REACTION OF STREPTOCOCCAL ANTISERA WITH VARIOUS TISSUES OF THE GUINEA PIG REVEALED BY IMMUNOFLUORESCENCE

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UDC 616.981.21-092.9-097.3-078.73.073.4

Investigations have shown [3-5, 9-11] that a common antigen is found in a culture of type 5 group A streptococcus and the heart tissue of man and experimental animals. Sera obtained by immunizing animals with cultures containing this antigen reacted in immunofluorescence experiments with the sarcolemma and the subsarcolemma of the muscle fibers of the myocardium and the skeletal musculature. These sera did not react with the tissues of other organs.

In addition, a second common antigen has been found in the type 1 streptococcus and the heart tissue of man and experimental animals. The sera of rabbits immunized with type 1 streptococcus specifically stained the intercalated disks of the myocardium. These sera did not react with sections of skeletal muscle [6].

The object of this investigation was to study the reaction of rabbit antisera against type 1 streptococcus on sections of the tissues of the liver and other organs of the guinea pig, rabbit, rat, mouse, and man.

EXPERIMENTAL METHOD

The investigation was carried out by means of the indirect immunofluorescence method [13] using pure antibodies against rabbit γ-globulin [7].

Antisera against type 1 streptococcus (strain No. 2/55) were obtained by intravenous immunization of rabbits with killed or living cultures of streptococci grown on a combined meat or casein medium. As controls, sera against type 5 streptococci, sera of unimmunized rabbits (normal), and whooping cough antiserum* were used.

The antiserum against type 1 streptococcus was absorbed by cultures of streptococci of types 1 and 5 and Haemophilus pertussis at the rate of 360 billion bacterial cells per ml serum.

To study the specificity of the reactions, the sera were absorbed with homogenates of liver tissues and guinea pig's heart tissue (0.1 ml homogenate to 0.2 ml serum), and also with sheep's erythrocytes.

To isolate pure antibodies against rabbit γ-globulin from a goat anti-rabbit serum, an immunosorbent was used by A. E. Gurvich's method [2]. The pure antibodies were conjugated with fluorescein isothicyanate by the method of Riggs and co-workers [12] modified by V. A. Blagoveshchenskii and A. Ya. Kul'berg [1]. The technique of preparing the rabbit antiserum and of obtaining the pure antibodies, and other technical data are described fully in the previous paper [3]. Most of the experiments were carried out on sections of the liver tissue of 22 normal guinea pigs. In addition, sections of the kidney, adrenals, and lymph gland of guinea pigs and also sections of human, rabbit, rat, and mouse liver tissue were also studied. The pieces of tissue were frozen at -70°. Sections were cut to a thickness of 4μ in a cryostat at -20°. Unfixed sections and sections fixed in 90° ethanol were investigated.

The sections were treated at room temperature for 40 min with serum and for 35 min with labeled antibodies (unfixed sections), and also for 1 h with serum and for 50 min with labeled antibodies (fixed sections). To remove nonspecific fluorescence, the tested sera and labeled antibodies were twice absorbed with mouse liver powder.

* The whooping cough antiserum was kindly made available by Professor M. S. Zakharova's collaborators.

Division of Streptococcal Infections, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow (Presented by Active Member of the Academy of Medical Sciences of the USSR G. V. Vygodchikov). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 63, No. 5, pp. 70-72, May, 1967. Original article submitted August 6, 1965.

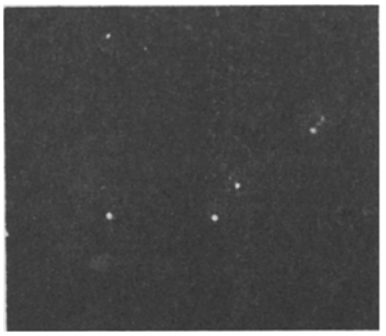

Fig. 1. Section through the liver tissue of a guinea pig treated with antiserum against type 1 streptococcus. Fluorescent inclusions in the cell nuclei. Objective 90×, Homal 3×.

