NEUTRALIZATION OF THE AGENT CAUSING LEUKOSIS AND SARCOMA OF FOWLS BY RABBIT ANTISERA*

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Shortly after the discovery that tumors and leukosis of fowls could be transmitted by cell-free filtrates Rous and Murphy (1) observed that fowls in which tumors had regressed were resistant to subsequent inoculation, and that the sera of these fowls neutralized the transmitting agent. No precipitins or complement-fixing antibodies could be demonstrated in these sera. Rous, Robertson, and Oliver (2) injected geese with tumor material and produced antisera which neutralized the agent. The complement-fixing antibodies present in these sera were unrelated to the neutralizing activity, since absorption with erythrocytes from normal chicken sera failed to remove the neutralizing antibodies, but removed the complement-fixing antibodies. These authors also immunized rabbits with tumor material and obtained hemolysins and hemagglutinins, but no neutralizing antibodies. Twelve years later, Andrews (3) studied neutralizing antibodies produced in fowls against several different tumor agents and found that three histologically different filterable fowl tumors showed a close immunological relationship. By the use of pheasant antisera Andrews (4) could distinguish differences among several filterable fowl tumors although some cross-neutralization occurred. Immune duck sera, however, did not show antigenic differences among the various strains studied.

Similar observations were made by Furth (5) and Uhl, Engelbreth-Holm, and Rothe-Meyer (6) with fowls recovered from leukosis. Recently, Ruffilli (7) described experiments suggesting that injection of fowl leukosis virus which had been inactivated by oxidation protects fowls against subsequent injection of the active agent. The immunity phenomena in fowl leukosis have been reviewed by Storti and Mezzadra (8).

Recently Andrews observed (9) that pheasants inoculated with a non-filterable transmissible sarcoma induced originally by tar developed neutralizing antibodies against the Rous sarcoma agent, and that antisera against fowl protein did not neutralize this agent. The neutralizing power of the serum against tar tumors was not affected by absorption with chick embryo pulp. These sera did not neutralize the agent of Fujinami sarcoma. Andrews concluded that this non-filterable tar sarcoma contained a virus immunologically related to that of Rous Sarcoma I. This observation was confirmed by Foulds (10), who found that rabbit antisera against a non-fil-

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terable transmissible tumor induced in fowls by 1:2:5:6 dibenzanthracene would neutralize the Rous agent.

Recently it has been shown (11–13) that the agents causing fowl sarcoma and leukosis could be concentrated by the ultracentrifuge. The bulk of the sediment from tumor tissues, however, does not consist of virus, since large amounts of material can be sedimented at the same speed from normal tissues as well. Moreover, the materials obtained at high speed from chicken tumor and from normal chicken spleen were identical in complement fixation and precipitin tests using sera of rabbits that had been injected with these fractions (13). Studies on the purification of the agent of leukosis and sarcoma of fowls (strain 13 (14)), made it desirable to search for methods for differentiating the agent from the normal tissue protein. The experiments to be described show that virus-containing heavy materials induce the formation of neutralizing antibodies in the rabbit and that these antibodies are distinct from the complement-fixing antibodies. Similar sediments from normal chicken spleen produce no neutralizing antibodies.

EXPERIMENTAL

Heavy materials were prepared from extracts of normal chicken spleen and from tumors produced by strain 13, as previously described (13). Rabbits were inoculated intravenously four times weekly with alum-precipitated sediments of these materials, using a total of 15 to 40 mg. of protein in 16 to 30 injections. Bleedings were made 5 days after the last injection. Sera were inactivated and filtered through a Berkefeld filter and stored without preservative under sterile conditions. Complement fixation tests were performed using 0.2 ml. of antigen (0.10 mg. N per ml.), + 0.2 ml. of the varying dilutions of serum, + 0.2 ml. of guinea pig complement diluted to contain approximately 2 units of complement. After incubation for 1 hour at 37° followed by 1 hour at room temperature, 0.2 ml. of a 5 per cent suspension of sensitized sheep erythrocytes was added to each tube. Hemolysis was read after ½ hour at 37°C.

Crude tumor extracts were employed in the neutralization tests, since they were more stable than the purified preparations. The tumor was ground with sand and saline, centrifuged to remove debris, and the viscous solution warmed to 37° with addition of enzyme preparation from pneumococcus (13) to reduce the viscosity. The solution was then filtered through a Berkefeld filter and stored in small tubes frozen at −60°C. Using the same extract, the neutralizing potency of several sera could be compared.

In carrying out the neutralization tests a measured volume of tumor extract was mixed with serum or saline and incubated at 37° for 15 minutes and allowed to stand overnight in the ice box. These mixtures were then diluted and injected into the breast and leg muscles of Barred Rock chicks of from 2 days to 1 month of age. By injecting the right breast and leg with one mixture, and the left breast and leg with another, different antisera could be compared in the same chickens.

