THE ANTIGENIC RELATIONSHIP OF THE VIRUSES OF MENINGOPNEUMONITIS AND LYMPHOGRANULOMA VENEREUM*

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A previous paper from this laboratory (1) described the isolation of a virus from cases of atypical pneumonia. This agent was found to be antigenically related both to the virus of meningopneumonitis originally described by Francis and Magill (2) and to the virus of psittacosis, but in other properties significant differences from the virus of psittacosis were noted. The pneumonitis virus was similar to the virus of lymphogranuloma venereum in that it formed aggregates of elementary bodies in infected cells and produced meningitis and pneumonia in mice (3), but failed to kill by the intraperitoneal route. The related meningopneumonitis strain produced glandular enlargement and other lesions in guinea pigs not unlike those produced by the virus of lymphogranuloma venereum (4). Francis and Magill (2) have noted the similarity of meningopneumonitis virus to the virus of lymphogranuloma venereum but they were unable to demonstrate any immunologic relationship of the two viruses by intracerebral cross-infection of recovered mice.

Recent studies from this and other laboratories (5) have shown that sera from human cases of psittacosis, lymphogranuloma venereum, and some cases of pneumonitis give definite cross-reactions when tested by complement fixation with antigens from the respective causative viruses. These studies also revealed that some cases of atypical pneumonia show a slightly to moderately positive dermal reaction with Frei antigen.

On the basis of active immunity tests and complement fixation studies on experimental animals, this paper will present evidence that the virus of lymphogranuloma venereum is related to other viruses which produce meningitis and pneumonia. Although previously thought to be distinct, these viruses appear to be related and as a group may represent variants descended from the same parent strain which have become adapted to various tissues and species.

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Materials and Methods

Strains of Virus.—The virus isolated from four cases of atypical pneumonia (1) and designated strain S-F was used in suspensions of mouse brain or lung for immunization and tests for immunity. The M.L.D. for mice intranasally was 0.05 cc. of a dilution of 10^{-2} or 10^{-3}, intracerebrally 0.03 cc. of a dilution of 10^{-5}.

Two strains of the virus of meningopneumonitis originally isolated by Francis and Magill (2) were used in the present studies. One strain MP-F97, isolated in 1934 and recently sent us by Dr. T. Francis, Jr., was shown to be antigenically related to the strain S-F (1). The other strain, MP-Cal 10 isolated in 1936, was supplied to us through the courtesy of Dr. T. B. Turner of the Johns Hopkins School of Hygiene and Public Health (13). The strain MP-F97 was used for immunization of animals in the form of suspensions of mouse lung or mouse brain. The strain MP-Cal 10 after several ferret passages and 16 intracerebral mouse passages was found to grow readily in developing eggs by inoculation into the allantoic cavity according to the method of Nigg, Crowley, and Wilson (6), and this strain also infected chick embryos by inoculation on the chorioallantoic membrane, into the yolk sac, or into the amniotic sac (7). For most of the immunity tests with the strain MP-Cal 10, the allantoic fluid of infected chick embryos was used. The M.L.D. of this material for mice was 0.05 cc. of a dilution 10^{-5} intranasally and 0.03 cc. of 10^{-7} intracerebrally.

The "LC" strain of the virus of lymphogranuloma venereum (LGV-LC) was sent us by Dr. Turner (13). It was passed in mice by intracerebral and intranasal inoculation, and in developing eggs by inoculation into the yolk sac (8). Unlike the virus of meningopneumonitis, it failed to multiply in the allantoic or amniotic sacs of developing eggs, and material obtained after inoculating the chorioallantoic membrane was barely infectious for mice. Mouse brain, mouse lung, and yolk sac infected with the strain LGV-LC were used in cross-immunity tests. The M.L.D. of these materials rarely exceeded 10^{-2} intranasally or intracerebrally. For comparison, a strain of the virus of lymphogranuloma venereum isolated in New Haven by Dr. Marion Howard was used for immunization of mice. This strain will be designated LGV-ST.[1] In its general properties it was similar to the strain LGV-LC.

A few experiments were done with two strains of psittacosis virus recently isolated from parakeets. These strains were used for immunity tests as suspensions of infected mouse liver and spleen. A strain of the virus of lymphocytic choriomeningitis isolated from monkeys (15) and strain 17D of yellow fever virus were used in certain immunity experiments.

