Cross Immunity Among Strains of *Chlamydia psittaci* (35176)

JOHN R. MITZEL,[1] GEORGE G. WRIGHT, AND NORMAN S. SWACK[2]
Medical Sciences Laboratory, Fort Detrick, Frederick, Maryland 21701

Previous reports have described growth of chlamydia in human diploid cell strain WI-38 and the preparation of vaccines by treatment of clarified tissue culture fluids with formaldehyde (1, 2). These preparations protected mice so that they survived appreciable levels of intraperitoneal challenge and lower but significant levels of respiratory challenge (2). Vaccines killed by exposure to ionizing radiation were more antigenic for mice than the formaldehyde-inactivated preparations (3). Exploratory experiments indicated that vaccines derived from Borg and 6BC strains gave only partial cross protection (2).

Previous observations on cross protection among chlamydia, involving immunization of mice with sublethal doses of virulent strains and challenge by the intracerebral route, revealed different degrees of relationship among agents associated with disease in various hosts (4–6). Interpretation of the results is complicated by the possibility that differences in infectivity of the immunizing agents influenced the level of immunity. Interference effects associated with persistant infection may also have contributed to the resistance observed. These studies provide little basis for inferences regarding the degree of cross protection obtainable with nonviable vaccines; experiments bearing on this question are presented below.

Materials and Methods. Tissue culture. Cell cultures used for the propagation of strains of *Chlamydia psittaci* for preparation of vaccines were 4- to 6-day-old monolayers of the WI-38 strain of human embryonic lung (7).

Chlamydia strains. Strains isolated from various host species were obtained from Dr. L. A. Page, Dr. Paul Arnstein, and the Virus and Rickettsia Division, Fort Detrick. Working seeds were prepared as 50% chicken yolk-sac suspensions and stored at —70°.

Vaccine preparation. Methods for growth of *C. psittaci* strains and for preparation of formalin-inactivated vaccines have been described (1, 2). Inactivation was carried out at pH 7.0 in the presence of 0.02% formaldehyde. The vaccines were held at 20° for 4 days and then stored at 4° for a minimum of 10 days before injection into mice.

Immunization and challenge of mice. Groups of Bagg strain Swiss-Webster mice from the Fort Detrick Colony were immunized at weekly intervals with three intraperitoneal 0.5-ml injections of undiluted vaccine. Three weeks after the last injection, the mice were divided into groups of 10 animals and challenged intraperitoneally with serial tenfold dilutions of the working seeds of the various strains. Groups of 10 normal mice were challenged at the same time. Deaths were recorded for 14 days and the LD_{50} was calculated (10). The differences between the $\log_{10}$ LD_{50}/ml of each challenge suspensions in normal and in immunized mice are reported as the protective index.

Results and Discussion. Characterization of chlamydia strains. A summary of the origin of the strains and the pathogenicity of the working seeds for embryonated chicken eggs, mice, and guinea pigs are shown in Table I.

The study included three strains isolated from human pneumonitis: Borg, SF, and MN. Of these, Borg was highly pathogenic for embryonated eggs, mice, and guinea pigs. SF and MN killed mice at low dilutions, and SF also killed guinea pigs. The three strains of turkey ornithosis, NJ-1, VT-1, and

[1] Present address: Naval Medical Field Research Laboratory, Camp Lejeune, Jacksonville, North Carolina 28542.

[2] Present address: Virology Laboratory, Veterans Administration Hospital, West Haven, Connecticut 06516.

TABLE I. Characteristics of Working Seeds of Chlamydial Strains.

Strain	Source	Log_{10} mouse $IPLD_{50}$/ml	Lethality for guinea pigs[a]	Log_{10} yolk sac LD_{50}/ml	
				Untreated eggs	Sulfadiazine-treated eggs[b]
Borg	Human pneumonitis	8.6	++	9.1	9.2
SF	Human pneumonitis (San Francisco)	1.1	+	7.6	8.2
MN (Cal-10)	Human meningopneumonitis	1.8	—	9.4	9.4
NJ-1	Turkey ornithosis (New Jersey)	8.8	++	8.5	8.4
VT-1	Turkey ornithosis (Virginia)	8.5	++	8.7	8.6
Havlik	Turkey ornithosis (Oregon)	8.8	++	8.4	8.0
6BC	Parakeet ornithosis	7.4	+	9.5	<6.0
CP-3	Pigeon ornithosis (California)	2.1	—	7.6	7.7
WC	Bovine enteritis	8.3	—	8.5	8.6
FP	Feline pneumonitis	<1.0	—	8.9	9.3
MoPn	Mouse pneumonitis		—	7.3	3.6

[a] For each strain 3 animals were injected ip with 1 ml of undiluted working seed, 3 animals with a 1:10 dilution. Death of 4 or more animals is recorded as ++, of <4 as +, of none as —. Survivors were held 21 days.

[b] Eggs received 1 mg of sodium sulfadiazine.

Havlik were all pathogenic for eggs, mice, and guinea pigs. Of the remaining strains, 6BC, CP-3, and WC were pathogenic for eggs and mice but not for guinea pigs. FP was pathogenic for eggs but not for mice or guinea pigs.

