Antibody for *Chlamydia psittaci* in Ascitic Fluids of Immunized Mice Implanted with Sarcoma 180 (34722)

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Neutralizing antibodies to *Chlamydia psittaci* have been demonstrated in the sera of various laboratory animals (1, 2). However, routine preparation of suitable antisera is attended by practical difficulties, and high-titer, homotypic neutralizing antisera for the various strains of this group of agents have been available in very limited quantities. Methods in use for the production of immune ascitic fluids in mice (3–5) suggested a means for obtaining antisera for *Chlamydia psittaci*. The present paper describes application of this procedure for preparation of useful amounts of antibody to several strains of *Chlamydia psittaci*.

Materials and Methods. Sarcoma cells. The strain of Sarcoma 180 tumor cells used in these studies was maintained by routine implant passage in Swiss Webster mice obtained from the Fort Detrick colony. Additional studies showed that sarcoma cells stored at −70° for approximately 3 years produced abdominal distention in approximately 2 weeks, but after storage for 5 years, the time required to produce swelling was increased considerably.

Vaccines. Formalinized vaccines for the Borg, 6BC, New Jersey (NJ), and California Pigeon (CP) strains of *Chlamydia psittaci* were used (6). The author is indebted to Dr. L. A. Page for the NJ and CP strains.

Production of antibody. Mice that survived vaccine antigenicity titrations were used. The mice had received three ip injections of 0.5 ml of vaccine and had been challenged with 0.5 ml of a range of dilutions of yolk sac suspension of the pertinent viable agent. After about 2 weeks, survivors that had received the highest challenge doses (1000–100,000 $YSLD_{50}$) were implanted intraperitoneally with approximately 2.5×10^6 sarcoma cells. Ascitic fluids were collected at 2- to 3-week intervals following implantations; 20–30 ml of ascitic fluid was obtained from each mouse. Fluids from groups that had been challenged with the same strain were pooled, clarified by centrifugation at 10,000 rpm, inactivated at 56° for 30 min, and stored at −20°. Titers did not change appreciably over a period of 2 years or more.

Neutralization titers. The tests were conducted by the varying virus-constant serum method, using infected yolk sac suspensions of the respective strains of *C. psittaci*. Equal amounts of the dilutions of agent and ascitic fluid were mixed, held at room temperature for 4 hr, then injected intraperitoneally into 18- to 20-g mice. The mice were examined daily and deaths were recorded over a 14-day period. End points were determined according to Reed and Muench (7). The log neutralizing capacity was found by subtracting the log of the mouse intraperitoneal LD_{50}/ml ($MIPLD_{50}$) of the immune ascitic fluid from that of the normal control ascitic fluid. Normal ascitic fluids had no apparent effect on the infectivity of yolk sac suspensions.

Complement fixation titrations. CF tests with immune ascitic fluids were carried out using the Standard Diagnostic Complement Fixation Method Adaptation to Microtest (8). Cell wall preparations of each strain were used as antigens (9). The dilution of ascitic fluid showing 30% hemolysis was taken as the end point. Negative results were obtained with normal ascitic fluids; anticomplementary activity of the fluids was low.

Results. Antibody in ascitic fluids. Neutralization titrations on four Borg immune ascitic fluid drainages obtained over a period of 39 days and experiments on the effect of dilution

TABLE I. Persistence of Antibody in Borg Immune Ascitic Fluid and Final Dilution End Points.

Type ascitic fluid[a]	After implantation (days)	Final dilution of ascitic fluid	Log $MIPLD_{50}$/ml	Log neutralizing capacity
Immune, drainage 1	16	1:2	<1.3	>6.2
Normal control		1:2	7.5	
Immune, drainage 2	22	1:2	<1.3	>6.4
		1:8	4.3	3.4
		1:32	6.6	1.1
Normal control		1:2	7.7	
		1:8		
Immune, drainage 3	29	1:2	<1.3	>4.9
4	39	1:2	>5.3	<0.9
Normal control		1:2	6.2	

[a] Ascitic fluids from 4 to 8 mice were pooled.