Methods of Immunizing Mice and Other Animals.—Animals were immunized by intracerebral, intranasal, or intraperitoneal injection of active virus in all experiments. Most of the work was done with mice of the Swiss strain bred locally. White rats, kangaroo rats (*Dipodomys deserti deserti*), and Syrian hamsters (*Cricetus auratus*) were found to be susceptible to infection with the viruses of meningopneumonitis and

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[1] We are indebted to Dr. Marion Howard of the Yale University School of Medicine and Dr. Geoffrey Rake of The Squibb Institute for Medical Research for sending two different lines of this strain.

lymphogranuloma venereum by intracerebral or intranasal inoculation, and these animals were also used in cross-immunity tests.

Mice, rats, and hamsters were immunized by the intracerebral and intranasal routes by giving initial doses containing approximately 1/10 to 1/100 M.L.D. This was followed 2 to 3 weeks later with a dose of 1/10 to 1 M.L.D. and usually at a similar interval by a third dose containing 1 to 10 M.L.D. In some experiments the animals received a fourth injection similar to the third. The strains S-F, LGV-LC, and LGV-ST were injected intraperitoneally into mice as 10 per cent suspensions of mouse brain or lung and the strains MP-F97 and MP-Cal 10 as 1 per cent suspensions of mouse tissue or 10 per cent allantoic fluid. Part of the mice receiving the strains MP-Cal 10, MP-F97, or S-F intraperitoneally were reinoculated 2 weeks later by the intracerebral route with 1 M.L.D. of the same strains.

Mice and hamsters being immunized with the meningopneumonitis strains were kept in a room apart from animals receiving the virus of lymphogranuloma venereum. Animals inoculated with psittacosis virus and the pneumonitis virus strain S-F were kept in strict isolation in a laboratory unit separate from that in which work was being done with the other strains.

Controls.—Normal mice of the same age as those being immunized were kept in boxes with wire mesh tops in the rooms with animals receiving the viruses of meningopneumonitis and lymphogranuloma venereum. In no case were these control mice found to be immune.

Tests for Homologous and Heterologous Immunity.—Tests for immunity were done by injection of approximately 10 M.L.D. of virus of known titer diluted from a suspension which had been previously tested and stored at −70°C. in a solid CO_2 ice box. The route of injection was the same as that of the last immunizing inoculation unless otherwise noted.

In accordance with previous observations the production of solid immunity even against the homologous strain of virus in animals inoculated by the intranasal or intracerebral routes was found to be rather uncertain (1). For this reason homologous immunity was demonstrated in each lot of animals by inoculating some of them with a lethal dose of the homologous strain either before or concurrently with the tests for heterologous immunity.

Complement Fixation.—Antigens for complement fixation were prepared from the allantoic fluid of 10 day chick embryos inoculated with the strain MP-Cal 10 into the allantoic sac, and incubated another 5 days. In general, these antigens were found to be superior to the mouse lung antigens used in earlier work.

Antigen from the lymphogranuloma venereum virus was prepared according to the method of Rake, McKee, and Shaffer (8) by growing the virus in the yolk sac. Antigens were used at dilutions of 1:4 to 1:8 of allantoic fluid or 1:40 to 1:80 of yolk sac. Normal control antigens were similarly prepared from the allantoic fluid and yolk sacs of uninoculated embryos. For the production of experimental immune sera, guinea pigs, rabbits, and mice were immunized by intraperitoneal or intranasal inoculation with active virus of the strains MP-F97, MP-Cal 10, and S-F. Mice and white rats were similarly immunized with the virus of lymphogranuloma venereum. To avoid the possibility of producing heterophile antibodies, all animals with the exception of the rabbit were immunized with infected tissues from the corresponding

species. The method of performing the complement fixation test was the same as that described for influenza (9).

