EFFECTIVE METHOD OF OBTAINING ANTISERA AGAINST HUMAN AND ANIMAL EMBRYONIC α-GLOBULINS

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Antisera with a high titer of antibodies against human and mouse α_F-globulins have been obtained from rabbits immunized with pure preparations of these antigens by injection directly into the popliteal lymph glands.

The subject of embryo-specific antigens has not assumed great theoretical and practical importance. Renewal of synthesis of α_F-globulins in malignant tumors of the liver [1] has served as the basis for development of an immunochemical diagnostic test [2, 3, 6]. To carry the study of α_F-globulins further, it was essential to have monospecific antisera with a high content of antibodies yet not giving crossed reactions with other serum proteins or tissue proteins.

Usually, in order to detect α_F-globulins, antisera against embryonic human and animal serum proteins, absorbed by sera of adult individuals, are used [1-3]. The preparation of such antisera requires prolonged and repeated cycles of immunization, and as a rule the titer of antibodies against α_F-globulin in these antisera is low. In addition, it is impossible to exhaust these antisera completely relative to certain components of the embryonic serum which differ from α_F-globulin.

In the investigation described below, antisera against α_F-globulins were obtained by immunizing animals by direct injection of individual arcs of antigen – antibody precipitate [8], and also of specimens of human and mouse α_F-globulins isolated by the writers' method of preparative disk electrophoresis in polyacrylamide gel [4], into a lymph gland [7].

Antigens. Animals were immunized with a precipitate consisting of human embryo-specific α-globulin (α_F-globulin) and the corresponding antibodies against it, and also with purified preparations of human and mouse α_F-globulin.

The precipitate was obtained by an immunodiffusion method [5]. The source of α_F-globulin was human fetal serum, and the source of antibodies was a rabbit antiserum against human fetal serum exhausted with adult human serum. After formation of the precipitation line, it was cut out together with the agar, washed in several portions of physiological saline for 48 h, broken up in a test tube with a glass rod, and treated with an equal volume of Freund's complete adjuvant (Difco). The adjuvant was carefully mixed with the crushed agar by means of a syringe until a thin, whitish emulsion was obtained (AG-1), and this was injected into rabbits.

In other experiments, purified preparations of human and mouse α_F-globulins were used. These were obtained by preliminary disk electrophoresis in polyacrylamide gel from sera of human fetuses and newborn animals. Solutions of human α_F-globulin (AG-2) and mouse α_F-globulin (AG-3) were treated with equal volumes of Freund's adjuvant and carefully mixed by means of a syringe.

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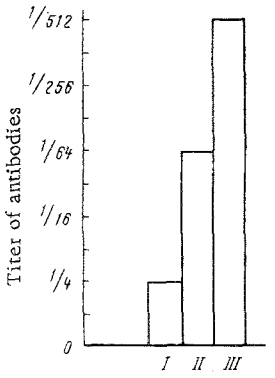

Fig. 1

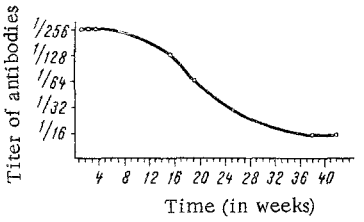

Fig. 2

Fig. 1. Relationship between antibody titer and site of injection of antigen. I-III) Injection of AG-1 and AG-2, respectively, into lymph gland; II) injection of AG-2 into region of lymph gland.

Fig. 2. Dynamics of titer of antibodies against AG-3 in sera of immunized animals.