on the neutralizing activity are shown in Table I. Ascitic fluids obtained from mice that had not been immunized or challenged were used as controls. Ascitic fluids obtained during the first three drainages from the immune group of mice exhibited a high neutralizing capacity. Greater than 10^6 $MIPLD_{50}$ was neutralized with day-16 fluid and greater than 10^5 $MIPLD_{50}$ was neutralized by fluid obtained on days 22 and 29. By the fourth drainage on day 39 the antibody level was considerably lower. Immune ascitic fluid diluted 1:32 neutralized approximately 10 $MIPLD_{50}$ of the Borg agent. Ascitic fluid from mice immunized with 6BC, NJ, and CP vaccines and challenged with the homologous strains neutralized 2.4, >5.8, and >0.4 log $MIPLD_{50}$/ml, respectively (Table II). Ascitic fluid from mice that had been immunized with vaccine but not challenged did not have neutralizing capacity.

Passive immunity. The duration of passive protection obtained in mice by intraperitoneal injection of 0.5 ml of undiluted Borg immune ascitic fluid is shown in Table III. Groups of animals were challenged intraperitoneally with various dilutions of infected yolk sac material of the Borg agent 1, 5, and 15 days following immunization. Passive protection was strongly evident on the first and fifth days following administration of ascitic fluid. No end point was obtained in the 15-day challenge, but it is evident that immunity had declined between 5 and 15 days.

Specificity of neutralization and complement fixation. The extent of cross reaction of the Borg and 6BC immune ascitic fluids was investigated (Table II). Borg immune fluid

TABLE II. Complement Fixation and Neutralization Titers of Ascitic Fluids.

Type ascitic fluid	Test strain	CF titer (cell wall antigen)	Log $MIPLD_{50}$/ml		Log neutralizing capacity
			Immune fluid	Normal fluid	
Borg	Borg	256	<3.3	7.1	>3.8
	6BC	4	7.1	5.7	—1.4
6BC	6BC	16	3.3	5.7	2.4
	Borg	8	6.3	7.1	0.8
NJ	NJ	64	2.5	>8.3	>5.8
CP	CP	4	<1.3	1.7	>0.4

TABLE III. Persistence of Passive Immunity Produced by Borg Immune Ascitic Fluid.

Interval between immunization and challenge (days)	Group	Mice surviving/total inoculated; dilution of Borg agent (log_{10})						
		—1	—2	—3	—4	—5	—6	—7
1	Immune	1/3		3/3		3/3		3/3
	Control			0/3		1/3		3/3
5	Immune	2/3		3/3		3/3		
	Control			0/2		1/3		3/3
15	Immune	0/2	0/3	0/3	1/3			
	Control				0/2	0/3	0/3	2/3

did not neutralize 6BC agent, and 6BC immune fluid neutralized less than 10 $MIPLD_{50}$ of Borg agent. The neutralizing capacity of the 6BC preparations was less than that of the Borg fluids. Complement fixation titers of the various ascitic fluids are also summarized in Table II.

Effect of host or route. No neutralization was obtained in embryonated eggs inoculated by the yolk sac route, and only a small amount of neutralization was obtained in mice inoculated by the ic route.

Discussion. Previous investigators had found that sera of immunized rabbits, mice, guinea pigs, and monkeys demonstrated little or no neutralizing capacity (10). Hilleman (11), using a system of infectivity scores based on extent of lung consolidation in mice, recorded a high neutralizing capacity in the serum of roosters immunized with mouse pneumonitis agent; no protection was obtained with the serum of similarily immunized rabbits. Although ascitic fluid titers were not as high as those obtained with convalescent rooster serum, preparation of ascitic fluids is more convenient and less hazardous than collection of serum from infected avian hosts. The ascitic fluids appear useful for studies on the antigenic and immunogenic structure of this group of microorganisms, and may prove applicable to other chlamydial agents. The ascitic fluids did not neutralize the agents in eggs, or protect mice inoculated intracerebrally. This indicates that host susceptibility and route of inoculation are important considerations in determining neutralizing capacity.

Summary. Ascitic fluids were collected from mice after immunization with nonviable vaccines for *Chlamydia psittaci,* challenge with the respective viable agent, and implantation of Sarcoma 180. The fluids produced against the Borg, 6BC, and New Jersey strains contained useful titers of antibody demonstrable by neutralization and complement fixation titrations. Neutralization was demonstrable against intraperitoneal challenge in mice, but was slight or negligible against intracerebral challenge in mice and against inoculation into the yolk sac of embryonated eggs.

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