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ANTIBODIES TO BRAIN AND OTHER TISSUES IN CASES OF *MYCOPLASMA PNEUMONIAE* INFECTION

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SUMMARY

Complement-fixing (CF) antibodies against brain tissue antigens were demonstrated in seven cases of *Mycoplasma pneumoniae* infection with neurological complications. However, forty-three out of fifty-four cases (80%) without neurological symptoms also acquired antibodies to brain during the course of *M. pneumoniae* infection. 75% of the patients with antibodies to brain also had antibodies against human lung and liver, but the antibody titres to the latter tissue antigens were usually lower than against brain. The reactivity of *M. pneumoniae* antisera to brain and other tissues was significantly reduced by prior absorption with *M. pneumoniae* antigen, indicating the presence of a related antigen in *M. pneumoniae* organisms and these tissues. Fractionation by density gradient centrifugation of thirty-three sera from twenty-six cases showed the antibodies to brain to be of the IgM class exclusively in twenty-four cases while the CF antibodies to *M. pneumoniae* were associated with both IgM and IgG.

The brain antigen reacting in the CF test with *M. pneumoniae* antisera could be extracted with chloroform–methanol in the same way as the crude lipid CF antigen of *M. pneumoniae*.

INTRODUCTION

Respiratory illness due to *Mycoplasma pneumoniae* may be complicated by involvement from the central nervous system (CNS) (Yesnick, 1956; Sköldenberg, 1965; Taylor *et al.*, 1967; Sterner & Biberfeld, 1969). The mechanism responsible for the neurological complications is not known, but several explanations have been discussed; among those the possibility that an immunologic reaction against nervous tissue may be involved (Taylor *et al.*, 1967). It, therefore, seemed of interest to examine sera from cases of *M. pneumoniae* infection with CNS complications for the presence of antibodies to brain. The present paper describes the occurrence of complement-fixing antibodies to brain and some other

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tissue antigens, both in cases with, and in cases without neurological complications. The specificity, immunoglobulin class and temporal appearance and persistence of these antibodies were studied.

Part of this work has been previously described in a brief report (Biberfeld, 1969).

MATERIALS AND METHODS

Sera from seven cases of *M. pneumoniae* infection with respiratory illness and neurological complications were available (Table 1). The case reports of these patients have been presented previously (cases 1–6 by Sterner & Biberfeld, 1969 and case 7 by Berglund & Wikberg 1969). Three of these cases had aseptic meningitis, two had meningo-encephalitis, and the remaining two cases acute psychosis. The neurological symptoms began 4–14 days after the onset of respiratory illness. A four-fold or greater change in titre or a high titre ($\geqslant 1/128$) of CF antibodies to *M. pneumoniae* was demonstrated in all seven cases. *M. pneumoniae* was isolated from throat specimens in six of these cases.

Fifty-four hospitalized cases of lower respiratory tract illness without neurological symptoms which showed a four-fold or greater increase in CF antibody titre to *M. pneumoniae* were also examined for antibodies to tissue antigens.

Three groups of sera served as controls: (1) sera from thirty-one apparently healthy blood donors, (2) sera from thirteen cases of acute respiratory illness (ARI) with a four-fold rise in CF antibody titre to adenovirus and finally (3) seven sera of ARI with a four-fold rise to influenza B.

Preparation of M. pneumoniae antigen

M. pneumoniae, strain FH, obtained from Dr R.M. Chanock, NIH, Bethesda, was grown as described by Somerson *et al.* (1967) in 500 ml of the PPLO broth medium devised by Hayflick (1965) containing 20% horse serum, 2·5% yeast extract, 1% glucose, 0·002% phenol red and 1000 units/ml of penicillin, contained in Povitsky bottles. After incubation at 37°C for 4–5 days the broth was discarded and the *M. pneumoniae* organisms attached to the glass surface were washed three times with phosphate buffered saline (PBS) before being removed by shaking the bottles with sterile glass beads in PBS. After removal of the glass beads the suspension of organisms was centrifuged at 15,000 rev/min (Spinco rotor 40) for 30 min and the pellet resuspended in 5 or 10 ml PBS. The CF antigen was prepared from this concentrated suspension by chloroform–methanol extraction and KCl partition according to the procedure developed by Kenny & Grayston (1965). 4 units of antigen, as determined by 'checkerboard' titration, were employed in the CF test.