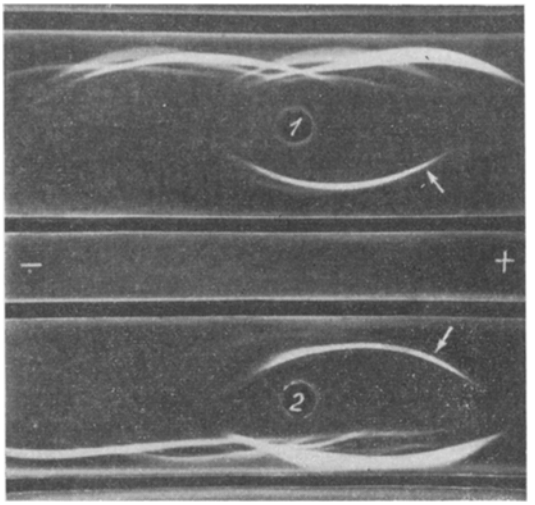

Fig. 3. Immunoelectrophoretic characteristics of specificity of rabbit antisera. 1) Serum of newborn mice (SNM); 2) human fetal serum (HFS). In gutters from top to bottom: rabbit antisera against SNM, AG-3, AG-2, and HFS. Arrows indicate precipitation arcs of α_F-globulins with corresponding antibodies.

Immunization of the animals. The experimental rabbits were divided into four groups, with two animals in each group. The first three groups of animals received injections of 0.1 ml of AG-1, AG-2, or AG-3 directly into both popliteal lymph glands, while those of group 4 received 0.1 ml of AG-2 into the region of the popliteal lymph glands, but without incising the skin. A repeated injection of antigens in the same dose, but without adjuvant, was given one month later. However, the antigens were diluted with a larger volume of physiological saline, because they were injected into the rabbits intravenously (0.2 ml), intramuscularly (into the forelimb; 0.4 ml), and into the region of one popliteal gland (0.4 ml).

In the full course of immunization each rabbit received about 20-30 μg of AG-1, 80-100 μg of AG-3, and 600 μg of AG-2.

Blood was taken from the marginal vein of the ear of the rabbits in a volume of 45-60 ml, 10-12 and 14 days after the second injection of antigen.

The specificity and activity of the antisera were tested by the methods of agar diffusion and immunoelectrophoresis [6].

Activity of the antisera was determined by a semiquantitative method. The final titer was taken as the highest dilution of antiserum which still reacted with antigen (25 and 50 μg human and mouse α_F-globulins), with the formation of a precipitation line visible to the unaided eye.

Of the eight experimental rabbits, one did not react. No antibodies either to α_F-globulin or to any other antigens were found in the serum of this rabbit, when immunized with AG-1, by the agar diffusion method. The rabbits of each of the remaining three groups reacted by identical antibody production.

Immunization of the animals directly into the lymph glands proved to be the most effective method. Comparison of antisera obtained from animals immunized with the same dose of antigen directly into the lymph glands and into the region of the popliteal glands showed (Fig. 1) that rabbit sera obtained after the first method of injection of antigen contained 8 times more antibodies than those obtained by the second method. The weakest immune response was observed to injection of precipitate into the lymph glands. The titer of antibodies in the serum of such rabbits was 128 times lower than the antibody titer in the sera of animals immunized with the purified preparation of the same antigen, in a larger dose. Consequently, the strength of the immune response is determined not by the character of the antigen injected (antigen – antibody precipitate), but by its dose and also by the site of its injection.

The length of time during which antibodies remain in the sera of the animals is extremely important with this method of immunization. It is clear from Fig. 2 that the titer of antibodies fell by only half in four months, and antibodies were still present even 10 months even after the second injection of antigen. The experiments showed that up to 200-250 ml of antiserum with a high titer of antibodies can be obtained from rabbits immunized by direct injection into the lymph glands, over a long period of time. This is of considerable practical importance, especially for the commercial preparation of diagnostic antisera.

The specificity of the antisera thus obtained was tested by immunodiffusion methods. One of the characteristic experiments to test specificity is illustrated in Fig. 3. Antisera against purified preparations of α_F-globulins clearly detected human and mouse α_F-globulins among a wide range of human fetal sera and sera of newborn mice. However, besides antibodies against α_F-globulins, they also contained traces of antibodies against human transferrin and mouse albumin, respectively. The presence of antibodies of this type in the antisera suggests that the preparations of α_F-globulins with which the animals were immunized also contained traces of impurities. These additional antibodies can easily be removed by the addition of traces of sera from adult individuals.

The antisera thus obtained have been used successfully for the diagnosis of carcinoma of the liver, for the study of crossed reactions of α_F-globulins, and for immunohistochemical investigations.

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