TECHNICAL MANUSCRIPT 219

PSITTACOSIS GROUP VACCINE PREPARED IN A HUMAN DIPLOID CELL STRAIN

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PSITTACOSIS GROUP VACCINE PREPARED IN A HUMAN DIPLOID CELL STRAIN

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In conducting the research reported here, the investigators adhered to "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

ABSTRACT

Growth characteristics of three agents of the psittacosis group in the L cell line and in human diploid cell strains have been described previously. Applications of these methods of cultivation to preparation of improved psittacosis group vaccines have been investigated. The Borg strain of human pneumonitis agent was used to infect cell cultures of human diploid lung strain, WI-38. Eagle basal medium (BME), which contained 3% calf serum, was removed from infected cell monolayers after 18 hours' incubation at 37 C, and replaced with BME (without serum) or BME containing 0.5% bovine serum albumin. Supernatant fluids were collected after 2 to 3 days at 37 C, and formalin inactivation rates were investigated. The conditions selected for routine inactivation were 0.05% formalin, pH 7.0 to 7.5, and 20 C. Mice injected intraperitoneally with single or multiple injections of vaccine and challenged by the same route at various time intervals after the last injection withstood large doses of homologous active agent. The immunized mice also were protected against a lethal challenge with the heterologous 6BC strain of the psittacosis agent. Cross-protection was further evidenced by protection against the Borg strain using a formalin-inactivated 6BC vaccine.

PSITTACOSIS GROUP VACCINE PREPARED IN A HUMAN DIPLOID CELL STRAIN

The properties of the human diploid cell strains and the potential application of this type of cell system for viral vaccine production have been described by Hayflick and associates.[1-4] Pearson et al.[5] have presented a study of the growth and morphological development of three agents of the psittacosis group in the L cell line and several human embryonic diploid cell strains. Comparative titrations of infected supernatant fluids in embryonated eggs indicated that the agents grew readily in diploid cell strains. The results were essentially the same as those obtained in the L cell line. Methods are described here for the preparation of formalin-inactivated vaccines from the Borg strain of the human pneumonitis agent grown in human diploid cell strains. Results of assessing these vaccines as immunizing antigens in mice are presented.

The tissue cultures used to prepare vaccine for the Borg agent were 4- to 6-day-old monolayers of the WI-38 strain of human embryonic diploid lung cells grown in one-liter Blake bottles or obtained commercially in 32-oz prescription bottles. Cell monolayers were washed with balanced salt solution (BSS), infected with a tissue culture stock seed of the Borg strain of the human pneumonitis agent, and incubated for 1 to 2 hr at 37 C. The inoculum was removed and maintenance medium, which contained 3% calf serum, was added. After 18 hours' incubation at 37 C, the maintenance medium was removed, and the monolayers were washed with BSS to remove the serum. Serum-free Eagle basal medium (BME) or, in a few of the preparations, BME containing 0.5% bovine serum albumin (BSA) was added. The BSA was added as a possible stabilizer. The cultures were incubated at 37 C for 2 to 3 days, at which time a 2 to 3+ cytopathic effect (cpe) was observed. At that time the supernatant fluids were collected, cellular debris was removed by low-speed centrifugation, and formalin was added. A single vaccine using the 6BC strain of the psittacosis agent was prepared in a similar manner and will be referred to later in the cross-protection studies.

Figure 1 shows the inactivation rates for the Borg agent in serum-free BME medium with 0.05% formalin, pH 7.0 to 7.5, and at three different temperatures. All infectivity assays were carried out in embryonated eggs.

In the absence of 0.05% formalin, the agent remained fairly stable at 4 C and 10 C over a 5- to 6-day period, at which time the assays were discontinued. At 20 C, there was a marked decrease in the stability of the agent after 12 hours. Temperatures above 20 C have not been investigated. At 4 C, and in the presence of 0.05% formalin, inactivation proceeded slowly. Assays on day 5 still showed the presence of the agent; however, on day 15, the fluids were no longer lethal for eggs. Inactivation

proceeded more rapidly at 10 C and was almost complete by 66 hours. At that time, 1 of 10 eggs died when undiluted material was used. All eggs survived in an assay conducted on day 6.

The majority of the subsequent preparations were inactivated at 20 C. Although inactivation (as measured in eggs) was complete in less than 24 hours, all preparations used for immunogenicity studies were held at this temperature an additional 2 to 3 days and then stored at 4 C for at least a week before being injected into mice. Vaccines prepared at 4 C were held at least 30 days at this temperature before being used for immunogenicity studies in mice.