Preparation of tissue antigens

Human brain, lung and liver were obtained at autopsy. The corresponding organs were removed from monkeys, rabbits and mice immediately after the animals had been killed. After rinsing the tissue carefully in PBS to remove blood it was weighed, minced with scissors and then homogenized in an Omnimixer for 2 min with borate buffered saline (Trotter, Belyavin & Waddams, 1957) in amounts to produce a 20% suspension (w/v). The suspension was filtered through gauze and then centrifuged at 2000 rev/min for 15 min. The supernatant was divided in small aliquots and stored at −70°C. The tissue extracts were tested by 'checkerboard' titration and were diluted 1/2 or 1/4 for use as CF antigens (see also

Fig. 2). Brain tissue antigens were seldom anti-complementary, but several lots of lung and liver antigens had to be discarded because of anti-complementarity.

In some experiments brain tissue was extracted with chloroform–methanol by the same procedure used for the preparation of the lipid CF antigen of *M. pneumoniae* (Kenny & Grayston, 1965). Briefly 5 ml of crude brain tissue antigen was extracted with 100 ml of chloroform and 50 ml of methanol. Then 37·5 ml of 0·1 M aqueous KCl were added. Two phases were obtained. The chloroform phase was evaporated and the residue reconstituted with 2·5 ml of ethanol, which was then mixed with 2·5 ml PBS containing 5% bovine albumin. The water–methanol phase was dialysed against PBS and concentrated to a volume of 5 ml. These preparations were then examined for antigen activity.

Wasserman (WR) antigen

Cardiolipin was prepared at the chemical department of the National Bacteriological laboratory as described by Pangborn (1947). Cardiolipin, 0·03%, was mixed with 0·05% lecithin and 0·3% cholesterol in alcohol. This antigen was used in the CF test in dilution 1/250 which is the antigen concentration employed for routine testing of WR antibodies.

Complement fixation test (CFT)

Sera to be tested were inactivated at 56°C for 30 min. The CFT was performed with the microtitre system (Sever, 1962). 2 units of complement were used. When testing for antibodies to *M. pneumoniae* and WR antigen the plates with the mixture of diluted sera, antigen and complement were always kept overnight in the refrigerator before addition of the haemolytic system consisting of 4 units of amboceptor and a 2% suspension of sheep erythrocytes. In the early phase of the studies incubation with tissue antigens was done at 37°C for 1 hr. However, it was found that higher antibody titres were obtained if fixation was allowed to proceed overnight at 4°C. The CF antibody titres presented are those obtained after incubation overnight unless otherwise stated.

Cold agglutinin (CA) test

The procedure described by Feller & Hilleman (1956) was followed. Two-fold serum dilutions were mixed with an equal volume of a 0·2% suspension of human O erythrocytes and the mixture was incubated at 4°C overnight.

Density gradient ultracentrifugation

A gradient of sucrose ranging from 10 to 37% was prepared. 0·4 ml or 0·6 ml of inactivated serum diluted 1/2 in PBS was layered over the gradient, to obtain a final volume of 5 ml, which was then centrifuged in a Spinco centrifuge using an SW 50 rotor at 35,000 rev/min for 18 hr at 5°C. Eleven to twelve serial fractions of 0·4 to 0·45 ml were collected dropwise from the bottom of the tubes and examined for antibody activity. In some instances the separation of IgM and IgG was checked by testing the fractions by the single radial diffusion method (Mancini, Carbonara & Heremans, 1965) against a monospecific antiserum to IgM and by immune diffusion, according to Ouchterlony, against a monospecific antiserum to IgG and IgA respectively.

Absorption with M. pneumoniae antigen

Sera were usually diluted 1/4 and divided into two portions. One portion of diluted

serum was absorbed with antigen and the other portion was incubated, centrifuged and heated at 56°C together with the absorbed sample. For the absorption the pellet obtained, after centrifugation at 15,000 rev/min of 0·5 ml of a suspension of *M. pneumoniae* organisms (see preparation of *M. pneumoniae* antigen), was resuspended in 0·5 ml of diluted serum. The mixture was kept 1–2 hr in room temperature and then at 4°C overnight before being centrifuged at 15,000 rev/min for 30 min. The supernalant was collected and absorbed once more. The supernatant obtained after the second absorption was inactivated at 56°C for 30 min and then examined for antibody activity together with the unabsorbed control sample.