Cross-Immunity Tests in Mice

Intracerebral Tests.—Mice immunized by intracerebral or intraperitoneal inoculation or a combination of the two were tested for active immunity 2 to 3 weeks after the last immunizing inoculation by intracerebral injection of the virus of meningopneumonitis strain Cal 10 or the virus of lymphogranuloma

TABLE I

Results of Intracerebral Tests for Active Immunity in Mice

Immunized with* strain	Route*	Intracerebral test with strain‡		
		MP-Cal 10		LGV-LC
		Deaths	Paralysis in survivors	Deaths
MP-Cal 10	I.P., I.C.	0/17	0/17	4/24
MP-F97	I.P., I.C.	1/11	0/10	2/10
S-F	I.P., I.C.	3/14	5/11	1/9
Psittacosis	I.P., I.C.	2/6§	2/4§	8/10
LGV-LC (M.L.)	I.C.	5/30	16/25	2/14
LGV-LC (Y.S.)	I.C.	7/19	8/12	0/17
LGV-ST (Y.S.)	I.C.	5/14	5/9	2/15
MP-Cal 10	I.P.	2/20	1/18	7/11
MP-F97	I.P.	0/7§	1/6§	3/5
LGV-LC (M.L.)	I.P.	4/5	1/1	7/7
Nil (controls)	—	58/58	—	39/44

* M.L., mouse lung; Y.S., yolk sac; I.P., intraperitoneal; I.C., intracerebral.

‡ In 3rd and 5th columns numerator is number of mice dead; denominator is number of mice tested. In 4th column numerator is number of mice showing definite paralysis of hind legs; denominator is number of mice surviving.

§ Tests done with strain MP-F97 instead of MP-Cal 10. All controls died.

venereum strain LC. The strain MP-Cal 10 was used for the tests at a dilution of 10^{-5} or 10^{-6} of allantoic fluid from infected chick embryos. Normal control mice receiving this amount of virus regularly died 6 or 7 days after inoculation. The strain LGV-LC was used as a 10 per cent suspension of infected mouse lung or yolk sac which killed the control mice in 2 to 4 days. Mouse brain preparations of this strain failed to kill all of the controls. The virus suspensions used in the tests were demonstrated to be free of bacterial contamination by the usual culture methods.

The results of 16 separate experiments are summarized in Table I. As shown in the 3rd and 4th columns of the table, mice immunized by intraperitoneal and intracerebral inoculation of the strains MP-Cal 10 and MP-F97 were solidly immune to intracerebral inoculation with the homologous

virus (MP-Cal 10) and survived without paralysis. About 70 to 85 per cent of mice immunized by intracerebral injection of psittacosis virus or the strains S-F, LGV-LC, or LGV-ST survived inoculation with the meningopneumonitis virus, but well over half of the survivors became paralyzed in the hind legs. Two strains of the virus of lymphogranuloma venereum propagated in mouse lungs or in yolk sac produced about an equal degree of immunity. In the group of mice immunized with strains MP-Cal 10, MP-F97, or S-F and tested by intracerebral inoculation with the virus of lymphogranuloma venereum, the immunity produced by the meningopneumonitis virus was almost as definite as that produced by the homologous strain. Mice immunized by intraperitoneal and intracerebral injection of psittacosis virus failed to survive intracerebral inoculation with the virus of lymphogranuloma venereum.

Mice receiving intraperitoneal inoculations of the strains MP-F97 and MP-Cal 10 were resistant to intracerebral inoculation with the meningopneumonitis virus, but did not show definite immunity to the virus of lymphogranuloma venereum injected intracerebrally. Mice receiving the latter virus intraperitoneally possessed no demonstrable immunity to intracerebral infection with either the homologous or heterologous viruses.

As shown in the last line of Table I, all of the control mice inoculated with the strain MP-Cal 10 died, and all but 5 of the controls receiving LGV-LC died. The results of 2 experiments with this virus, in which less than 80 per cent of the control mice died, have been omitted from the summary given in Table I.

Mice immune to intracerebral inoculation with the viruses of lymphogranuloma venereum and meningopneumonitis were not resistant to infection by the same route with the virus of yellow fever or lymphocytic choriomeningitis, and mice immunized with the latter agent were not immune to the lymphogranuloma virus. Two groups of mice inoculated with normal mouse brain and sterile broth had no immunity to intracerebral infection with MP-Cal 10 and mice of a third group similarly inoculated with a chick embryo tissue culture of the virus of influenza B were not resistant to LGV-LC.

Intranasal Tests.—Mice were immunized by multiple intranasal inoculations with increasing amounts of virus of the strains MP-Cal 10, MP-F97, LGV-LC, and LGV-ST. Tests for active immunity were done 2 to 3 weeks after the last immunizing inoculation.

