Tumor Cell-Induced Mouse Ascites Fluid as a Source of Viral Antibodies. (24009)

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(Introduced by Preston L. Perlman)
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In studying the immune response of mice to viral antigens, there are severe limitations to the volume of blood serum obtainable. As one possible solution to this problem Munoz (1) reported that considerable quantities of mouse peritoneal fluid result from injections of Freund's adjuvant and that this fluid is rich in specific antibodies induced by injections of certain albumins. The present report will show that Sarcoma 180 cells are also convenient for inducing large volumes of ascites and that such peritoneal fluid contains virus-induced antibodies. Wagner(2) has stated that passively immunized mice exhibit viral antibody levels in Ehrlich tumor cell-induced ascites similar to those found in the blood serum. This report will show that a similar result can be obtained with Sarcoma 180 cell-induced ascites produced in mice actively immunized with viral antigens.

Materials and methods. The viruses used were chick embryo adapted lines (American Type Culture Collection) of the PR8 strain of influenza A virus and the California strain (#11914) of Newcastle disease virus (NDV). A single allantoic fluid pool of each of these viruses was used for immunizing procedures as well as for subsequent hemagglutination-inhibition tests. Groups of 20 Swiss mice (Webster strain, 18 to 20 g) were immunized by injecting into the peritoneal cavity 0.2 ml of undiluted allantoic fluid pools of either NDV or influenza virus. These injections were repeated twice weekly every other week for 6 weeks. Immediately after the last immunizing injection, all mice were injected intraperitoneally with 0.5 ml of a 1/20 balanced salt solution dilution of S-180 cells obtained from a mouse exhibiting marked abdominal distention resulting from prior injection of tumor cells. In these experiments each mouse received approximately 1 million tumor cells, although the number of such cells implanted does not seem to be critical. When the mice became markedly swollen by accumulation of peritoneal fluid (in 7-10 days), they were anesthetized with ether and heart blood as well as ascites were collected separately. Since it was not known what effect clotting might have on antibody content of the ascites, heparin was used to prevent clotting of blood and of ascites. In that antibody content of blood was to be compared to that of the ascites, it was necessary that a uniform volume to volume ratio of these 2 fluids be maintained during their collection. For example, in a group of 20 mice, one mouse could have a high antibody titer, while another might have a low one. Collection of a comparatively large volume of blood and a small volume of ascites from the mouse with the high antibody titer and the converse with the low titered mouse, would lead to a serum sampling error when all blood samples from a single group were pooled. Thus, it was arbitrarily decided that for each 0.1 ml of heart blood obtained from a mouse, 1 ml of ascites would also be collected from the same mouse. Blood from all mice in a single group was combined and centrifuged at 2000 rpm for ½ hour to remove the cells. A similar procedure was followed for the ascites samples. Both fluids were ampuled and stored at dry-ice temperatures. The above procedure can be complicated by the unpredictable death of mice that received S-180 cells. This problem was largely overcome, however, by use of large groups of animals. For example, in a total of 40 mice only 8 died before they could be utilized. This disadvantage may be further counterbalanced by using pools of fluids from large groups of mice, where the results cannot easily be influenced by an unusual response of a single animal. Eventually, of course, all mice will die over a period of time that depends on the number of S-180 cells

TABLE I. Hemagglutination-Inhibition of Immune Mouse Blood Plasma and Ascites Fluid against Newcastle Disease and Influenza A Viruses.

Antibody source	Immunizing virus	Test virus	Dilutions of plasma or ascites fluid (Exp. I) 1/80	1/160	1/320	1/640	1/1280	1/2560	Red cell control
Blood plasma	Influenza	Influenza	—	—	—	—	+	+	—
Ascites		"	—	—	—	±	+	+	—
"		NDV	+	+	+	+	+	+	—
Blood plasma (2 tests)	NDV	NDV	—	—	+	+	+	+	—
Ascites (2 tests)		"	—	—	+	+	+	+	—
Ascites		Influenza	—	+	+	+	+	+	—

			Dilutions of plasma or ascites fluid (Exp. II) 1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	
Unheated ascites	Influenza	Influenza	—	—	—	—	—	+	+	+	+	—
Heated " *		"	—	—	—	—	—	+	+	+	+	—
Unheated "		NDV	+	+	+	+	+	+	+	+	+	—
Heated "		"	+	+	+	+	+	+	+	+	+	—
Unheated blood plasma		Influenza	—	—	—	—	—	—	+	+	+	—
Heated " "		"	—	—	—	—	—	±	+	+	+	—
Unheated " "		NDV	+	+	+	+	+	+	+	+	+	—
Heated " "		"	+	+	+	+	+	+	+	+	+	—
Unheated ascites	NDV	NDV	—	—	—	—	+	+	+	+	+	—
Heated "		"	—	—	—	—	+	+	+	+	+	—
Unheated "		Influenza	—	+	+	+	+	+	+	+	+	—
Heated "		"	±	+	+	+	+	+	+	+	+	—
Unheated blood plasma		NDV		—	—	—	—	+	+	+	+	—
Heated " "		"	—	—	—	—	+	+	+	+	+	—
Unheated " "		Influenza		+	+	+	+	+	+	+	+	—
Heated " "		"	—	±	+	+	+	+	+	+	+	—

