Complement-Fixing Antimouse Antibodies Found in Mice after Inoculation of Brain, Freund's Complete Adjuvant, and Sarcoma 180/TG Cells[1] (36022)

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(Introduced by J. Casals)

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Ascitic fluid, prepared in mice by various methods, has been used in place of mouse serum as a source of antibodies because larger volumes can be obtained. Munoz (1) induced ascitic fluid containing antibodies to egg albumin and to bovine albumin by injecting mixtures of these antigens and Freund's complete adjuvant (FCA) into mice intraperitoneally (ip). Similarly, by propagating sarcoma 180 cells in the peritoneal cavity of mice adequately immunized against either influenza A or Newcastle disease virus, Herrmann and Engle (2) obtained ascitic fluid containing antibodies to these viruses. The advantage of hyperimmunizing mice with antigen in Freund's adjuvant before inducing ascitic fluid has been described by Sartorelli *et al.* (3).

In this laboratory, certain hyperimmune ascitic fluids induced by the sarcoma 180/TG technique (3) gave false positive reactions in complement-fixation (CF) test when tested against arboviruses grown in mouse brain. The studies reported here indicate that the sarcoma 180/TG strain and mouse brain have antigens in common, as a consequence of which, some of these ascitic fluids contain antimouse tissue antibodies.

Materials and Methods. Immunization. Three groups of one hundred 12-week-old randomized female Swiss mice, ICR/Kp strain, began the immunization schedules shown in Table I. All injections consisted of 0.2 ml and were given ip. Ascitic fluids were harvested by paracentesis; and the cellular sediment was removed by centrifugation. The supernate was stored at −70° until thawed for use. Ascitic fluids to be tested for antibody stability were stored at −15° for 11 months.

Adjuvant emulsions. Brain, harvested from apparently normal 6-day-old ICR/Kp mice, was prepared as a 10% suspension in phosphate-buffered saline. One part of FCA (Difco, Detroit, MI) was ejected from a glass syringe into 1 part of 10% brain suspension, and the mixture was then drawn up and down in the syringe to create an emulsion. Emulsions of saline and FCA were prepared in the same way.

Sarcoma 180/TG. The sarcoma 180/TG cells, certified free of selected specific murine viruses, were supplied by the Research Reference Reagents Branch of the National Institutes of Health. The cells underwent 23 additional mouse passages at this laboratory before use.

CF tests. Testing was done by the micromethod of Casals (4), based on the 100% hemolysis end point and adapted to a 4-drop technique. Lyophilized guinea pig complement was titrated to determine 1.8–2.0 units of complement. Ascitic fluids, diluted 1:4, were inactivated at 60° for 20 min. Antigen, in the form of brains from apparently normal infant mice of several sources (the ICR/Kp strain and mouse colonies maintained at the Center for Disease Control, Atlanta, and the Middle America Research Unit, Panama), was prepared by sucrose–acetone extraction

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TABLE I. Schedules for Immunization and Induction of Ascites in Three Mouse Groups.[a]

Day	Group I	Group II	Group III
0	MB + FCA	—	MB + FCA
7	MB + FCA	—	MB + FCA
15	—	S-180/TG	S-180/TG
18	FCA + saline	—	FCA + saline
22	MB + FCA	—	MB + FCA
25	Paracentesis	Paracentesis	Paracentesis
31	Paracentesis	Paracentesis	Paracentesis
36–37	Paracentesis	Paracentesis	Paracentesis

[a] MB, mouse brain; FCA, Freund's complete adjuvant; S-180/TG, sarcoma 180/TG cells.

(5), freeze-dried, and stored at —70° until rehydrated and diluted 1:4 in Veronal buffer for testing. Antigen alone and ascitic fluid alone were used as controls. A 1:4 dilution of ascitic fluid that fixed complement on a scale of 1+, 2+, 3+, or 4+ was considered positive.

Sucrose gradient centrifugation. Sucrose gradients were prepared by the method of Schluederberg (6). From each of five solutions of sucrose in Veronal buffer (pH 7.4) ranging from 36% downward in 6% decrements, 0.9 ml was slowly pipetted into cellulose nitrate tubes (0.5-in. diam × 2 in.). After refrigeration of the gradients for several hours, 0.2 ml of ascitic fluid samples from mice, which in preliminary experiments had developed CF antibody after schedule II or III, was pipetted onto each gradient.

TABLE II. Antimouse CF Antibody in Ascitic Fluids Induced in Three Groups of Mice by Different Schedules.[a]

	No. mice positive/no. tested	
	Day 31	Day 36–37
Group I MB + FCA	1/64 (2%)	1/52 (2%)
Group II S-180/TG only	4/48 (8%)[b]	7/36 (19.4%)
Group III MB + FCA + S-180/TG	22/88 (25%)[c]	24/64 (37.5%)

[a] For abbreviations, see Table I.
[b] $0.25 > p > 0.10$.
[c] $0.05 > p > 0.025$.

