FIXATION OF ANTITISSUE ANTIBODIES ON TISSUE CELL STRUCTURES STUDIED BY THE MIXED AGGLUTINATION METHOD

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UDC 616.008.9-092.18-097.3

Antitissue antibodies were studied by the mixed-agglutination method. The sera of albino rats with an experimental infarction-like condition were tested. Sections of the heart, skeletal muscle, and kidney of the rat were used. Fixation of antitissue antibodies on tissue sections of the organs was detected by means of an indicator system consisting of tanninized sheep's red cells sensitized with normal rat serum globulins and treated with anti-γ-globulin serum.

Mixed agglutination has been used by several workers to study antibodies against surface and intracellular cell antigens [1, 4]. The principle of the method is that cells of a culture or tissue sections are treated with antiserum containing antibodies against the test antigen. After a short time the cells or tissue sections are washed with buffer to remove excess antibodies and then treated with red cells sensitized with a globulin homologous with the test serum and treated with anti-γ-globulin serum. The antibodies induce agglutination of the sensitized red cells with the cell cultures or tissue sections. Some workers have used cell cultures [2, 3, 5, 6] in the mixed agglutination method, others histological tissue sections [8]. By the use of tissue cultures it was possible to study the surface antigens of the cells, whereas by the use of tissue sections both surface and intracellular antigens can be studied.

The object of the investigation described below was to use the mixed-agglutination method in order to detect antitissue antibodies. Tissue sections were used to study the antitissue antibodies. The sera of albino rats with an experimental infarction-like condition were tested.

EXPERIMENTAL METHOD

Histological sections were prepared from the organs of rats (heart, skeletal muscle, kidney). Pieces of the organs were taken through alcohols and xylol and embedded in paraffin wax [7]. Sections 5μ in thickness were cut from the paraffin blocks, placed in the center of a cover slip, straightened out with needles, and incubated at 37°C. The sections were then dewaxed, washed with buffer solution, and the test serum was applied, after which the specimens were placed in a moist chamber and incubated for 1 h. After incubation the excess of test serum was removed by dipping the cover slips with the sections vertically in a beaker containing buffer. Phosphate buffer, pH 7.2, was used for all manipulations.

The indicator system was prepared from sheep's red cells. A 2% suspension was prepared from thrice-washed red cells. The suspension was mixed with an equal volume of 1:20,000 tannin solution. Tanninization was carried out at room temperature for 10 min, after which the red cells were washed 3 times and made up to their original volume. The resulting suspension of tanninized red cells was mixed with an equal volume of the globulin fraction of normal rat serum with a protein concentration of 3 mg/ml. The mixture was incubated for 30 min at room temperature, after which the sensitized red cells were washed 3 times with buffer and resuspended to a concentration of 0.5%. Equal volumes of this suspension and of rabbit antiserum against rat globulins in a dilution of 1:10 were mixed and incubated for 30 min at room

Allergologic Research Laboratory, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 75, No. 4, pp. 74-76, April, 1973. Original article submitted December 3, 1971.

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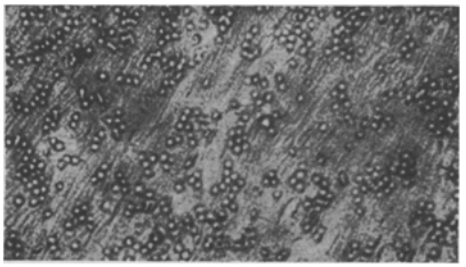

Fig. 1. Positive mixed-agglutination reaction: many adherent red cells of indicator system on section of rat heart tissue (256×).

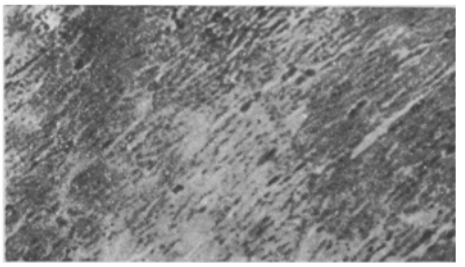

Fig. 2. Negative mixed agglutination reaction: solitary red cells of indicator system on section of rat heart tissue (256×).

temperature. The red cells agglutinated into small clumps. The agglutinated erythrocytes were washed 3 times with buffer and resuspended to 0.5% concentration. This was the final indicator system as used in the experiments.

