcal antibodies. As a result of prolonged immunization of 20 rabbits with human heart tissue, in 2 of the animals the titer of complement-fixing antibodies in the blood was 1:640, in 5 animals – 1:320, in 9 animals – 1:160; in 3 animals – 1:80, and in 1 animal – 1:40. The maximal reaction was observed in the test with the antiserum and the saline extract or the globulin fraction, while hardly any reaction took place with DNP or with the nuclear fractions. Meanwhile the sera against heart tissue (15 of 20 cases), diluted 1:40-1:160, reacted with the streptococcal antigens. They were active not only against the membranes of the streptococci, in which the crossed antigen has been shown to be located [7], but also against the fraction of intracellular antigens. The sera of half the animals gave a positive reaction with polysaccharide C, but in smaller dilutions than with the other streptococcal fractions.

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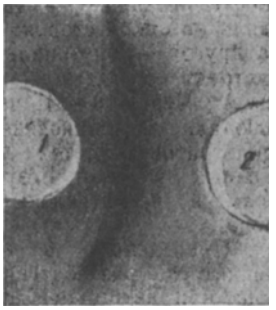

Fig. 1. Antigenic composition of human heart tissue. 1) Antiserum against human heart tissue; 2) extract of heart tissue.

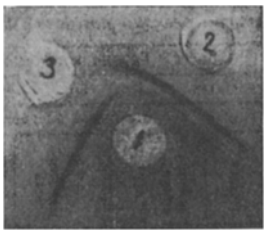

Fig. 2. Common antigen in human heart tissue and the streptococcus. 1) antiserium against human heart tissue; 2) extract of heart tissue; 3) extract of streptococci.

In the crossed CRF the sera of the animals of groups 1 and 2, tested with heart tissue from the cadavers of a patient with rheumatic fever and a healthy person, did not reveal the presence of the specific antigen. No differences likewise were observed in the activity of these sera against the test fractions of streptococcal antigens.

To detect streptococcal antibodies in the blood of the animals immunized with human heart tissue, the technically simpler latex agglutination reaction also was used. In this reaction the sera of intact animals, diluted 1:100, gave nonspecific agglutination of latex particles loaded with streptococcal or tissue antigens. The immune sera reacted with heart extracts in dilutions of 1:8000-1:12 000, and with streptococcal preparations in dilutions of 1:900-1:1500.

The precipitation reaction in agar was used to investigate the antigenic composition of the heart tissue and the possibility that it may also contain components in common with streptococci. When this reaction was carried out between a saline extract of heart tissue and homologous antisera, 4 precipitation lines were observed, corresponding to four antigens (Fig. 1). The sera of 15 rabbits gave precipitation lines in the reaction with streptococcal antigens, while the sera of 7 rabbits gave a common precipitation line with the heart and streptococcal antigens (Fig. 2). In a few cases (in 5 of 20) a very weak precipitation line was observed between the test sera and polysaccharide C, but merging of the precipitation lines of the polysaccharide and tissue antigens was never observed.

In the passive anaphylaxis reaction, two groups of guinea pigs (10 animals in each group) were injected intracardially with heart (group 1) or specific (against human serum proteins, group 2) antiserum. Each experimental guinea pig and also 5 control animals (group 3) were injected intradermally on one side of the abdominal wall with tissue extract of human heart, and on the other, with intracellular extract of group A streptococci. The development of areas of infiltration at the sites of the intradermal injections was observed 18-24 h later. The results of the reactions are given in the table.

Results of the Passive Anaphylaxis Reaction

No. of animal	Group of animals					
	1		2		3	
	extract injected					
	heart	streptococcus	heart	streptococcus	heart	streptococcus
1	+	+	+	±	−	−
2	+	+	+	±	−	−
3	+	+	±	±	−	−
4	±	±	±	±	−	−
5	±	−	±	±	−	−
6	±	±	±	±		
7	±	±	±	±		
8	±	±	±	±		
9	±	±	±	±		
10	+	+	+	±		

Legend. + diameter of infiltration 5 mm or over; ± diameter of infiltration less than 5 mm; - no infiltration present.

The results given in the table give additional confirmation of a common factor in the antigenic structure of human heart tissue and streptococci, for local signs of anaphylaxis were found in the guinea pigs of group 1 at the sites of injection of both the tissue and the streptococcal extracts. These results show that the common antigen belongs to the organ-specific group, because the foci of infiltration developed mainly in animals receiving injection of heart antiserum.

It may thus be concluded from the results of these experiments that a least four antigens are present in human heart tissue, one of which is common to the streptococcus. In the heart tissue

this antigen is evidently bound to the cytoplasmic components of the cell. In the streptoccoccus it is probably found not only in the membrane, but also inside the cell.

The common antigen is evidently organ-specific. No specific rheumatic antigen could be detected.

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