Absorption with tissue antigens

The IgM fraction of serum or whole serum was diluted 1/2 in PBS containing fresh guinea-pig serum in dilution 1/10 and divided in two portions. One portion, usually 0·4–0·6 ml, was absorbed once or twice with the pellet obtained after centrifugation at 20,000 rev/min of 1 ml of a tissue extract. The mixture was kept for 2 hr at 37°C with occasional mixing and then at 4°C overnight followed by centrifugation at 20,000 rev/min for 30 min. The supernatant was inactivated at 56°C for 30 min and examined for antibody activity together with the unabsorbed control portion which also had been similarly incubated, centrifuged and inactivated.

RESULTS

All seven cases with neurological complications after *M. pneumoniae* infection were found to have CF antibodies against brain tissue. The antibody titres against human brain and lung tissue in these cases are presented in Table 1. It appears that four cases had antibodies to lung in addition to brain. In cases 1–4 antibodies to brain were present in the earliest available sera collected on the day of or a few days after the onset of neurologic symptoms. However, case 6 had no demonstrable antibodies to brain at the onset of neurological symptoms. Only in one (No. 6) of the cases with CNS symptoms was the first serum sample taken early enough to allow the demonstration of a rise of antibodies to brain during the course of illness. In the other cases a fall in antibody titre was demonstrable.

Examination of fifty-four cases of *M. pneumoniae* infection without neurologic complications, all of which developed a significant ($\geqslant$ four-fold) titre rise of CF antibodies to *M. pneumoniae* during the course of respiratory illness, showed that forty-three (80%) of these cases also had a four-fold or greater increase in CF antibody titre to human brain tissue.

None of the cases in a control group, comprising thirteen patients with adenovirus infection and seven cases of influenza B infection acquired antibodies against brain during the infection.

Sera from three rabbits immunized with *M. pneumoniae* antigen were also examined in the CF test with brain antigen. All the rabbits showed a four-fold rise in antibody titre against brain. However, pre-immunization sera from all three rabbits reacted with brain tissue antigen to a titre of 1/8–1/16.

Organ and species specificity

The tissue antibodies present in sera of patients with *M. pneumoniae* infection were studied with regard to organ and species specificity. Paired sera from thirteen cases with antibodies to human brain were examined in the CF test with two or three of the following

antigens: monkey, rabbit and mouse brain, and found to be equally reactive with all the brain antigens tested. The results of some of these tests are shown in Table 2.

A number of sera were also tested for antibodies against lung and liver antigens from different species. Table 2 shows the results of CF antibody tests with various tissue antigens

TABLE 1. CF antibody titres against human brain and lung tissue antigens in seven cases of *M. pneumoniae* infection with neurological complications

Case No. Neurologic complication	Time after onset of		Throat culture of *M. pneumoniae*	CF antibody titre against		
	Respiratory illness	Neurologic symptoms		*M. pneumoniae*	Human brain	Human lung
223 (1)	8d	1d	+	128	16	<2
Meningitis	15d	8d	nd	128	8	<2
	21m		nd	4	<2	nd
229 (2)	11d	Day of onset	−	512	128	<2
Meningitis	20d	9d	+	512	32	<2
	32d	21d	+	512	32	<2
	100d	89d	−	64	2	<2
	116d	105d	−	64	2	<2
71 (3)	17d	3d	−	128	16	<2
Meningitis	34d	20d	+	256	16	<2
	24m		nd	32	<2	nd
402 (4)	12d	3d	−	512	32	4
Meningo-	23d	14d	−	512	32	4
encephalitis	39d	30d	+	128	16	<2
	54d	45d	−	128	8	<2
30 (5)	21d	12d	−	512	32	8
Acute psychosis	33d	24d	+	256	16	4
	42d	33d	+	256	4	<2
	72d	83d	−	512	<2	<2
	100d	91d	−	256	<2	<2
139 (6)	4d	Day of onset	+	<4	<2	nd
Acute psychosis	13d	9d	−	64	4	nd
	19d	15d	−	128	8	nd
	32d	28d	+	128	16	8
	48d	44d	+	64	4	<2
	72d	68d	+	64	4	nd
	110d	106d	−	32	<2	nd
614 (7)	19d	13d	−	512	16	8
Meningo-	35d	29d	−	256	<2	<2
encephalitis	49d	43d	nd	128	<2	nd

The antibody titre is expressed as the reciprocal of the dilution. The case numbers in parentheses refer to the case numbers used in a previous publication (case Nos 1–6, Sterner & Biberfeld, 1969; case No. 7, Berglund & Wikberg, 1969). nd = Not done, d = days, m = months.

