Infrequent C-F Antibody Response in Mice with Adenovirus Type 12 Tumors.* (31921)

G. L. VAN HOOSIER, JR., J. J. TRENTIN, S. S. CHENAULT, M. E. BRYAN, AND K. J. MCCORMICK
Division of Experimental Biology, Baylor University, College of Medicine, Houston, Texas

Reports on the development of specific complement fixing (C-F) antibodies against tumor antigens in animals bearing adenovirus induced tumors have been limited to the golden hamster *(Mesocricetus auratus)* and the rat *(Rattus norvegicus)*(1-3). Examination of sera from humans with cancer for C-F antibodies to adenovirus tumor antigens is suggested as the results would possibly indicate individuals with tumors of adenovirus etiology. Observations on the antibody response in different species should prove helpful in interpreting any results obtained with human materials. In previous studies in this laboratory, newborn mice of inbred strains DBAf and Af were found resistant to tumor induction by adenovirus type 12 while the C3Hf/Gs strain was found susceptible, although the percent of animals developing tumors was lower and the latent period longer than that observed in hamsters(4). The serial transplantation of these tumors in isologous mice offered an opportunity to compare the C-F antibody response of the mouse with that of the hamster.

Materials and methods. Serum was obtained from mice by cardiac puncture and immediately diluted 1:4 with chilled saline and tested at an initial serum dilution of 1:8. Complement fixation tests were performed similar to standard virus laboratory procedures(5). Tumor antigens were prepared by homogenizing viable tumor tissue in a Virtis homogenizer with enough C-F saline to make a 10% suspension. Homogenization was followed by slow speed centrifugation and the supernatant was frozen at —70°C until tested.

Results. Sera from tumor bearing mice were tested by the complement fixation test for antibodies against adenovirus type 12 induced mouse tumor, adenovirus type 12 induced hamster tumor, and tissue culture grown adenovirus type 12, all of which were known to be reactive by complement fixation with tumorous hamster sera. Reactions observed were as follows: 7 of 40 mouse sera reacted with mouse tumor antigen, 5 of 69 reacted with hamster tumor antigen, and 4 of 20 reacted with adenovirus type 12 tissue culture grown antigen, while appropriate control antigens were negative. Serum titers ranged from 1:8 to 1:32.

Fig. 1 illustrates the absence of a correlation between tumor size and serum titer in the mouse which was in contrast to the positive correlation found in hamsters and illustrated in Fig. 2.

The presence of antigen in 23 of 24 mouse tumors tested against positive tumor bearing hamster sera indicates that the failure of a high percent of mice to respond is not attributable to the complete absence of antigen(s) in the tumor. Table I shows the distribution of mouse tumor antigen titers, and, for comparative purposes, the antigen titers of 8 hamster tumors tested against one of the positive hamster serum pools used for testing 6 of the mouse tumors. The results suggest that the titer of antigen in the mouse tumors may be less than in the hamster tumors.

Discussion. One possible explanation for this observation is that the tumor mass, which appears to represent a larger percent of the body weight in a mouse than in a hamster, may absorb out the circulating antibody so that the remaining levels in the sera are undetectable. Habel was able to detect C-F antibodies in sera from tumorous C57BL mice against polyoma tumor C-F antigens although the percent of mice positive was not given (6). It is also noteworthy that C-F antibodies were not found against homologous tumor antigens in hamsters bearing tumors induced by an avian adenovirus (CELO)(7). Another

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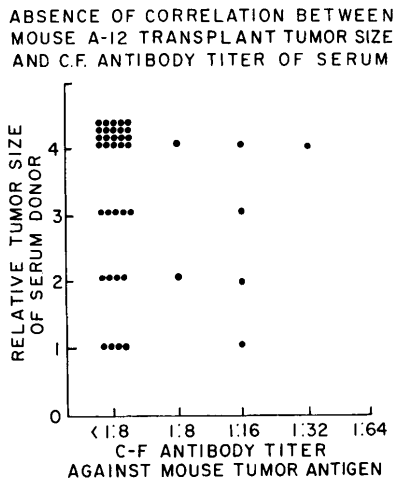

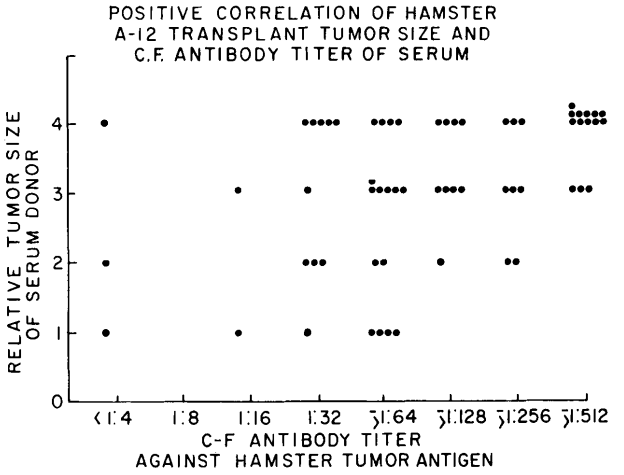

FIG. 1 and FIG. 2.

