An Outbreak of *Mycoplasma pneumoniae* Infection in a Community

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NAKAO, T., UMETSU, M., WATANABE, N. and KIKUCHI, N. *An Outbreak of Mycoplasma pneumoniae Infection in a Community.* Tohoku J. exp. Med., 1970, 102(1), 23-31 — An increasing number of patients with atypical pneumonia were seen at the residents of the collective houses provided by a paper manufacturing company in the summer of 1967. *Mycoplasma pneumoniae* was established as the etiological agent in 80.5% of 36 patients who were subjected to serologic study. Familial infection was common. Outstanding symptom was cough. Skin rashes were noticed in 3 cases. Complement-fixing (CF) and indirect-hemagglutinating (IHA) antibody titers rose about 10 days after infection and remained unchanged for at least 2 months, but declined 9 to 12 months later. CF and IHA antibodies to *Mycoplasma pneumoniae* were both of the 19S and 7S types. There was an increase of 7S relative to 19S CF antibodies with time after the onset of illness, and almost all CF antibodies were 7S a year later. IHA antibodies were predominantly 19S globulins in various stages of illness. — atypical pneumonia; *Mycoplasma pneumoniae*; Complement-fixing antibody; indirect hemagglutinating antibody

During the period from April to November, 1967, an outbreak of respiratory tract disease occurred in a collective housing population in the suburbs of Kushiro City, which is in the eastern part of Hokkaido. In about 30% of the patients pulmonary infiltrations developed. Laboratory studies revealed that this outbreak was mainly caused by *Mycoplasma pneumoniae* (*M. pneumoniae*).

There have been only a few reports on the outbreak of *M. pneumoniae* infection in a community in the past.¹⁻³ The present paper constitutes the first report on the outbreak of *M. pneumoniae* infection in Japan.

MATERIALS AND METHODS

Population. An outbreak of *M. pneumoniae* infection occurred in the collective houses provided by a paper manufacturing company in the suburbs of Kushiro City. These houses were away from the middle of the City. Residents kept little contact with the people of Kushiro City, but they had close contact mutually. The population in the company houses was 1,210 in 405 families. Most parents were in their thirties and most of their children were under ten years of age.

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Specimens. Laboratory investigations were carried out on 67 members in 29 families in whom paired sera were available. Throat swab specimens were collected and extracted into bouillon. They were kept at -20°C until examination. One-tenth ml of the throat specimen was inoculated onto two PPLO agar plates. The plates were incubated at 37°C . One plate was held under aerobic conditions and the other under anaerobic. Each plate was inspected every other day for 4 weeks. When a mycoplasma colony appeared, it was subcultured on a PPLO agar plate.

Serum specimens were collected from patients at the time of visit to the clinic and again two to three weeks later. Specimens were also collected from 35 patients nine to twelve months after the onset of symptoms.

Complement fixation test. Antigen for the test was made with the Mac strain of *M. pneumoniae* which was donated by the Department of Bacteriology, Tohoku University School of Medicine, and the method for preparation of the CF antigen was performed according to the method described by Chanock *et al.*⁴ CF antibody was measured by the micromethod described by Sever⁵ with two exact units of complement and four units of antigen.

Indirect hemagglutination test. The same antigen as that for the complement-fixation test was used. The method of Dowdle and Robinson⁶ with a modification by Taylor-Robinson *et al.*⁷ was followed. Horse serum was used in substitution for rabbit serum as standard diluent. A broth culture of *M. pneumoniae* which had been grown for 5 to 6 days was centrifuged at $10,000\times g$ for 45 minutes, and the pellets were washed and resuspended in 20 volumes of a phosphate-buffered saline (PBS, pH 6.4). This suspension was sonicated in ultrasonic disintegrator for 30 minutes, and kept at -20°C until use. A 4% suspension of the washed sheep erythrocyte was incubated at 37°C for 10 minutes with an equal volume of the tannic acid solution diluted 1:2,000 in PBS and the cells were washed again. One volume of antigen was mixed with one volume of the 2% suspension of the tannic acid-treated cells in 4 volumes of PBS, pH 6.4, and the mixture was incubated for 20 minutes at 37°C . The cells were washed three times in PBS containing 1% horse serum and resuspended in the diluent to produce a 1% suspension of sensitized red cells.