in seven cases. In these cases the mixtures of serum dilutions, antigen and complement had been incubated at 37°C for 1 hr before addition of the haemolytic system. In some instances the titres obtained after incubation at 4°C overnight are given also. It appears

that five of the patients with brain antibodies had no demonstrable antibodies against lung and/or liver from man and monkey when short-time incubation was used although they had antibodies against lung and liver antigens from mouse. Antibodies against lung and liver from man could be demonstrated when the CF test was performed with overnight incubation, but the antibody titres were usually lower than against brain (Tables 1 and 2). Seventeen (74%) out of twenty-three cases with a four-fold or greater change in brain antibody titre had a significant change of antibodies against human lung and eleven out of fourteen (79%) cases with antibodies to brain showed a four-fold titre rise of antibodies against liver.

Table 2. CF antibody titres against various organ antigens in seven cases of *M. pneumoniae* infection without neurological complications

Case No.	Days after onset of respiratory illness	CF antibody titre against											
		M. pneumoniae	Human			Monkey			Mouse			Rabbit	
			brain	lung	liver	brain	lung	liver	brain	lung	liver	brain	liver
460	5	32	4	nd	nd	4	<2	<2	8	4	nd	2	<2
	15	1024	64	<4	<4	64	<2	<2	64	64	64	64	<2
428	9	8	<2	<2	<2	<2	<2	<2	2	2	2	2	<2
	20	1024	32	8	32	32	8	8	32	16	32	32	16
565	6	16	<2(<2)	<2(4)	<2(4)	<2	<2	<2	4	2	2	<2	<2
	27	256	64(32)	<2(16)	<2(8)	16	<2	8	64	8	8	32	2
	52	128	16(32)	<2(16)	<2(8)	4	<2	<2	16	8	8	16	<2
307	7	32	<2(2)	<2(<2)	<2(<2)	<2	<2	nd	<2	<2	<2	<2	<2
	18	256	8(16)	<2(8)	<2(8)	8	<2	nd	8	4	4	16	<2
	39	256	4(16)	<2(4)	<2(8)	4	<2	nd	8	nd	nd	8	nd
515	7	8	<2(<2)	<2(<2)	<2(<2)	<2	<2	nd	<2	nd	nd	<2	nd
	29	512	8(16)	<2(8)	<2(4)	4	<2	nd	8	nd	nd	4	nd
35	12	32	<2(8)	<2(16)	nd(8)	<2	<2	nd	4	4	2	2	nd
	21	512	32(64)	<2(32)	8(16)	16	<2	nd	32	8	8	8	nd
266	7	<4	<2(<2)	<2(<2)	<2(<2)	<2	<2	nd	<2	2	2	<2	nd
	21	256	16(64)	8(16)	8(32)	8	4	nd	8	8	4	16	nd

The antibody titre is expressed as the reciprocal of the dilution. The CF test was performed with incubation of serum dilutions, antigen and complement at 37°C for 1 hr. Titres obtained after incubation at 4°C overnight are given in some instances within parentheses. nd = Not done.

The binding time also influenced the antibody titre against human brain. Two convalescent phase sera which were negative (titre < 1/2) when tested with short time binding had a titre of 1/8 and 1/16 respectively after incubation with brain antigen and complement overnight, but most sera showed only a two- to four-fold increase in antibody titre to brain after prolonged incubation (Table 2).

Sera from thirty-one blood donors were tested for CF antibodies against human brain, lung and liver with incubation overnight. Antibodies against brain were found in three cases (10%), antibodies against liver in six cases (19%) and against lung in seven cases (23%). The antibody titres were in no case higher than 1/4. When short time incubation

was used for testing of the same sera against human brain antigen all sera were negative (titre $<1/2$).

Absorption experiments

In order to determine if the reactivity of *M. pneumoniae* antisera with brain and other organs was due to a serological cross reaction, absorption experiments were performed. The results of some of the absorption experiments are shown in Table 3. Absorption of human antisera with *M. pneumoniae* antigen removed or reduced the CF antibodies against brain and lung as well as those against *M. pneumoniae*. The titres of cold agglutinins were not significantly changed by the absorption. In the absorption experiments with brain

TABLE 3. Results of absorptions of whole sera or IgM fractions of sera from cases of *M. pneumoniae* infection with *M. pneumoniae* antigen (M.pn.) and human brain tissue antigen. The antibody titre is expressed as the reciprocal of the dilution.