* 56°C for 30 min.
+ = complete hemagglutination; ± = partial hemagglutination; — = no hemagglutination.

originally implanted. When 1 million cells were implanted, the average day of death for a group of 10 mice was 14. The level of viral antibody in blood plasma and ascites was determined by hemagglutination-inhibition procedure(3). It was first necessary to titrate both virus pools to determine the highest saline dilution that would completely agglutinate a 2% suspension of washed, chicken erythrocytes. Based on this, a standard virus suspension was prepared so that 1 ml contained 8 times more virus than necessary for complete hemagglutination. The antibody-containing fluid to be tested was diluted in 2-fold steps in 0.85% NaCl solution. The standard virus suspension was mixed with each plasma dilution in a 1:1 volume ratio. The antibody-antigen mixtures were incubated 1 hour at room temperature and 1 ml of each mixture was added to 0.5 ml of a 2% suspension of red blood cells in a plastic depression plate, which was rotated vigorously to insure mixing of the reagents. The resultant pattern of the agglutinated erythrocytes was read after 45 min.(3).

Results. The data (Table I) indicate that ascites contains antibodies in amounts which are comparable to those found in blood plasma. There seems little doubt as to the specificity of the influenza virus neutralizing antibodies, since essentially no non-specific neutralization of NDV was observed. This was not true, however, in one portion of Exp. I, where dilute NDV-specific ascites appeared to neutralize influenza virus. In this experiment neither the ascites nor blood plasma was heated to remove heat-labile non-specific hemagglutination-inhibitors. Exp. II (Table I), however, resolved this point, since heated (56°C for 30 min.) as well as unheated ascites were tested. In this experiment, non-specific inhibitors were not observed at dilutions greater than 1/16. It can also be noted that the antibody titers observed in the first

TABLE II. Neutralization of Infectivity of Influenza A (PR8) Virus for Mice with Specific Immune S-180 Induced Ascites.

Dilution of ascites	Survivors* / Total mice
1/ 80	4/5
1/ 160	5/5
1/ 320	5/5
1/ 640	2/5
1/1280	5/5
1/2560	4/5
Control	0/5

* Experiment was terminated 14 days after infection.

experiment were higher than those in the second. There is no explanation at present for this result other than there may have been differences between the chicken red blood cells used.

Additional preliminary data (Table II) indicate that dilute, specific immune peritoneal fluid will neutralize the infectivity of influenza A virus for mice. In this experiment, a mouse lung-adapted strain of influenza A virus was diluted so that 0.1 ml contained 100 LD_{50}'s. The virus suspension was then mixed with dilutions of the ascites in a 1:1 volume ratio and incubated at room temperature for 1 hr. The antibody-antigen mixtures were then instilled intranasally in 0.1 ml volumes into the mice. Control mice received virus diluted 1:1 with saline. The data show that the peritoneal fluid neutralized the infectivity of the virus at a dilution greater than 1/2560. It is apparent that when a small amount of virus is used, as in this experiment, the antibody titer of the ascites is very high. Other experiments indicate that specific immune ascites for influenza B (LEE) virus will also neutralize the interfering capacity of this virus.

Discussion. It is a simple matter to obtain large quantities of ascites from mice, injected with S-180 cells. It is frequently possible to obtain 10 ml from a single mouse, and not unusual to collect 15 and 20 ml. In our experiments, all mice accumulated significant amounts of peritoneal fluid and an average of 5 ml was conveniently obtained from surviving mice. This amounted to at least 10 times as much antibody as could be obtained by cardiac puncture. Thus, it would seem desirable, in certain cases, to use mice in place of those larger animals now maintained only for occasional use as an antibody source.

Mice that have been injected with S-180 cells frequently contain significant volumes of ascites over protracted periods of time. This would suggest the possibility of infecting the mice with a virus and then following the antibody rise by periodic intraperitoneal tappings.

Summary. Mice immunized by multiple injections of either influenza A or Newcastle disease virus produced antibody levels in the Sarcoma 180 cell-induced ascites comparable to levels found in blood plasma as measured by hemagglutination-inhibition test. The infectivity and interfering capacity of influenza viruses could also be neutralized by the specific immune ascites. It was possible to collect at least 10 times more ascites than heart blood.

1. Munoz, J., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 757.

2. Wagner, R. R., *J. Immunol.*, 1955, v74, 439.

3. Salk, J. E., *ibid.*, 1944, v49, 87.

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