From the results presented in Table II it is apparent that the strain MP-Cal 10 produces in the respiratory tract of mice much better immunity to itself than it does to the virus of lymphogranuloma venereum. By intranasal inoculation, the strains LGV-LC and LGV-ST produced a more solid immunity to the homologous virus than to the strain MP-Cal 10. The results suggest a partial cross-immunity in mice immunized and tested by the intranasal route since about half the test animals survived while nearly all of the controls died. However, this cross-immunity was not as definite as that observed in the in-

tracerebral tests. Mice immunized by intraperitoneal and intranasal inoculation with the viruses of influenza A or B did not survive intranasal inoculation with the virus of lymphogranuloma venereum or the virus of meningopneumonitis.

Intraperitoneal Tests.—Mice receiving a single intraperitoneal inoculation of the strain LGV-LC were not resistant to intraperitoneal inoculation of a 10 per cent mouse lung suspension of the strain MP-F97. One other similar experiment in which 3 immunizing injections of the strain LGV-LC were given was inconclusive because only 1 of the controls died.

In 2 experiments 14 out of 17 mice immunized by 3 intraperitoneal injections with the virus of lymphogranuloma venereum survived infection with a dose of psittacosis virus which killed all but one of 7 controls. In a third experiment a larger test dose of psittacosis virus (approximately 1,000 M.L.D.) was used and all

TABLE II

Results of Intranasal Tests for Active Immunity in Mice

Immunized with	Intranasal tests with strain			
	MP-Cal 10		LGV-LC	
	Deaths	Lesions in survivors	Deaths	Lesions in survivors
MP-Cal 10	1/20	*	8/18	7/10
MP-F97	—	—	4/18	8/14
LGV-LC	3/7	2/4	5/19	*
LGV-ST	8/12	4/4	0/7	0/7
Nil (controls)	26/27	1/1	27/27	—

* Surviving mice were not autopsied because they were used in other tests.

but 1 of 22 mice immunized with the strain LGV-LC died while mice immunized with the strain MP-Cal 10 survived. A fourth experiment was inconclusive because less than half of the controls died. As previously reported (1) the strains S-F and MP-F97 after intraperitoneal inoculation produced sufficient immunity to protect most mice against death from 1,000 M.L.D. of psittacosis virus.

Immunity Tests in Other Animals

The strain MP-Cal 10 when inoculated intracerebrally or intranasally as an undiluted allantoic fluid culture regularly produced fatal infection in white rats, kangaroo rats (*Dipodomys deserti deserti*), and Syrian hamsters (*Cricetus auratus*). In hamsters and rats inoculated intracerebrally, striking neurological symptoms were seen. Animals with fatal infections appeared only slightly ill 24 hours after inoculation, but later became comatose, often with marked tremors in the extremities, and died within 48 or 72 hours. A sticky white discharge appeared around the eyes. Animals with less severe infections

were lethargic or even comatose for a few days and later became paralyzed in the hind legs and lumbar region. In such animals extreme emaciation was the rule, but recovery occurred in about half of them after 2 to 3 weeks. Convulsive seizures were noted in some cases. The virus of lymphogranuloma venereum failed to kill hamsters regularly after intracerebral inoculation and symptoms of involvement of the central nervous system were much less pronounced than with the strain MP-Cal 10. Consequently challenge tests for immunity in rats and hamsters were done only with the strain MP-Cal 10.

TABLE III

Intracerebral Immunity Tests with Meningopneumonitis Virus in White Rats and Hamsters

Experiment No.	Animal No.	Virus	Immunization procedure			Results of intracerebral test with strain MP-Cal 10
			Material	Inoculated	Route	
1	White rat 4	LGV-LC	R.L., M.B.	10 per cent 2×	I.C.	Survived, no symptoms
	White rat 1 a	MP-Cal 10	A.F.	1:1 1×	I.N.	Survived, no symptoms
	White rat 2 a	MP-Cal 10	A.F.	1:1 1×	I.N.	Comatose, recovered
	Hamster 11	LGV-LC	H.B., M.B.	10 per cent 2×	I.C.	Partial paralysis, recovered
	Hamster 12	LGV-LC	H.B.	10 per cent 1×	I.C.	Partial paralysis, recovered
	White rat 5	nil	(Control)			Died 2 days, brain congested
	White rat 6	nil	(Control)			Died 3 days, brain congested
	Hamster 13	nil	(Control)			Died 4 days, brain congested
2	Hamster 11 a	MP-Cal 10	A.F.	1:1 1×	I.C.	Paralysis, recovered
	Hamster 12 a	MP-Cal 10	A.F.	1:1 1×	I.C.	Paralysis, recovered
	Hamster 14	LGV-LC	H.B., M.L.	10 per cent 3×	I.C.	Severe paralysis, died 8 days
	Hamster 15	LGV-LC	H.B., M.L.	10 per cent 3×	I.C.	Severe paralysis, died 7 days
	Hamster 16	LGV-LC	H.B., M.L.	10 per cent 3×	I.C.	Severe paralysis, died 10 days
	Hamster 17	LGV-LC	H.B., M.L.	10 per cent 3×	I.C.	Paralysis, recovered
	Hamster 18	nil	(Control)			Died 2 days
	Hamster 19	nil	(Control)			Died 2 days