Sera were incubated at 56°C for 30 minutes, then added with 0.1 ml of packed sheep erythrocytes per 0.1 ml serum and incubated for another 30 minutes at 4°C to accomplish the absorption. Treated sera were titrated to give 2-fold dilution using 0.4 ml horse diluent in the tubes and an equal volume of 1% suspension of sensitized erythrocytes was added. The tubes were incubated at 37°C and read for hemagglutination when controls showed well formed buttons, usually after 2 to 3 hours. The reciprocal of the highest initial dilution yielding a clearly positive agglutination of the sensitized erythrocyte was considered as an end-point.

A four-fold or greater rise in antibody measured by either technique was considered to be serologic evidence of *M. pneumoniae* infection. But in some cases, the acute stage was over when the first serum specimen was obtained, so that CF titers of 1:64 or more and or IHA titers of 1:160 or more in both serum specimens were also considered to be serologic evidence of infection.

Treatment by 2-mercaptoethanol (2-ME). Sera were incubated for 2 hours at 37°C with an equal volume of 0.2 M 2-ME in 0.02 M PBS and with the buffer alone. Mercaptoethanol-treated sera and control sera were dialyzed against PBS at 4°C for 48 hours. Antibody titers were measured by CF and IHA tests on 2-ME treated sera and control sera.

Antibody titers against viruses. CF antibody for adenovirus was measured by the micro-method. Antibody titers to influenza A2 and B were determined on all sera by the hemagglutination inhibition test.

Cold agglutinin test. A standard macro cold-agglutinin (CA) test employing 0.5 ml of serum dilution and 0.5 ml of 0.2% suspension of human O red blood cells was used.

RESULTS

Isolation. Four strains were obtained under aerobic conditions in primary culture, but subcultivations of the isolated strains on artificial medium were unsuccessful. These strains seemed to be *M. pneumoniae* based on the characteristic colony formation. Under anaerobic conditions 18 strains of mycoplasma were isolated. Species identification by the growth inhibition test was not done, but they were suspected to be composed of 7 strains of *M. hominis* type 1, 1 of *M. salivarium* and 10 of *M. orale*, based on the formation and appearance of colonies after the subcultivation on artificial medium.

TABLE 1. Age distribution of the patients with *M. pneumoniae* infection

Age (years)	Number of cases tested	Number showing increased antibody titer		
		CF	IHA	CF and/or IHA
0-4	6	2	3	3
5-9	24+ (1)	16+ (1)	15	17+ (1)
10-14	6	5	5	5
15-29	1			
30-39	12+ (9)	7+ (1)	7	8+ (1)
40-49	4+ (4)	4	3	4
Total	53+ (14)	34+ (2)	33	37+ (2)

Numerals in parentheses indicate the numbers of persons with no clinical symptom.

Fig. 1. Comparison of antibody titers measured by complement-fixing (CF) and indirect-hemagglutination (IHA) tests in sera from patients with *M. pneumoniae* infection

Serologic results. Thirty-nine out of 67 cases (74%) were due to *M. pneumoniae* as shown in Table 1. Thirty-seven out of 53 patients (70%) with respiratory tract disease had positive antibodies against *M. pneumoniae*. IHA titers were, in general, higher than CF titers in sera from persons with serologically positive antibodies. CF titers ran parallel with IHA titers in cases with rise of antibody, but in cases with low titers of antibody no relationship between CF and IHA titers was noticed (Fig. 1).