possibility is that the cell-virus relationship in the murine system is different from that in the hamster system and the resultant expression of antigen(s) is quantitatively or qualitatively different. However, mice have been found to develop immunity to transplants of virus free adenovirus type 12 tumors following virus immunization(8), although it is probable that the antigen(s) involved in tumor transplantation immunity are different from those reactive in the C-F test. C-F antibodies have been demonstrated in C3Hf/Gs mice in our colony as a result of natural infection with murine adenoviruses(9), and this would suggest that the results observed with C-F antibodies to tumors are not due to some peculiarity of the C3H mouse in our colony to respond to antigens with C-F antibodies. The time period between tumor transplant and collection of sera does not appear significantly different in the mouse and hamster and thus does not seem to be a probable explanation for the results obtained. It does not appear that heterophil antigens and antibodies present in the random bred hamster but absent in the inbred mouse could explain the differences observed because of the evidence for the specificity of the reaction.

Thus it appears that in the mouse, and perhaps in other species as well, the presence of specific antigens in the tumor is a more reliable indication of the etiological virus than the presence of antibodies in the sera.

Summary. In contrast to tumor bearing hamsters, in which 95% (59 of 62) of the animals in this series developed complement fixing (C-F) antibodies against homologous adenovirus type 12 tumors, only 18% (7 of 40) of the C3Hf/Gs mice bearing adenovirus type 12 transplant tumors developed C-F antibodies against homologous mouse tumors. Only 7% (5 of 69) of the tumor bearing mice developed C-F antibody when tested against adenovirus type 12 hamster tumor antigen. Also, there was no significant correlation in the mouse between tumor size and antibody titer as there was in the hamster. The disparity between the development of antibodies

TABLE I

Antigen		Antigen titer *vs* 1:16 dilution of positive hamster serum					
		Antigen dilution					
	No. tested	<1:4	1:4	1:8	1:16	1:32	1:64
Mouse tumors *vs* positive pool C	18	1*	4	5	8	0	0
Mouse tumors *vs* positive pool D	6	0	2	1	2	1	0
Hamster tumors *vs* positive pool D	8	0	0	0	1	5	2

* Number with indicated titer.

in the mouse and the hamster is not accounted for by absence of antigen in the mouse tumors because most contained antigen as determined by reactivity with positive hamster sera.

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Effect of Pituitary and Gonadal Hormones on Friend Virus Disease in Mice.* (31922)

E. A. MIRAND, N. BACK, J. T. GRACE, JR., AND RITA BUFFET
Roswell Park Memorial Institute, New York State Department of Health, and State University of New York at Buffalo

Friend Virus Disease (FVD) as described by Mirand *et al*(1) is associated with malignant reticulum cell proliferation, a pronounced hypervolemic polycythemia, and progressive hepatosplenomegaly. Although the precise role of an endogenous erythropoietic stimulating factor (ESF) in this disease has not been determined fully, exogenous ESF greatly accelerates the erythropoietic response induced by this viral infection(2). The effect of chemotherapeutic agents on the disease has been studied(3,4,5), but little attention has been given to the effect of hormones. Erythropoiesis was found to be stimulated by androgens(6,7) and adrenal corticosteroids (8), depressed by estrogens(6,9). A mechanism for the depression of erythropoiesis by estrogens in normal and plethoric rodents has been suggested recently(10). The present study reports the effect of several hormones on the erythropoietic response in Friend Virus Disease.

Materials and methods. Ha/ICR Swiss female weanling mice, weighing 24-26 g, were inoculated intraperitoneally with a standard 0.2 ml inoculum of a 10% cell-free spleen homogenate filtrate obtained from Friend virus-infected mice. Methods for preparing the splenic homogenates have been described previously(1).

Mice were divided into groups of 6 mice per group. Drug-treated groups received the hormones subcutaneously once or three times a week for 2 weeks prior to infection with FVD. Drugs then were injected once or three times a week for four consecutive weeks. At weekly intervals, the per cent 24-hour Fe^{59} uptake in blood, liver, spleen, and femur was measured following intravenous administration of the isotope(1). The following measurements also were made weekly: hematocrit, body weight, spleen weight, and liver weight. Mice receiving no drug or virus (Table I), the drug alone or only the virus inoculum comprised the control groups. Mice were maintained in a 24°C temperature-controlled environment on a diet of Derwood-Morris mouse chow and water *ad libitum*.

The following hormones were administered: (a) adrenocorticotropic hormone (ACTH) I.M., 38 mg/kg in saline 3 times/week. The potency of ACTH is expressed in milligrams. An arbitrary standard has been selected (Lot. No. LA-1-A) and all preparations are assayed in terms of milligrams of this standard. The

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