RESULTS

The results of the neutralization experiments are summarized in Table I which shows that the rabbit antisera against heavy material from tumor neu-

TABLE I

Neutralization of Agents 13 and 11 with Rabbit Antisera against Heavy Materials from Chicken Tumor and Spleen

Antiserum		Tumor extract	Ratio of tumors produced to number of sites inoculated		
	ml.	*ml.*			
Experiment 1. Sarcoma 13					
Dilution of mixture			1:2500	1:250	1:25
Saline	2.4	0.1	9/10	8/10	9/10
Spleen 49	1.9	0.1	5/6	8/8	9/10
Experiment 2. Sarcoma 13					
Dilution of mixture			1:20,000	1:1000	1:50
Saline	2.45	0.05	0/10	3/10	8/8
Spleen 49	2.45	0.05	0/10	4/10	8/8
Tumor 70_1	2.45	0.05	0/10	1/10	4/8
Experiment 3. Sarcoma 13					
Dilution of mixture				1:1000	1:50
Saline	2.45	0.05		6/10	8/8
Spleen 48	2.45	0.05		5/10	6/10
Tumor 70_2	2.00	0.05		0/10	0/10
Tumor 71	2.45	0.05		0/10	0/8
Experiment 4. Sarcoma 13					
Dilution of mixture			1:5000	1:1000	1:60
Spleen 48	2.5	0.05	3/8	3/10	10/10
Tumor 71	0.1	0.05	4/10	3/10	8/10*
Tumor 71	0.5	0.05	0/10	1/10	5/10†
Tumor 71	2.5	0.05	0/8	0/10	0/10
Experiment 5. Sarcoma 13					
Dilution of mixture				1:1000	1:60
Spleen 49	2.5	0.05		3/8	8/10
Tumor 70_2 absorbed with spleen cell suspension	2.6	0.05		0/8	0/10
Tumor 70_2	2.5	0.05		0/10	0/10
Tumor 70_2	0.5	0.05		1/10	0/10
Experiment 6. Sarcoma 13					
Dilution of mixture			1:5000	1:1000	1:60
Spleen 48	1.0	0.06	0/8	6/10	8/10
Tumor 73	0.10	0.06	0/10	0/10	0/10
Tumor 73	0.50	0.06	0/10	0/10	0/10
Tumor 73	2.50	0.06	0/10	0/10	0/10
Experiment 7. Sarcoma 11					
Dilution of mixture			1:5000	1:500	1:50
Spleen 48	2.4	0.10	0/10	4/10	6/10
Tumor 71	2.4	0.10	0/10	0/10	4/10
Experiment 8. Sarcoma 11					
Dilution of mixture			1:2500	1:250	1:25
Spleen 49	2.4	0.10	2/10	5/10	7/9
Tumor 70_2	2.4	0.10	0/10	1/10	1/10

The total volume of the mixtures in Experiments 1, 2, 7, and 8 was 2.5 cc., in other experiments 3 cc.

* Large tumors.

† Small tumors.

tralize the agent. Antisera against heavy material from normal chicken spleen do not contain these neutralizing antibodies. The strength of the neutralizing antibodies in different sera can be determined by titration, using various amounts of immune serum with a constant amount of virus.

The neutralizing antibodies in the anti-tumor sera are unrelated to the complement-fixing antibodies. This is indicated by the observation that serum 71 contained neutralizing antibodies, but no complement-fixing antibodies. The complement-fixing antibodies in serum 70 could be removed by absorption with a suspension of cells from normal chicken spleen without any detectable effect on the neutralizing potency of the serum. Moreover, antisera against heavy materials from normal spleen do not neutralize the agent but fix complement in high dilutions. Thus, injection of heavy materials from tumor may give rise to antibodies specific for the agent, to complement-fixing antibodies against chicken tissue, or to both. It is noteworthy that sera of chickens immune to the viruses of leukosis and tumors contain neutralizing (3, 5, 6) but no complement-fixing antibodies (13).

Under the conditions of these experiments the neutralizing antibodies themselves do not fix complement. This may be either because the neutralization test will detect smaller amounts of antibody than the complement fixation reaction or because the neutralizing antibodies are unable to fix complement. Instances of the latter are well known; the antiflagellar (H) antibodies to the typhoid bacillus in rabbits (15) and antipneumococcus horse serum (16) are outstanding examples. An instance of the former has recently been observed by Kidd (17) who found that most antisera against the Shope papilloma virus contained virus-neutralizing and complement-binding antibodies in the same relative proportion, but a few sera neutralized small amounts of virus, yet failed to bind complement.

These observations furnish additional evidence that the agent is only a small part of the heavy material obtainable from tumor tissue and that preparations of the agent hitherto regarded by several investigators as pure contain large amounts of normal heavy materials.

The observations of Amies (18) are not in agreement with this conclusion. Amies obtained by repeated fractional centrifugation a suspension of the agent which was apparently free from fowl protein and could be agglutinated specifically by sera of fowls bearing the corresponding tumor. The sera also contained neutralizing antibodies for the agent but hyperimmune rabbit antifowl sera also neutralized the agent. From this finding it is inferred that the tumor agent contains an antigen which is normally present in fowl tissue.

Experiments 7 and 8 show partial neutralization of agent 11 by antiserum against agent 13. This indicates some degree of serological relationship between agent 11, which causes only sarcoma, and agent 13, which has potentialities of producing both sarcoma and erythroleukosis.

SUMMARY

Neutralizing antibodies against fowl tumor agents can be produced in rabbits by injection of heavy materials obtained from chicken tumor. Similar sediments from normal chicken spleen produce no neutralizing antibodies. The complement-fixing antibodies produced by both materials are unrelated to the neutralizing antibodies.

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