CF and IHA titers against *M. pneumoniae* were found to be declined 9 to 12 months after *M. pneumoniae* infection as shown in Figs. 2 and 3. CF and IHA titers rose about 10 days after infection and remained unchanged for at least 2 months in most cases. There was individual difference in rise and fall of antibody

Fig. 2. Rise and fall of complement-fixing (CF) antibody titers in 19 patients with respiratory tract disease proven serologically to be *M. pneumoniae* infection

Fig. 3. Rise and fall of indirect-hemagglutinating (IHA) titers in 20 patients with respiratory disease proven serologically to be *M. pneumoniae* infection

TABLE 2. *Effect of 2-mercaptoethanol (2-ME) on complement-fixing (CF) and indirect-hemagglutinating (IHA) antibody titers to Mycoplasma pneumoniae in sera from patients with Mycoplasma pneumoniae infections*

Case No.	Age (years)	Time after clinical onset	CF antibody		IHA antibody	
			Control	2-ME treated	control	2-ME treated
1	4	4 d.*	32	4	<10	<10
		17 d.	64	64	320	10
		12 m.**	32	32	20	10
2	5	2 d.	8	N.T.***	<10	N.T.
		22 d.	64	16	320	20
		9 m.	8	8	40	10
3	8	6 d.	32	32	<10	<10
		27 d.	128	64	320	20
4	9	15 d.	16	16	<10	<10
		35 d.	512	256	2,560	20
5	11	7 d.	16	8	20	20
		28 d.	64	32	160	<10
6	31	10 d.	129	16	160	10
		59 d.	32	8	320	<10
		10 m.	16	8	20	20
		2 d.	8	N.T.	10	N.T.
8	34	39 d.	128	128	320	160
		9 m.	64	32	40	<10
		7 d.	16	8	80	40
		28 d.	128	16	640	20
	36	11 m.	64	64	40	20
		13 d.	256	64	1,280	160
		34 d.	2,048	258	5,120	160
		10 m.	64	64	160	20

*d, days.

**m, months.

***N.T., not tested.

titers, but no significant relationship could be observed between the severity of illness and antibody titers.

There was no significant rise of antibody titer against influenza A2, B and adenovirus.

Effect of 2-ME treatment. Treatment with 2-ME did not give rise to any significant decrease in CF titers in sera obtained during the period from 9 to 12 months after *M. pneumoniae* infection in all cases, but a marked decrease was noticed in some cases in sera taken within 2 months after onset of illness. IHA titers markedly decreased in most cases regardless of the stadium of disease (Table 2).

EPIDEMIOLOGY

An outbreak of respiratory tract disease occurred among collective housing populations during 8 months period from May to November, 1965, with high incidence in summer. In total 274 patients in 203 families were affected with

Fig. 4. Distribution of 274 patients with respiratory infection by month.
(%, percentage of atypical pneumonia to acute respiratory infection in month concerned)

respiratory tract disease. The distribution of 274 patients with respiratory infection by month is shown in Fig. 4. And, 28% of the 274 patients with respiratory infection had pneumonia.

CLINICAL FEATURES

Symptoms and signs in 37 patients with respiratory tract infection associated with *M. pneumoniae* are listed in Table 3. Coughing was noticed in all cases and persisted for a long time. Coughing persisted over 2 weeks in 47% of the cases. Generally coughing persisted much longer in children than in adults. Relationship between clinical symptoms and antibody response to *M. pneumoniae* in 53 patients with respiratory tract infection is shown in Table 4. Twenty nine out of 36 patients (81%) with pneumonia had positive antibody titers measured by CF and/or IHA tests, and 29 out of 37 patients (78%) with respiratory tract infection had pneumonia. The lower portion of the right lobe of the lung in a roentgenogram of the chest was most commonly involved. Bilateral sides of the lung were

TABLE 3. *Symptoms and signs of 37 patients with Mycoplasma pneumoniae infection*

Symptoms and signs	Percentage
Cough	100.0
Fever	94.0
Sore throat	9.4
Headache	7.7
Sputum	7.7
Nasal discharge	9.4
Nausea	7.7
Vomiting	5.7
Abdominal pain	3.8

TABLE 4. *Relation between clinical diagnosis and results of serologic tests in patients with respiratory tract infection*