As the control parallel with the complete indicator system, the separate components were used, such as tanninized red cells only or tanninized red cells treated with rat globulins but not treated with anti-γ-globulin serum.

The prepared indicator system was placed at the bottom of the well on a slide. A coverslip with a section was placed above the well with the section face downward. The coverslip was smeared with petrolatum at the edges and the slide was then turned over so that the indicator system covered the section. The specimens thus formed were incubated for 1 h. At the end of this time the indicator system had settled, covering the section. After 1 h the specimens were taken from the incubator, the slides were turned with the coverslips face upward, and they were examined under the microscope. A strongly positive reaction was characterized by complete covering of the section with red cells (Fig. 1). Moderate covering of the section with red cells was assessed as 2+, and slight covering as 1+. The highest dilution of the serum giving a reaction of 1+ was taken as the titer. If only isolated cells of the indicator system remained on the section, the reaction was regarded as negative (Fig. 2). The writer's modification of the mixed-agglutination method is that paraffin tissue sections were used, and the indicator system consisted of tanninized sheep's red cells sensitized with rat globulin and treated with rabbit antiserum against rat γ-globulin.

EXPERIMENTAL RESULTS

The sera of 63 rats with an experimental infarct-like condition were studied at various times after ligation of branches of the left coronary artery, and the sera of 20 normal rats were studied as the control.

The greatest number of positive reactions was observed when the sera of experimental animals killed from 11 to 15 days after production of the experimental infarct were tested. Of the 63 sera tested 30 (47.7%) gave positive results.

All the sera tested were studied in parallel by the passive hemagglutination (PHT) and complement-fixation (CFT) tests.

The largest number of positive results was obtained by the use of the mixed-agglutination method, while the PHT and CFT gave approximately equal numbers of positive results, namely 31.25 and 30.7%, respectively. The antibody titers both in the PHT and CFT, moreover, were lower than in the mixed agglutination reaction. The titer of hemagglutinating antiheart antibodies in the serum reached a dilution of 1:1280, compared with 1:40 for complement-fixing antibodies and 1:1600 for antibodies detected by the mixed agglutination method.

To verify the organ specificity of the antitissue antibodies studied, besides sections from the normal rat heart, sections from rat skeletal muscle and kidney, which have common antigenic determinants with heart antigens, also were used. The use of these control sections showed that fixation of globulin adsorbed on the cells of the indicator system was particularly marked on sections of the heart and less marked on sections of the kidney and skeletal muscle, thus indicating the relative organ specificity of the antitissue antibodies studied. Exhausted sera gave a negative mixed agglutination reaction.

Of the 20 normal rat sera, antiheart antibodies were found in 4 in titers of 1:10-1:100, whereas no antiheart antibodies could be found in these rats by the PHT or CFT. The discovery of antiheart antibodies in the serum of the normal animals is evidence of naturally occurring antiorgan antibodies.

The method used thus enabled the fixation of antiheart antibodies on myocardial tissue sections to be detected. Fixation of heart tissue cell antigens with antiheart antibodies was achieved by means of an indicator system consisting of sheep's red cells treated in the appropriate way. Mixed agglutination enabled antitissue antibodies against organ-specific antigens located within the cells to be demonstrated, for tissue sections were used in the experiments. When sections are cut, cell structures are disturbed and the intracellular components, which are concerned with organ-specific antigens, are exposed. Since the method described enabled antitissue antibodies to be detected in sufficiently high titers, it can be used to detect antibodies appearing during autosensitization, which characteristically give a comparatively weak immune response.

It can be concluded from the facts described above that the mixed-agglutination method enables antitissue antibodies to be found in the sera of normal animals and it is a valuable method for the study of fixation of antiorgan antibodies.

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