Explanation of symbols: R.L., rat lung; M.B., mouse brain; H.B., hamster brain; A.F., allantoic fluid; I. N., intranasal. Others as in Table I.

One, two, or three successive inoculations at intervals of 2 to 4 weeks indicated by 1×, 2×, or 3× in 4th column.

Experiment 1.—White rat 4 and 2 hamsters, Nos. 11 and 12, were immunized by intracerebral injection of 10 per cent brain suspensions containing the strain LGV-LC and tested for immunity by intracerebral inoculation of 0.10 to 0.15 cc. of undiluted allantoic fluid culture of the strain MP-Cal 10. Hamsters 1 a and 2 a which had received both LGV-LC and MP-Cal 10 by the intranasal route (see Experiment 4) were also tested by intracerebral inoculation. The results are presented in Table III.

Experiment 2.—Hamsters were inoculated intracerebrally with the fourth hamster-brain passage of the strain LGV-LC followed 2 weeks later by the same strain in a 10 per cent mouse lung suspension which killed 2 normal hamsters. After 2 weeks another injection of the mouse lung preparation was given. Four immune hamsters, Nos. 14, 15, 16, and 17, were tested after an interval of 3 weeks by intracerebral injection of undiluted allantoic fluid containing the meningopneumonitis virus strain Cal 10. Hamsters 11 a and 12 a, which had been immunized with LGV-LC and tested

intracerebrally with MP-Cal 10, were retested at the same time by intracerebral inoculation. The results are presented in Table III.

Experiment 3.—In a third intracerebral experiment, not shown in Table III, a smaller test dose was used and consequently all the controls did not die. Six hamsters immunized by 2 or 3 intracerebral injections of the strain LGV-LC and 6 hamsters similarly immunized with the strain MP-Cal 10 were tested for immunity by intracerebral inoculation of 3 per cent allantoic fluid from chick embryos infected with the strain MP-Cal 10. One of the hamsters immunized with LGV-LC died in 2 days, 2

TABLE IV

Intranasal Immunity Test with Meningopneumonitis Virus in Animals Immunized with the Virus of Lymphogranuloma Venereum

Experiment No.	Animal No.	Immunization procedure			Result of intranasal test with strain MP-Cal 10
		Material	Inoculated	Route	
4	White rat 1	M.L.	10 per cent 3×	I.N.	Survived, no symptoms
	White rat 2	R.L.	10 per cent 1×	I.N.	Survived, no symptoms
	Kangaroo rat 1	M.L.	10 per cent 3×	I.N.	Survived, no symptoms
	Hamster 1	M.L.	10 per cent 3×	I.N.	Survived, no symptoms
	White rat 3	Nil	(Control)		Died 3 days lungs ++++
	Kangaroo rat 2	Nil	(Control)		Died 3 days lungs ++++
	Hamster 2	Nil	(Control)		Died 3 days lungs +++
5	Hamster 3	H.L., M.L.	10 per cent 3×	I.N.	Died 3 days lungs ++++
	Hamster 4	H.L., M.L.	10 per cent 3×	I.N.	Died 4 days lungs ++++
	Hamster 5	H.L., M.L.	10 per cent 3×	I.N.	Died 7 days lungs ++++
	Hamster 6	H.L., M.L.	10 per cent 3×	I.N.	Died 8 days lungs ++++
	Hamster 7	Nil	(Control)		Died 3 days lungs ++++
	Hamster 8	Nil	(Control)		Died 3 days lungs ++++
	Hamster 9	Nil	(Control)		Died 3 days lungs ++++
	Hamster 10	Nil	(Control)		Died 39 days lungs ++++*

Explanation of symbols: M.L., mouse lung; H.L., hamster lung; R.L., rat lung.
* Died, possibly with secondary infection or relapse.

others died in 9 and 12 days with paralysis, and 3 recovered after having been comatose and paralyzed for about 1 week. Three hamsters immunized with the homologous strain MP-Cal 10 developed severe paralysis and died, and 3 survived without symptoms. Three control hamsters died in 5, 9, and 12 days respectively and 1 survived.