Case No.	Specimen	Absorbed with	CF antibody titre against			Titre of CA*
			M. pneumoniae	Human brain	Mouse lung	
460	Whole serum	—	1024	64†	64	512
		M.pn.	64	4†	4	512
646	Whole serum	—	128	32		
		M.pn.	<4	<4		
353	Whole serum	—	64	32		32
		M.pn.	8	4		64
428	Whole serum	—	512	32†	32	256
		M.pn.	4	<4†	<4	128
565	Whole serum	—	512	64	16	128
		M.pn.	<4	<4	<4	128
460	IgM fraction	—	128	16		
		Brain	128	4		
646	IgM fraction	—	16	8		
		Brain	16	<2		
353	Whole serum	—	128	32		
		Brain	128	<4		
499	IgM fraction	—	128	16		
		Brain	256	4		
379	IgM fraction	—	256	32		
		Brain	256	8		

* = CA = cold agglutinins

† = Antibody titre against monkey brain antigen

antigen, the IgM fractions of human *M. pneumoniae* antisera were used in most instances since the antibodies reacting with brain were of IgM class (see below). It was frequently difficult to remove the antibodies against brain by absorption with brain antigen. However, addition of fresh guinea-pig serum seemed to facilitate the absorption. Absorption with brain tissue reduced the antibody titre against this antigen but did not significantly lower the antibody titre to *M. pneumoniae*. Two human antisera to *M. pneumoniae* containing CF antibodies against brain were absorbed with human lung and liver antigens, which removed or decreased the antibodies against brain.

TABLE 4. Temporal change of antibody titres against human brain and *M. pneumoniae* antigen in nine cases of *M. pneumoniae* infection without neurological complications

Case No.	Time after onset of respiratory illness	Antibody titre against	
		Brain	*M. pneumoniae*
292	11d	<2	<4
	19d	32	1024
	60d	16	1024
	5m	2	1024
	17m	<2	128
28	5d	<2	<4
	12d	16	64
	31d	32	256
	4m	8	128
	7m	2	128
	14m	<2	32
138	9d	<2	32
	13d	8	128
	23d	16	256
	58d	4	256
	14m	<2	32
129	9d	2	32
	16d	16	256
	28d	8	256
	95d	4	256
	6m	<2	128
654	10d	<2	<4
	25d	64	512
	34d	16	512
	4m	4	256
336	8d	<2	16
	27d	8	128
	41d	8	128
	4m	4	64
	16m	2	64
107	8d	2	8
	28d	32	512
	44d	32	256
	84d	16	256
	9m	8	128
	22m	8	128
290	4d	<4	<4
	12d	16	128
	24d	32	128
	79d	16	128
	10m	16	64

d = Days, m = months.

Temporal appearance and persistence of antibodies to brain

The temporal changes of antibody titres against brain and *M. pneumoniae* antigen in relation to time after onset of illness are shown in Tables 1 and 4. The brain antibodies appeared during the 2nd week of illness and reached a peak titre 2–4 weeks after clinical onset. The antibody titres were always lower against brain than against *M. pneumoniae*. The titre of brain antibodies usually decayed more rapidly than the titre of *M. pneumoniae* antibodies. For example in case 292 (Table 4) the titre of *M. pneumoniae* antibodies was still at the maximum level 5 months after onset of illness whereas the titre of brain antibodies had decreased sixteen-fold. Similar results were seen in several other cases (for instance Nos 28, 138, 129 and 654, Table 4; Nos 5 and 7, Table 1). However, eight out of fifteen cases still had brain antibodies in titre $\geqslant 1/2$ 5–6 months after the onset of illness and in four out of thirteen cases brain antibodies persisted for more than 12 months (for example case 107, Table 4).

Immunoglobulin class of antibodies to brain

For determination of the molecular class of the brain antibodies sera were fractionated by density gradient centrifugation. The result of a representative fractionation experiment of two sera from one patient (No. 107) is illustrated in Fig. 1. The CF antibodies to *M. pneumoniae* were distributed in two peaks, the first peak (fractions 1–4) containing IgM and the second peak (fractions 5–9) IgG and IgA as determined by immunodiffusion tests with monospecific antisera to these immunoglobulins. The CF antibodies forming the second peak could only belong to the IgG class, since IgA antibodies do not fix complement. It appears from Fig. 1 that the brain antibodies were present exclusively in the IgM fractions both in serum collected 28 days and 84 days after clinical onset.