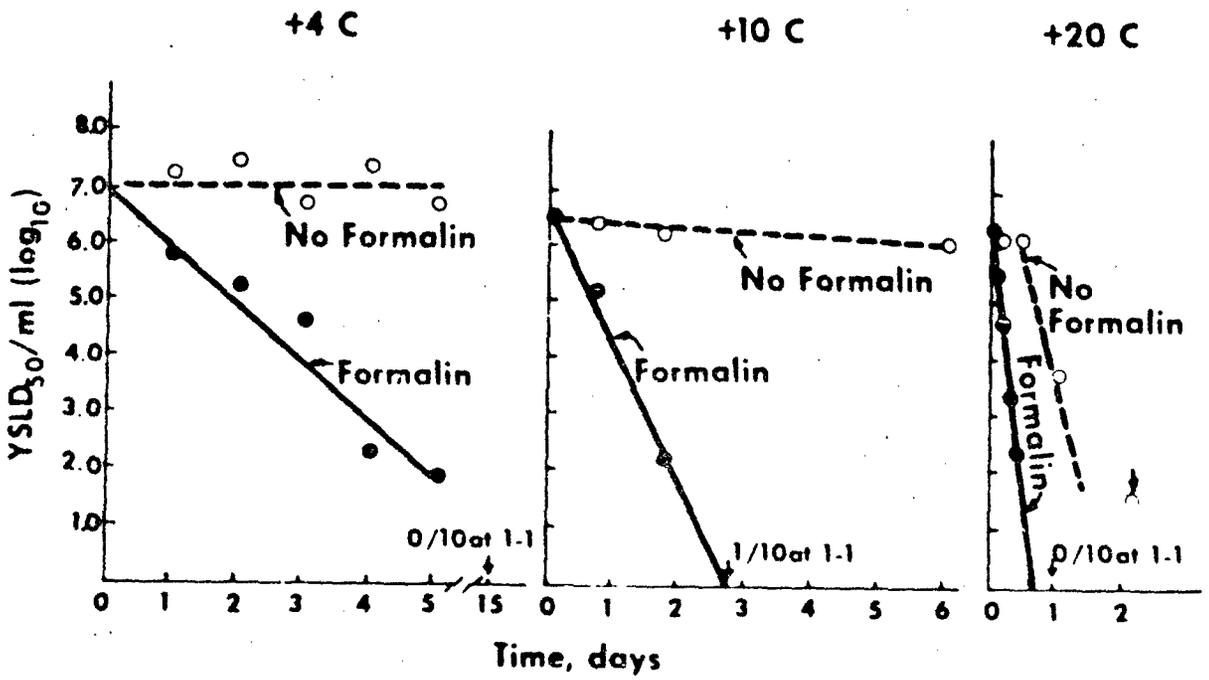

Figure 1. Inactivation of Borg Agent in Serum Free MEM with 0.05% Formalin and at pH 7.0 to 7.5.

Additional infectivity assays were carried out on a few of the preparations in suckling and in 10- to 14-g mice via the intracerebral route; none of the inoculated animals died. Also, two subsequent passages of egg yolk sacs or mouse brain suspensions in eggs or mice did not indicate the presence of virulent agent.

Table 1 shows the results obtained with three different vaccine preparations of formalin-inactivated Borg agent. These three preparations contained 0.5% bovine serum albumin, and were inactivated with 0.05% formalin. Vaccine 7 was inactivated at 20 C and vaccines 8 and 9 were inactivated at 4 C. With vaccine 7, a group of mice received one ip injection, and the mice were challenged via the same route 6 weeks later. A second group of mice received 3 ip injections at weekly intervals and was challenged 21 days after the last injection. The challenge material consisted of an infected yolk sac suspension diluted in brain heart infusion broth. With a challenge dose of 4×10^4 LD_{50} (or 4×10^6 LD_{50} for vaccines 8 and 9), 80 to 100% of the challenged animals survived. The scattered deaths, which occurred frequently whenever a single injection schedule was followed, were not observed when three injections of the vaccine were used. Since inactivation occurred more rapidly at 20 C, and the resulting vaccine appeared to provide adequate immunity, this temperature was selected for the remaining studies.