Summary of Intracerebral Tests (Experiments 1, 2, and 3).—Of 13 animals immunized with the virus of lymphogranuloma venereum and tested with meningopneumonitis virus, 1 survived without symptoms, 6 developed paralysis of varying degree but recovered, 5 died later than 1 week after inoculation with severe paralysis and inanition, and 1 died in less than 1 week. Of 10 animals immunized with the homologous meningopneumonitis strain, 4 survived with-

out paralysis, 3 developed paralysis but recovered, 2 became paralyzed and died more than a week after inoculation, and 1 died in less than a week. Of 9 controls 1 survived, 2 died in more than a week, and 6 died in less than a week. The results indicate that hamsters and rats inoculated intracerebrally with the virus of lymphogranuloma are partially immune to the virus of meningopneumonitis given by the same route.

Experiment 4.—White rat 1, kangaroo rat 1, and hamster 1 were immunized by 3 intranasal inoculations with mouse lung preparations of increasing titer of the strain LGV-LC. White rat 2 received a single intranasal inoculation with a 10 per cent suspension of lung from the first white rat passage of the above strain. The animals were tested approximately 2 weeks after the last immunizing dose by intranasal inoculation of 0.2 cc. of undiluted allantoic fluid from chick embryos infected with meningopneumonitis virus. The results are presented in Table IV.

Experiment 5.—Hamsters 3, 4, 5, and 6 were inoculated intranasally with 10 per cent hamster lung suspension of strain LGV-LC. Subsequently, at intervals of 2 weeks these animals received 2 intranasal inoculations of mouse lung material containing the same strain. Eighteen days after the last inoculation the animals were tested by intranasal inoculation of 0.2 cc. of undiluted allantoic fluid containing the strain MP-Cal 10. In this experiment the immunized animals survived only slightly longer than the controls (Table IV).

Summary of Intranasal Tests (Experiments 3 and 4).—Of 8 animals immunized intranasally with the virus of lymphogranuloma venereum and tested with meningopneumonitis, 4 survived without symptoms, 2 survived over twice as long as the controls but eventually died, and 2 died in the same length of time as the controls. Six control animals died in 3 days and 1 survived for 39 days. The results of these experiments are inconclusive, but suggest an increased resistance to the strain MP-Cal 10 in animals inoculated intranasally with the strain LGV-LC.

Complement Fixation Tests with Immune Animal Sera

Table V presents the results of complement fixation tests with sera from animals immunized with the pneumonitis virus strain S-F, meningopneumonitis virus, and the lymphogranuloma venereum virus. The sera of mice and guinea pigs immunized with the strains S-F, MP-F97, and Cal 10 gave strong complement fixation with the MP-Cal 10 antigen, but relatively weak, or negative, reactions with the LGV-LC antigen. An exception to this was rabbit 33, immunized with the strain S-F in mouse lung, which developed complement-fixing antibodies to both viruses in about equal titer. This serum gave slight reactions with antigen prepared from normal yolk sac and allantoic fluid. One serum (mouse 99) was slightly anticomplementary.

Mice and rats immunized with the virus of lymphogranuloma venereum developed complement-fixing antibodies of a similar titer to the homologous

and heterologous viruses. The apparently lower titer with the LGV antigen of some sera from animals immune to this virus was probably due to the fact that in this series of tests this antigen was somewhat less sensitive than the MP-Cal 10 antigen.