Analogous results were obtained with twenty-seven sera from twenty-one other cases of *M. pneumoniae* infection without neurological complications, all of which were found to have brain antibodies of IgM class only. Eleven of these sera had been collected later than a month after onset of respiratory illness. A serum collected 22 months after the infection (case 107, Table 4) still contained brain antibodies of IgM class exclusively. Two out of three patients with neurological complications possessed brain antibodies of IgM class only, whereas the third case (No. 229, Table 1) had brain antibodies of IgG class predominantly.

Sera with high levels of IgM antibodies to *M. pneumoniae* usually reacted in the CF test with brain antigen but there were a few exceptions. Thus one patient lacked antibodies to brain although she had CF antibodies of IgM class against *M. pneumoniae* to a maximum titre of 1/512. It also occurred that the titre of brain antibodies fell off while the titre of CF antibodies of IgM class to *M. pneumoniae* was stationary. For instance in case 30 (Table 1) 30–35% of the CF antibodies to *M. pneumoniae* (titre 1/512) were of IgM class both 21 and 72 days after onset of illness but the antibodies to brain decayed from a titre of 1/32 to $<1/2$ during this time.

Among seven cases which had CF antibodies to *M. pneumoniae* of IgG class exclusively there was one case which acquired antibodies to brain during the infection. Sera taken 19 and 40 days after onset of illness contained brain antibodies in titre 1/4 whereas the acute phase serum was negative (titre $<1/2$). In this case the brain antibodies were of IgG type. Absorption of this serum with *M. pneumoniae* antigen removed the antibodies against brain.

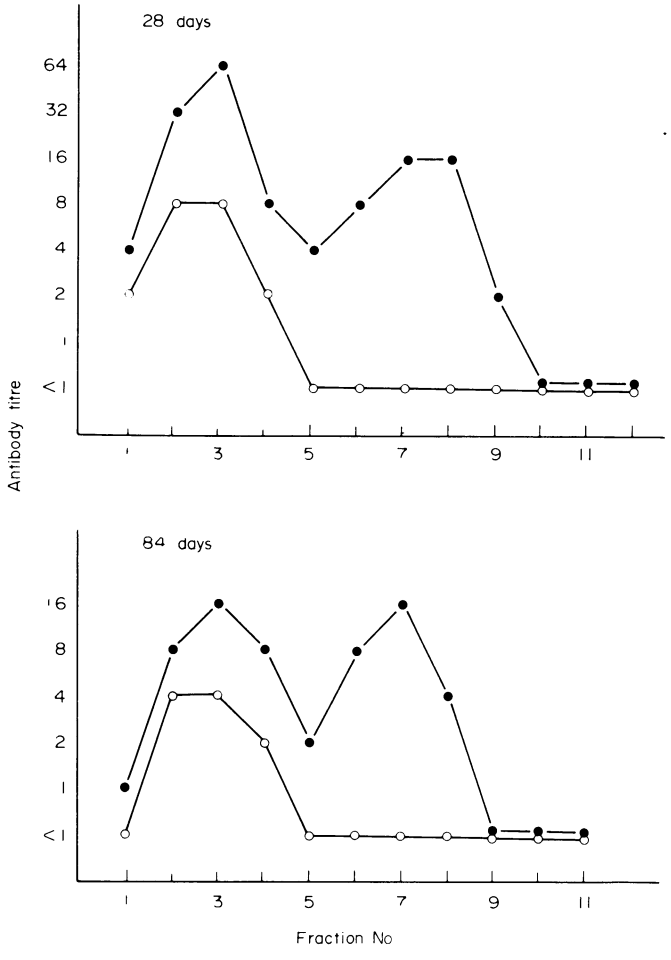

FIG. 1. Distribution of complement fixing antibodies against *Mycoplasma pneumoniae* (●) and human brain tissue antigen (○) in density gradient centrifugation fractions of two sera from the same patient (No. 107, Table 4) collected 28 and 84 days after onset of pneumonia. The fractions were collected from the bottom of the centrifuge tube.

Chloroform–methanol extraction of brain antigen

When the human brain tissue antigen was extracted by chloroform–methanol followed by KCl partition according to the procedure used for the preparation of the crude lipid CF antigen of *M. pneumoniae* the CF activity was recovered in the resuspended residue of the evaporated chloroform phase. No CF activity was detected in the concentrated methanol–aqueous phase. The results of simultaneous 'checkerboard' titrations of crude brain tissue antigen and chloroform–methanol extracted brain antigen with the same human antiserum to *M. pneumoniae* are shown in Fig. 2.