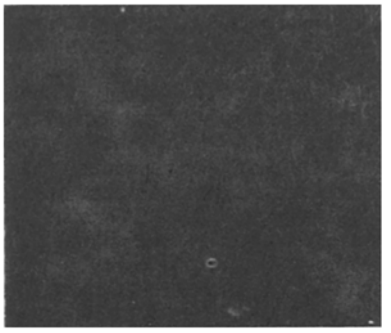

Fig. 2. Section of the liver tisuse of a guinea pig treated with antiserum against type 1 streptococcus, absorbed with type 1 streptococcus. Objective 90×, Homal 3×.

The preparations were examined by means of the ML-2 luminescence microscope with an objective of 90× (oil immersion), and a Homal 3× lense was used for photography.

EXPERIMENTAL RESULTS

The antiserum against type 1 streptococcus revealed brightly fluorescent circular inclusions in the sections of the guinea pig's liver, mainly localized in the cell nuclei (Fig. 1). On examination with phase contrast the localization of these inclusions did not coincide with the nucleolus. Fluorescence was observed in both the unfixed sections and the sections fixed with ethanol. Similar inclusions were found in the cell nuclei of the kidney, adrenal, and lymph gland of the guinea pig. Inclusions were observed equally in animals of different sexes. No fluorescence of any other tissue elements was observed.

In the sections treated with normal rabbit sera, sera against type 5 streptococuss and whooping cough antiserum, either no inclusions were found whatever or their fluorescence was hardly visible.

Absorption of the antiserum against type 1 streptococcus with the homologous culture completely abolished the reaction with the liver tissue and other organs. Dark nuclei were clearly visible in the sections (Fig. 2). Absorption of the serum with type 5 streptococcus did not affect the character or intensity of the reaction with liver tissue. When strains of streptococci grown on casein broth were used for absorption, the same results were obtained.

Absorption of antiserm against type 1 streptococcus with H. pertussis cells and with sheep's erythrocytes did not abolish the reaction with the inclusions.

After absorption with tissue homogenates, precise results were not obtained. Absorption with liver tissue homogenate completely abolished the reaction of the serum with the inclusions. Absorption with heart tissue homogenate slightly diminished the intensity of their fluorescence. If the intensity of fluorescence of the inclusions stained with serum in ordinary conditions was denoted by ++++, after absorption of the serum with guinea pig's liver tissue the intensity of fluorescence corresponded to ++, and after absorption by heart tissue, to +++. When sections of human, rabbit, rat, and mouse liver were investigated, no fluorescent inclusions were found.

Hence, in all the samples of guinea pig's liver tissue tested, antiserum against type 1 streptococcus specifically stained the inclusions in the cell nuclei. The use of pure antibodies against rabbit γ-globulin in the immunofluorescence experiments ruled out the possibility that other serum components than γ-globulin were participating in the reaction. The reaction was completely abolished after absorption of the serum with type 1 streptococcus. Evidently in these experiments a specific reaction took place, associated with the presence of a specific antigen in the type 1 streptococcus, in common with certain tissue elements of the guinea pig's liver. The specificity of this reaction was confirmed by the fact that type 5 streptococcus and H. pertussis did not remove the antibodies during absorption of the type 1 serum. The effect of a meat nutrient medium [8] in these experiments was also excluded by the fact that the fluorescence was completely

abolished by absorption of the serum with strains of type 1 streptococcus subjected to prolonged passage in casein broth, but was not abolished by absorption with type 5 streptococcus after similar passage. The incomplete abolition of the reaction during absorption with liver tissue may perhaps be attributed to the fact that a certain quantity of the specific antigen of the tissue was lost during preparation of the homogenate, so that it became inadequate for complete absorption of the antibodies from the streptococcal antiserum. The reaction was not associated with antibodies against Forssman's antigen.

It may be concluded from these results that type 1 streptococcus and certain components of the liver tissue, and also of other organs of the guinea pig, contain a common antigen. No common antigen was found in the liver tissue of other experimental animals and man. This antigen differs from the common antigen with the intercalated disks of the myocardium which has been detected in several animals and in man [6]. The nature of the inclusions which were found in the guinea pig suggests that they are more likely to be an antigenic component of the guinea pig's liver tissue itself than foreign elements.

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