TABLE 1. EFFECTIVENESS OF BORG VACCINES IN IMMUNIZING MICE AGAINST HOMOLOGOUS INTRAPERITONEAL CHALLENGE

Vaccine No.	Temperature of Inactivation, C	No. of Injections	Challenge Dose, $IPLD_{50}$ Injected				
			4×10^5	4×10^4	4×10^3	4×10^2	$4 \times$
			Survival, per cent				
7	20	1	Not done	100	80	60	10
		3	Not done	100	100	100	10
8	4	1	80	90	90	100	10
9	4	1	100	90	90	90	10

Table 2 shows the effectiveness of three serum-free vaccines in immunizing mice against ip challenge. These results indicated that 90 to 100% of the immunized mice survived a challenge dose of 2×10^6 LD_{50}, and 40 to 88% of the immune groups survived 2×10^8 LD_{50}. A more quantitative difference in the effectiveness of the vaccines as immunizing antigens was demonstrated with a challenge dose of 2×10^6 $MIPLD_{50}$/ml. With vaccine 21, changes in the immunization schedule and increasing the number of injections provided only a slight increase in the antigenic response.

TABLE 2. EFFECTIVENESS OF BORG VACCINES IN IMMUNIZING MICE AGAINST HOMOLOGOUS INTRAPERITONEAL CHALLENGE[a]

Vaccine No.	No. of Injections	Challenge Dose, $IPLD_{50}$ Injected: 2×10^8 Survival, per cent	Challenge Dose, $IPLD_{50}$ Injected: 2×10^6 Survival, per cent
18	4	88	100
19	3	67	90
21	3	40	90
	4	50	100

a. Immunization and challenge schedule (0.5 ml ip)
0, 4, 8, 22 days - challenge at 5 weeks
0, 7, 14 days - challenge at 4 weeks

Table 3 shows the response of mice given three ip injections of undiluted and diluted vaccines to ip challenge with homologous agent. The challenge doses ranged from 2×10^3 to 2×10^8 $MIPLD_{50}$. These results indicated that vaccine 19 could be diluted considerably and still elicit demonstrable protection against an ip challenge.

TABLE 3. EFFECTIVENESS OF DILUTED BORG VACCINE (19) IN IMMUNIZING MICE AGAINST HOMOLOGOUS INTRAPERITONEAL CHALLENGE[a]

Vaccine Dilution	Challenge Dose, $IPLD_{50}$ Injected			
	2×10^3	2×10^4	2×10^5	2×10^6
	Survival, per cent			
Undiluted	Not done	Not done	95	53
1:3	100	100	67	33
1:10	100	60	53	13
1:27	13	27	27	7
1:81	10	0	0	10
nonimmunized	0	5	0	0

a. Immunization and challenge schedule (0.5 ml ip) 0, 7, 14 days challenge at 4 weeks

Preliminary results on cross-protection with formalin-inactivated Borg and 6BC agents are shown in Table 4. Mice were given three intraperitoneal injections of the designated vaccine at weekly intervals. Mice receiving Borg vaccine were challenged intraperitoneally 10 weeks after the last injection; mice receiving 6BC vaccine were challenged 3 weeks following the last injection. The $IPLD_{50}$/ml calculated from the challenge suspensions for the immunized and control groups of mice are shown in the right column. The reduction of the LD_{50} in the immune group was used as the index of effectiveness. When mice received Borg vaccine, approximately 2 logs of protection were obtained when challenged with the 6BC agent, and greater than 5 logs of protection were obtained with the homologous agent. When mice received 6BC vaccine, approximately 2.5 logs of protection were obtained with both the heterologous and homologous agents.

TABLE 4. RESULTS IN MICE OF CROSS-IMMUNITY TESTS BETWEEN THE BORG AND 6BC AGENTS

Vaccine	Immunizing Injections	Challenge Agent	Calculated $MIPLD_{50}/ml$ (log_{10})
None	0	6BC	3.8
Borg	3	6BC	1.9
None	0	Borg	6.4
Borg	3	Borg	<0.7
None	0	Borg	6.6
6BC	3	Borg	3.8
None	0	6BC	4.2
6BC	3	6BC	1.6

In summary, these studies clearly demonstrated that supernatant fluids from cultures of the Borg agent that was grown in human diploid lung cells and inactivated in the presence of formalin were capable of eliciting an immune response in mice. Animals treated with this preparation were capable of resisting significant levels of homologous agent and the heterologous 6BC agent.

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