Extracted brain antigen dissolved in PBS retained its activity in the CF test after heating in a boiling water bath for 5 min.

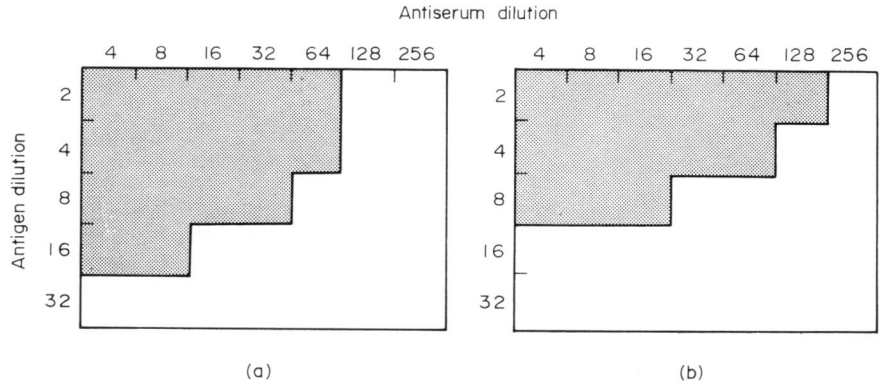

FIG. 2. 'Checkerboard titrations of (a) crude brain tissue antigen and (b) chloroform–methanol extracted brain antigen with a human antiserum to *M. pneumoniae*. The shaded area denotes the antigen and antiserum dilutions which gave complete or almost complete fixation of complement. The dilutions are expressed as reciprocal values.

Antibodies to cardiolipin

Paired sera from eighteen patients with *M. pneumoniae* infection without neurological complications who had developed antibodies against brain were examined in the CF test against WR antigen. Two of these cases showed an increase in antibody titre to cardiolipin from $<1/2$ to 1/32 and 1/64 respectively. When the sera were tested against a twenty-five times higher concentration of cardiolipin antigen, than that employed routinely in the CF test, a significant rise in antibody titre was detected in three additional cases.

DISCUSSION

The present study was initiated in an attempt to find evidence of an immunologic reaction against brain in cases of *M. pneumoniae* infection with neurological complications. CF antibodies to brain were found in such cases but also in cases without neurological symptoms. Absorption experiments showed that antibodies to *M. pneumoniae* cross-react with brain tissue from man and other species.

M. pneumoniae antisera reacted also with lung and liver antigens, but less frequently and in lower titres than with brain. The explanation of this might be that brain tissue contains more of the cross-reacting antigen per unit weight than the other tissues examined. However, it was not possible to test if higher antibody titres could be obtained with more concentrated extracts of lung and liver because such antigen preparations were anti-complementary.

During studies of primary atypical pneumonia (PAP) Thomas *et al.* (1943) found that a large proportion of patients with this illness developed antibodies reacting in the CF test with lung tissue antigens from mice. Sera from a few cases of PAP were tested also for CF antibodies against lung antigen from other species including man, with positive results. The demonstrated antibodies were considered to be directed primarily against lung, but

some reactivity with other tissues such as liver, heart and kidney occurred (Thomas, 1964). The reactivity with brain tissue antigen was not examined. In recent years it has been shown that *M. pneumoniae* is the most important cause of PAP (Hayflick & Chanock, 1965). The organ antibodies found in cases of PAP (Thomas *et al.*, 1943) probably correspond to the *M. pneumoniae* induced antibodies which cross-react with brain and other tissue antigens as shown by the present work.

Previously, serological cross-reactions between *M. pneumoniae* and some microorganisms have been demonstrated, namely *M. mycoides* (Lemcke, Shaw & Marmion, 1965) and *Streptococcus* MG (Marmion, Plackett & Lemcke, 1967; Lind, 1968). The cross-reaction between *M. pneumoniae* and *Streptococcus* MG, which seems to be due to sharing of a glycosyl diglyceride (Plackett & Shaw, 1967; Plackett *et al.*, 1969), offers an explanation for the known occurrence of agglutinins to *Streptococcus* MG in a proportion of cases with *M. pneumoniae* infection.