TABLE V

Results of Complement Fixation Tests with Sera of Animals Immunized with the Viruses of Lymphogranuloma Venereum and Meningopneumonitis

Serum No.	Immune to	Antigen	Serum dilution				Normal tissue Control 1:4	Serum control 1:4
			1:4	1:8	1:16	1:32		
G.P. 81	S-F	MP-Cal 10	+++	+++	+	0	0	0
G.P. 81	S-F	LGV-LC	0	0	0	0	0	
G.P. 87	MP-F97	MP-Cal 10	++++	++++	++++	++	0	0
G.P. 87	MP-F97	LGV-LC	0	0	0	0	0	
G.P. 88	MP-F97	MP-Cal 10	++++	+++	++	0	0	0
G.P. 88	MP-F97	LGV-LC	0	0	0	0	0	
Rabbit 33	S-F	MP-Cal 10	++++	++++	++++	+++	±	0
Rabbit 33	S-F	LGV-LC	++++	++++	++++	+++	++	
Mouse 99	S-F	MP-Cal 10	++++	++++	++++	++++	++	++
Mouse 99	S-F	LGV-LC	+++	+	0	0	++	
Mouse 115	MP-F97, Cal 10	MP-Cal 10	++++	++++	++++	++++	0	0
Mouse 115	MP-F97, Cal 10	LGV-LC	+++	+	0	0	0	
Mouse 102	MP-F97, S-F	MP-Cal 10	++++	++++	++++	++++	0	0
Mouse 102	MP-F97, Cal 10	LGV-LC	++	0	0	0	0	
Mouse 116	LGV-LC	MP-Cal 10	++++	++++	++++	++++	0	0
Mouse 116	LGV-LC	LGV-LC	++++	++++	+++	++	0	
Mouse 117	LGV-LC	MP-Cal 10	++++	++++	+++	++	0	0
Mouse 117	LGV-LC	LGV-LC	++++	+++	++	0	0	
Rat 12	LGV-LC	MP-Cal 10	++++	++++	++++	++++	0	0
Rat 12	LGV-LC	LGV-LC	++++	++++	++++	++++	0	
Rat 16	LGV-LC	MP-Cal 10	+++	++	±	0	0	0
Rat 16	LGV-LC	LGV-LC	+	0	0	0	0	

Various degrees of complement fixation indicated by plus signs. 0, no fixation.

Sera from normal guinea pigs, rabbits, rats, and mice, and sera from mice immunized with mouse lung suspensions of the virus of influenza A gave no reaction with the LGV-LC and MP-Cal 10 antigens. The sera of guinea pigs immune to the strains S-F and MP-F97 contained no complement-fixing or neutralizing antibodies for the virus of influenza A or B.

The antigens used in these tests gave complement fixation with the sera from cases of pneumonitis proved to be caused by the strain S-F by isolation of the virus (1) and with sera from known cases of lymphogranuloma venereum. These results have been reported elsewhere (5). The antigens gave no significant fixation with normal human sera or with sera from cases of influenza or other acute upper respiratory disease.

DISCUSSION

The apparent antigenic relationship noted between the virus of meningopneumonitis and the virus of lymphogranuloma venereum was probably not due to accidental contamination of the strains of virus used because cross-immunity was obtained with 3 strains of meningopneumonitis virus from 2 sources and with 2 strains of the virus of lymphogranuloma venereum from different sources. In the present work factors such as chance cross-infection of animals, diet, and age which might produce increased resistance have been controlled.

Francis and Magill (2) did not describe in detail their negative experiments in mice on cross-immunity between these viruses. We are unable to account for the difference in our results unless the mice used by the above authors received smaller immunizing doses or fewer injections. It must be remembered, in this connection, that with the viruses under investigation a single sublethal dose often failed to produce demonstrable immunity even to the homologous strain.

The possibility that the cross-immunity observed in recovered animals was due to a non-specific local resistance or to the so called "interference phenomenon" should be considered. Armstrong (10) has reported that mice receiving intranasal inoculations with saprophytic bacteria possess a slight and transient resistance to intranasal infection with the viruses of St. Louis encephalitis and influenza. This was attributed to local accumulation of leucocytes. In our experiments injection of broth, normal mouse tissue, or tissue suspensions infected with viruses unrelated to the strains under investigation presumably would produce a similar accumulation of leucocytes, but no increased resistance was observed. Other authors have reported increased resistance to experimental poliomyelitis in monkeys inoculated with lymphocytic choriomeningitis (11) and to Rift Valley fever in mice inoculated with yellow fever virus (12). In all of these experiments the immunity produced was less striking and more transient than that observed in the present work. Also, in the present studies it appears that the cross-relationship was confined to a group of viruses in which other similarities were demonstrable and was not elicited by the unrelated agents of influenza, yellow fever, or lymphocytic choriomeningitis.

