Complement-Fixing Antibodies (Brown-Pearce Carcinoma) in the Blood Serum and in the Aqueous Fluid of the Anterior Chamber of the Eye*

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Intracutaneous transplantation with the Brown-Pearce carcinoma results in a tumor which, after growing to a certain size, regresses, leaving the animal immune to subsequent growth of this tumor (2, 3, 9). We have recently shown (10) that such immunization does not confer protection against growth of this tumor in the anterior chamber of the eye, despite the fact that all other tissues tested are resistant following regression of the skin tumor.

The lack of immunity of the anterior chamber of the eye of the immune rabbit to this tumor may result from the barrier which exists between the blood plasma and the aqueous humor, preventing the passage of antibodies from the former into the latter (5). This may be explained by the fact that the aqueous humor is formed by a process of dialysis from the blood plasma. Crystalloids readily pass into the anterior chamber but larger molecules, the size of serum albumin or larger, are retained. The work of Becht and Greer (1), and also that of Hektoen and Carlson (7), who studied the concentration of antibodies in the various body fluids of animals immunized to bacterial and other foreign proteins, supports this assumption. They found that the titer of immune bodies was highest in the serum, and weakly positive or negative in the aqueous fluid of the anterior chamber of the eye.

Therefore it was thought advisable to study the comparative concentration of tumor antibodies in the blood serum and in the aqueous fluid of the anterior chamber of the eye of rabbits bearing the Brown-Pearce carcinoma. The presence of tumor antibodies in the serum and their absence from the aqueous fluid in the same animal at the same time would indicate that such a barrier does exist and would provide support for the assumption that the successful growth of the Brown-Pearce carcinoma in the anterior chamber of the eye of the tumor-immune animal is referable to the failure of antibodies to gain access to the aqueous fluid.

To determine the presence or absence of tumor antibodies in these fluids, the complement fixation test as described by Kidd (8) and by Cheever (4) was used. Kidd reported almost 100 per cent positive reactions. We performed complement fixation tests according to Kidd's technic on the sera from rabbits with tumor but did not obtain results as consistently positive as did Kidd. However, repeated tests on the blood serum during the course of the development of the tumor disclosed the presence of complement-fixing antibodies at some stage in its course. The positive complement fixation reaction was not maintained, however. Nevertheless, we decided to use this immune reaction to determine the relative concentrations of antibodies in the blood serum and in the aqueous fluid.

Twenty-four tumor-bearing rabbits, on which complement fixation tests were made at intervals of 1 to 3 days from the time of the appearance of the tumor, were used for this experiment. In all these animals a 3 to 4 plus complement fixation reaction was obtained in the blood serum at some time during the course of the development of the tumor. Though this usually corresponded with the height of development of the tumor, such was not always the case. As soon as complement-fixing antibodies were identified in the blood serum, aqueous fluid was withdrawn from the anterior chamber of the eye. Because of the decided variations in the titer of complement-fixing antibodies in the serum, it was considered essential that they be determined in the aqueous fluid on the same day on which a positive reaction was obtained in the serum. The technic of withdrawing aqueous fluid was as follows: The animals were anesthetized with intravenous nembutal. The eye was grasped...
with a fixation forceps and rotated so that the corneolimbal junction was readily accessible; then a long hypodermic needle, attached to a 1 cc. tuberculin syringe, was inserted through the corneolimbal junction into the chamber and fluid withdrawn, about 0.25 to 0.5 cc. from each eye.

The antigen used in the tests was prepared from tumor tissue removed aseptically from stock tumor animals. Only firm cellular portions of tumor were selected, necrotic portions being discarded. Tumor tissue was preserved in 50 per cent Locke-glycerol solution until ready for use. Portions were then mixed with sterile sand and saline and thoroughly ground in a mortar with a pestle. This mixture was allowed to stand in the icebox for 24 hours, centrifuged at 2,000 r.p.m. for 15 minutes, and the supernatant fluid separated and placed in a water bath at 60° C. for 30 minutes. The anticomplementary unit of the antigen thus prepared was determined and the antigen diluted so that 0.2 cc., the amount used in the tests, was equivalent to one-quarter of the anticomplementary unit. The titer of this antigen was then tested with a known positive serum. Only antigen which yielded a 4 plus reaction with a known positive blood serum was used for the tests. The strength of the antigen was found to diminish considerably after standing for 4 to 6 weeks at icebox temperature.