Among the heterogenetic antibodies occurring in cases of *M. pneumoniae* infection are also cold agglutinins and antibodies reacting with WR antigen. Recent results suggest that a reaction product of red blood cells with *M. pneumoniae* may be responsible for the production of cold agglutinins (Feizi *et al.*, 1969). However, the reaction of *M. pneumoniae* antisera with WR antigen might be due to a serological cross-reaction. Plackett *et al.* (1969) recently reported that *M. pneumoniae* synthesizes a phospholipid distinct from cardiolipin but it is reactive with some WR positive sera in the CF test. CF tests performed in the present study showed that antibodies to cardiolipin antigen were rare compared to antibodies against brain in patients with *M. pneumoniae* infection. Absorption of sera, containing antibodies both to brain and cardiolipin, with *M. pneumoniae* antigen did not reduce the antibody titre either to brain or to cardiolipin, although the CF antibodies to *M. pneumoniae* were absorbed out (Biberfeld, unpublished data). The possible relationship between the antibodies to cardiolipin and to brain is under investigation.

It was found that in twenty-four of twenty-six cases examined the CF antibodies reacting with brain were of the IgM class exclusively both in the early phase of illness and several weeks or months after infection. The present and previous studies have shown that CF antibodies to *M. pneumoniae* belong both to the IgM and the IgG class (Biberfeld, 1966, 1968; Schmidt, 1966; Fernald *et al.*, 1967) the proportion of IgG to IgM antibodies increasing with time after the onset of infection (Biberfeld, 1968).

The antibodies cross-reacting with brain, apparently constitute only a small part of the CF antibodies to *M. pneumoniae* since absorption of *M. pneumoniae* antisera with brain antigen did not significantly reduce the titre of CF antibodies to *M. pneumoniae*, not even of IgM class.

The CF antigen of *M. pneumoniae* is present in the lipid fraction of the organisms (Kenny & Grayston, 1965; Sobéslavský, Prescott & Chanock, 1967; Lemcke, Marmion & Plackett; 1967). Results of recent studies indicate that the active CF components of *M. pneumoniae* are associated with glyco- and phospholipids (Beckman & Kenny, 1968; Plackett *et al.*, 1969). The number of different antigenic components with CF activity is not known. Beckman & Kenny separated two chromatographically distinct lipid fractions with CF activity one of which consisted of five subfractions and Plackett *et al.* separated seven different components, but the serological relationship between these fractions has not been clarified. The finding that the brain antigen reacting in the CF test with *M. pneumoniae* antisera was heat-stable and extractable with chloroform–methanol suggests that this

antigen is also associated with lipids. Plackett *et al.* (1969) have shown that rabbit antisera to *M. pneumoniae* cross-react with glycolipids from spinach leaves and with cytolipin H, a glycolipid (ceramide lactoside) originally derived from human tumour tissue. The brain is rich in glycolipids (*cerebrosides*), which may be responsible for the cross-reaction between brain and *M. pneumoniae*. Examination of the sensitivity of the brain CF antigen to periodate treatment, which has been shown to reduce the CF activity of the *M. pneumoniae* antigen (Lemcke *et al.*, 1967), may help to clarify the role of carbohydrate groups in the cross-reaction between brain and *M. pneumoniae*.

There is at present no indication of a pathogenic role of the *M. pneumoniae* induced antibodies reacting with brain and other tissues. The fact that antibodies to brain were present both in cases with and in cases without neurological symptoms and reached approximately the same titre levels in both groups speaks against a pathogenic importance of these antibodies. However, in spite of this the possibilities should be considered that within the population of cross-reacting antibodies there may exist antibodies with cytotoxic activity that could damage nervous tissue. In order to further evaluate the possible pathogenic role of the cross-reacting antibodies their presence in the cerebro spinal-fluid (CSF) should also be investigated. Collection of CSF's from patients with *M. pneumoniae* infection for antibody examination is in progress.

It is of interest in this context that three of four cases of the Guillain-Barré syndrome associated with *M. pneumoniae* infection had CF antibodies to *M. pneumoniae* in the CSF (Steele *et al.*, 1969). These cases were not examined for antibodies to brain, but it is known from a previous study that serum antibodies to brain and nervous tissue occur in 50% of patients with the Guillain-Barré syndrome (Melnick, 1963). It is conceivable that in some cases of the Guillain-Barré syndrome the antibodies to brain and nervous tissue may actually be *M. pneumoniae* induced antibodies cross-reacting with brain, as discussed previously (Biberfeld, 1969).

Finally, the possibility should also be considered that the neurological disorders in *M. pneumoniae* infection may be the result of an immune reaction mediated by sensitized lymphocytes.

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ABBREVIATIONS

CA cold agglutinin
CF complement fixation
PAP primary atypical pneumonia

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