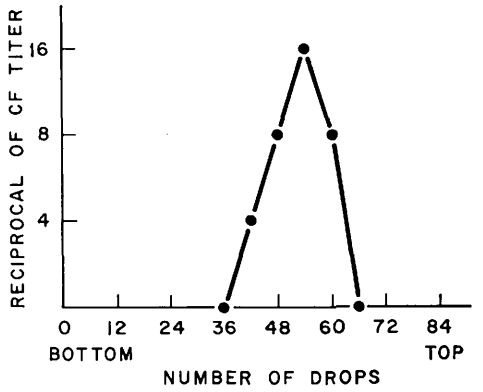

FIG. 1. Antimouse CF reactivity of fractions obtained by sucrose gradient centrifugation of ascitic fluid taken 16 days after inoculation of sarcoma 180/TG cells.

The gradients were then centrifuged in a Spinco SW-50.1 rotor at 31,000 rpm for 16.5 hr. Six-drop samples, collected by piercing the bottom of the tube with a needle, were diluted 1:4 and tested without inactivation by CF as described above. Positive fractions were retested in serial 2-fold dilutions.

Results. In mouse groups II and III, both given sarcoma 180/TG cells, CF antibodies to mouse brain were not detected in ascitic fluid taken 10 days after injection of the tumor but were detected 16 days after such injection (experiment day 31). Table II shows the proportion of mice in all three groups that were CF-test positive against mouse brain on experiment days 31 and 36-37. On day 31, 25% of group III mice (22/88) were CF-positive, 8% of group II (4/48), but only 2% of group I (1/64). All antigens reacted in the same manner with representative ascitic fluids, regardless of source. These antimouse CF antibodies were stable through 11 months' storage at —15°.

Analyses of sucrose gradient centrifugation of four CF-positive ascitic fluids, taken from mice at least 16 days after injection of sarcoma cells, showed the mean peak of antimouse brain CF activity to be 0.63 from the bottom of the gradient. Figure 1 presents the results obtained with one of these samples. Under the conditions used, this fraction contains the IgG type of immunoglobulin (6).

Discussion. Repeated injections of brain

and adjuvant have been shown to evoke CF antibrain antibodies in many animal species (7), including mice (8); this response varied with different strains of a species (9). It also has been demonstrated that sera from some animals sensitized to brain tissue can fix complement *in vitro* not only to brain but also to corpus luteum (7), testicular tissue (10), and placental tissue (11), which is evidence that each of these tissues shares antigenic constituents with brain.

The present data indicate that mouse brain shares antigens with the sarcoma 180/TG strain, although the precise nature of these antigens has not been determined. Most of the mice in groups II and III that formed CF antibodies to mouse brain did so by day 16 after sarcoma 180/TG inoculation. In the case of group I mice, only 2% of which were CF-positive on experiment day 31, it seems possible that the antibodies detected arose in response to the mouse brain emulsified in FCA. In group II mice, 8% of which had formed CF antibodies to mouse brain by day 31, the multiplying sarcoma cells may have provided a continuous antigenic stimulus. In group III mice, with 25% CF-positive on day 31, the enhanced effect may indicate that mouse brain emulsified in FCA acted as a primary stimulus, and sarcoma 180/TG cells as a secondary stimulus. The fact that antigens prepared from mice of two other colonies reacted similarly to those from ICR/Kp mice argues against a murine virus accounting for the reactions observed.

Since antimouse tissue antibody develops progressively with time after administration of sarcoma 180 cells, for practical purposes the presence of this antibody may be minimized by collection of ascitic fluids before day 10 after tumor inoculation. This procedure obviously entails giving up the larger volumes of ascitic fluid obtained with later and multiple paracenteses. Alternatively, ascitic fluid can be prepared by immunizing female mice with virus-infected mouse brain emulsified in FCA as described for group I mice—a method that, in this laboratory, has produced adequate quantities of hyperimmune mouse ascitic fluid with minimal antimouse tissue antibody.

Summary. A significant proportion of ascitic fluids induced in mice by the sarcoma 180/TG technique reacted positively in complement-fixation (CF) test against mouse brain antigen when tested 16 days after tumor injection. The proportion was increased when mice were previously immunized with mouse brain and Freund's complete adjuvant (FCA). Antimouse tissue reactions were minimal in mouse ascitic fluids induced by the FCA technique of Munoz. Antimouse CF antibody was not detected in ascitic fluids taken on day 10 after inoculation of sarcoma cells.

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