The experiments on active immunity in mice indicate quantitative antigenic differences between the strains studied. Thus the strain S-F from human cases of pneumonitis was apparently somewhat different from the strains LGV-LC, MP-Cal 10, and MP-F97 because only partial cross-protection was observed. A difference apparently similar in degree was found between the virus of lymphogranuloma venereum and the strain MP-Cal 10 (Tables I and II). The cross-immunity tests with the viruses of psittacosis and lymphogranuloma venereum were inconclusive, but an indirect antigenic relationship between

these two agents is indicated by the fact that meningopneumonitis virus immunized mice against both of them. Extensive studies on the relationship of psittacosis to other viruses were not attempted because similar investigations are being conducted in other laboratories.

Since the immune sera used in the complement fixation tests were produced by immunizing animals with infected tissues of the corresponding species, heterophile antibodies of normal tissues could not be concerned in the observed cross-reactions. The group specific complement-fixing antibodies in the sera of animals immunized with the virus of lymphogranuloma venereum probably resulted from an immune response either to an antigenic constituent of the virus particle or to an antigenic product of the interaction of virus and infected tissue. The corresponding group specific factor of the meningopneumonitis strains MP-Cal 10, MP-F97, and S-F apparently stimulated less antibody response because sera of mice and guinea pigs immunized with these strains, although showing a high titer with the meningopneumonitis virus, gave slight or no fixation with the virus of lymphogranuloma venereum (Table V). On the other hand, human sera from cases of pneumonitis often gave strong fixation with both antigens from lymphogranuloma venereum and meningopneumonitis (5). These differences in species response cannot be adequately explained on the basis of present observations.

Much additional work on differences in pathogenicity will be necessary before a definite classification of these viruses is possible, but a few basic criteria may be noted. Only one member of the group under consideration, psittacosis virus, is highly virulent for mice by the intraperitoneal route. The virus of meningopneumonitis kills mice only after intraperitoneal injection of massive doses. Strains of the agent of lymphogranuloma venereum were much less virulent by any route for mice than the other viruses. Differences in virulence for guinea pigs, hamsters, birds, and chick embryos have also been noted. Pinkerton (14) has observed that meningopneumonitis virus produces a fatal infection in pigeons after intracerebral inoculation while one strain of psittacosis virus does not. All of the strains studied except LGV-LC and LGV-ST produced paralysis of the hind legs of mice after an incubation period of 5 to 6 days following intracerebral injection and some caused paralysis irregularly after intraperitoneal inoculation.

The observations recorded in this paper suggest that the viruses causing certain forms of atypical pneumonia and lymphogranuloma venereum in human beings, psittacosis in birds, and other viruses which may have come from mice or ferrets should be classified together as representatives of a group of agents which in various modified forms are distributed widely in nature and cause infections of diverse character. The members of this group of viruses are characterized by the formation of elementary bodies which stain with basic aniline dyes, by the possession of antigenic components common to each, and by the

production of meningitis, pneumonia, and granulomatous infiltrations of the skin in experimental animals. Analogous groups of similar size are the *Rickettsiae*, and the pleuropneumonia-like organisms. It is also apparent that criteria previously used for classification of these agents, particularly with regard to the psittacosis group, have not been adequate.

SUMMARY

Animals recovered from infection with the viruses of lymphogranuloma venereum, meningopneumonitis, and psittacosis, were reinoculated in cross-immunity tests with these viruses.

In mice immunized by intracerebral or intranasal inoculation a reciprocal partial cross-immunity between the viruses of lymphogranuloma venereum and meningopneumonitis was demonstrated. In preliminary experiments, similar cross-immunity between the agents of lymphogranuloma venereum and psittacosis was not definitely demonstrated.

Hamsters, white rats, and kangaroo rats recovered from intracerebral or intranasal infection with the virus of lymphogranuloma venereum were more resistant than normal controls to inoculation with the virus of meningopneumonitis.

Sera of animals immunized with the viruses of lymphogranuloma venereum and meningopneumonitis showed cross-reactions by complement fixation with antigens of these viruses.

The results indicate an antigenic relationship between the viruses of lymphogranuloma venereum and meningopneumonitis.

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