To confirm the classification of the strains as *C. psittaci*, they were tested for sensitivity to sodium sulfadiazine and for production of glycogen in tissue culture as proposed by Page (8, 9). Mouse pneumonitis (MoPn), a strain of *Chlamydia trachomatis*, was included as a positive control and showed the expected sensitivity to sulfadiazine and production of glycogen. All test strains were insensitive to sulfadiazine with the exception of 6BC (Table I). The anomalous sensitivity to sulfadiazine of this strain had been noted previously (9). None of the test strains produced glycogen in L cells, although CPE was present in all cases.

Cross protection studies. Groups of mice were immunized with vaccines derived from each of the various strains and their resistance to challenge with each of the virulent strains was determined (Table II). The homologous protection ranged from the high level given by the Borg and NJ-1 vaccines to the low level given by CP-3, MN, and FP vaccines. Poor antigenicity of the latter probably results from the low infectivity titers of the vaccines prior to inactivation. Evidently the procedures developed for the Borg strain are applicable to many strains of *C. psittaci*. The relatively low infectivity of strains CP-3, MN, and FP for normal mice by the intraperitoneal route limited the attainable protective index.

Although some degree of cross protection was observed among most of the strains, the strains appear to fall into two groups. The first group contained Borg, NJ, VT-1, and Havlik strains. The vaccines derived from these strains all gave high levels of protection to other members of the group, except that Havlik was difficult to protect against with any vaccine. These vaccines gave lower levels of protection to the second group, which contained the WC, 6BC, SF, and CP-3 strains. Vaccines derived from this group gave their highest protective levels to members of the second group and irregular low level protection to the first group. The low protective activity of the Mn and FP vaccines made it difficult to assign these strains to either group. It would appear that no one strain of *C. psittaci* will yield a vaccine of optimum effectiveness against all strains, and

TABLE II. Protection of Mice by Vaccines Prepared from 10 Strains of *C. psittaci.*

Vaccine strain	Infectivity[a] before inactivation	Challenge strain; protective index							
		Borg	NJ-1	VT-1	Havlik	WC	6BC	CP-3	MN
Borg	7.2	6.0	6.1	5.7	2.1	1.7	2.3	0.6	0.6
NJ-1	7.1	3.5	4.8	5.1	0.0	3.3	3.6	0.0	1.1
VT-1	6.7	4.0	3.7	6.1	0.0	2.4	3.3	0.0	0.0
Havlik	6.2	3.2	4.3	5.6	0.9	3.0	2.7	0.3	0.6
WC	7.1	2.9	2.2	1.4	0.0	6.4	4.4	0.3	0.4
6BC	6.8	0.9	0.0	2.1	0.0	1.4	2.9	1.0	0.0
SF	7.2	0.8	0.0	1.3	0.0	1.6	3.2	0.6	1.5
CP-3	5.0	0.0	0.0	0.0	0.0	0.6	1.4	0.1	1.5
MN	6.0	1.2	0.0	0.0	0.7	0.9	1.1	0.0	0.0
FP	5.1	0.0	0.0	0.0	0.0	1.4	0.0	0.0	0.0

[a] Log_{10} yolk sac LD_{50}/ml before treatment with formaldehyde.

that at least a bivalent preparation will be necessary to provide a broad spectrum of protection.

Summary. The degree of cross immunity was investigated among 10 strains of *Chlamydia psittaci* isolated from various mammalian and avian hosts. Mice were immunized intraperitoneally with nonviable tissue-culture vaccines prepared from each strain and tested for their resistance to homologous and heterologous challenge. Although most strains provided some degree of cross protection, the strains appeared to fall into two antigenic groups. At least a bivalent vaccine will apparently be required to give protection against a broad spectrum of strains.

1. Pearson, J. W., Duff, J. T., Gearinger, N. F., and Robbins, M. L., J. Infec. Dis. **115**, 49 (1965).

2. Swack, N. S., and Duff, J. T., J. Infec. Dis. **118**, 468 (1968).

3. Wright, G. G., Swack, N. S., and Mitzel, J. R., Proc. Soc. Exp. Biol. Med. **131**, 858 (1969).

4. Eaton, M. D., Martin, W. P., and Beck, M. D., J. Exp. Med. **75**, 21 (1942)

5. Beck, M. D., Eaton, M. D., and O'Donnell, R., J. Exp. Med. **79**, 65 (1944).

6. Wagner, J. C., Golub, O. J., and Andrew, V. W., J. Infec. Dis. **84**, 41 (1949).

7. Hayflick, L., Amer. Rev. Resp. Dis. **88**, 387 (1963).

8. Page, L. A., Int. J. Syst. Bacteriol. **16**, 223 (1966).

9. Page, L. A., Int. J. Syst. Bacteriol. **18**, 51 (1968).

10. Reed, L. J., and Muench, H., Amer. J. Hyg. **27**, 493 (1938).

Received July 9, 1970. P.S.E.B.M., 1970, Vol. 135.