Four tube tests were set up with 0.2 cc. antigen and 0.02, 0.03, and 0.1 cc. of blood serum which had been inactivated for one-half hour at 56° C. One-tenth cc. of 10 per cent guinea pig serum was added to each tube and the mixture placed in a water bath at 37° C. for 1 hour. Two-tenths cc. of a mixture of equal parts of 5 per cent sheep cells and anatoceptor (2 units) were added and incubated again at 37° C. for 20 minutes, and the results of the tests noted.

Inasmuch as only relatively small amounts of aqueous humor could be obtained, the technic of the test had to be modified slightly when applied to this fluid. Only one amount of aqueous humor, 0.4 cc., was used in each test; otherwise the procedure was the same as that used for serum.

The results were quite clear-cut and consistent. As previously noted, all the blood serums gave at least on one occasion a 3 to 4 plus positive complement fixation reaction. Aqueous humor obtained from the eyes of these animals, at the height of complement fixation titer in the serum, invariably gave a negative complement fixation reaction. In not a single instance was it possible to demonstrate complement-fixing antibodies in the aqueous humor of tumor-bearing rabbits even though a high titer of such antibodies could be demonstrated at the same time in the blood serum of these animals.

It is known that if the eye is disturbed, so that the intraocular capillaries become dilated, the permeability of these capillaries becomes altered and the composition of the aqueous fluid changes so that it approximates more closely that of the blood plasma, particularly in respect to its protein content. Such an alteration in the permeability of the intraocular capillaries can be brought about by puncture of the anterior chamber and evacuation of the aqueous fluid. The fluid which refills the chamber under these circumstances has a protein content which approximates that of the blood, and apparently antibodies which were formerly not present, or present in very low concentration, now appear in the aqueous humor in definitely increased concentration. Immune bodies in newly formed aqueous fluid may be increased up to 100 times (6).

Accordingly, it was thought advisable to examine the antibody content of the re-formed aqueous fluid in 12 of the animals which had previously yielded negative complement fixation reactions in the aqueous fluid. It was found that in this re-formed aqueous a 1 to 3 plus complement fixation reaction was obtained.

The absence of immune bodies from the aqueous fluid of the eye while present in high titer in the serum of the same animal suggests that a barrier exists which prevents the passage of antibodies from the serum into the aqueous fluid. This provides a possible explanation for the successful growth of the Brown-Pearce carcinoma in the anterior chamber of the eye of the tumor-immune rabbit when the tumor will not grow in any of the other usual sites of transplantation following immunization. The appearance of complement-fixing antibodies in the re-formed aqueous is not surprising. This fact has been known to ophthalmologists for many years. A recognized therapeutic procedure in the treatment of eye infections is to perform an anterior chamber puncture, withdrawing aqueous fluid. Because of the increased antibody content in the re-formed fluid, these patients frequently show distinct improvement.

SUMMARY

Immunization of rabbits against the Brown-Pearce carcinoma does not confer protection against the growth of this tumor in the anterior chamber of the eye. Complement fixation tests made with the blood serum and the aqueous humor reveal that, although complement-fixing antibodies are present in the blood of tumor-bearing and tumor-immune rabbits, they do not gain access to the aqueous fluid. It is suggested that the barrier which exists between the blood and the aqueous humor, preventing the passage of antibodies from the former into the latter, is responsible for the successful growth of the Brown-Pearce carci-
noma in the anterior chamber of the eye of tumor-immune rabbits when it will not grow in any other location.

REFERENCES

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