Clinical diagnosis	Results of serologic tests		Total
	Number of cases with positive antibody	Number of cases without positive antibody	
Pneummonia	29	7	36
Bronchitis	4	1	5
Upper respiratory disease	4	8	12
Total	37	16	53

involved in 2 cases. Infiltration in the lung disappeared within 2 weeks in most cases. Infiltration disappeared more rapidly in children than in adults. There was only one patient with otitis media. There were 3 cases with exanthem. Two patients were 6 years of age and one was 40 years of age. Significant rise in antibody titers measured by CF and IHA tests was noticed in a child with exanthem. No rise of antibody titers measured by CF and IHA tests was noticed in the other 2 cases, but both CF and IHA titers in the first and second serum specimens gave evidence of *M. pneumoniae* infection. The rashes were described as urticarial in 3 cases, and vanished quickly without pigmentation.

LABORATORY ASPECTS

Leucocyte counts on 75% of the patients ranged from 5,000 to 8,000 per cubic millimeter. Sera from 15% of the patients with proved *M. pneumoniae* infections, when tested for CA, had titers of 1:64 or more. Erythrocyte sedimentation rates were elevated in most cases. The values over 20 mm in the first hour were obtained in 82% of the patients.

DISCUSSION

M. pneumoniae has been thought to be not highly contagious, so that few reports appeared in the past on the epidemic in a civilian population. But in a group having the opportunity for close contact, the infection spreads readily though slowly.^{2,10-13}

The failure to isolate the organism from patients may have been due to the freezing and thawing of all specimens before inoculation and due to the use of bouillon for the collection of throat swabs. But serologic studies revealed that main causative agent of the epidemic was *M. pneumoniae*. High incidence of pneumonia over 3 months period indicates that the spread of *M. pneumoniae* is slow but easy in close contact.

An outstanding symptom of *M. pneumoniae* was cough, particularly during night. Cough was very troublesome in children.

Skin rashes have been described by Foy *et al.*⁸ and Copps *et al.*³ Skin manifestations were recorded in 8% of illnesses due to *M. pneumoniae* in studies in families,⁸ and 11% in a community outbreak of *M. pneumoniae*.³ Skin rashes in the present 2 cases were probably due to *M. pneumoniae* infection.

Only 15% of 37 patients had CA titers of 1:64 or more. The low positive rate is probably because we were unable to obtain more than single early sample for testing.

CF and IHA titers rose about 10 days after the infection and remained unchanged for at least 2 months in most cases, but was declined 9 to 12 months later. Cordero *et al.*¹⁴ tested titers of the tetrazolium reduction inhibition (TRI), hemagglutination-inhibition (HI) and CF antibodies two years after the *M. pneumoniae* infection in individuals with known date of seroconversion whose clinical response had been documented. They reported that approximately half the patients who seroconverted following clinically apparent disease had TRI and HI antibody levels comparable to those during convalescence, and only 6 to 10% had lost detectable antibodies, while those who had inapparent infections showed marked declines in titers; analysis of CF results indicated similar trends.

A sequential synthesis of 19S and 7S antibodies upon antigenic stimulation has been demonstrated. Commonly there is a transient early 19S response followed by subsequent 7S production. Viral antigen has been found to induce CF antibodies almost exclusively of the 7S class, whereas CF antibodies against bacteria, rickettsiae and toxoplasma may involve both 19S and 7S antibodies. Also 19S and 7S antibodies may show different efficiencies in various serologic tests. Cold-agglutinins which often appear in *M. pneumoniae* infections belong to the 19S globulins.¹⁵

2-ME is known to reduce 19S but not 7S antibodies. Our studies revealed that CF and IHA antibodies to *M. pneumoniae* are of both the 19S and the 7S types. The proportion of 19S to 7S antibodies varied considerably between individuals even when the examinations were made at the same time after the clinical onset. However, in the majority of the cases there was an increase of 7S relative to 19S CF antibodies with time after onset, and almost all CF antibodies were 7S a year later. IHA antibodies were predominantly 19S globulins in various stages of illness. These results agree with the data reported by Biberfeld.¹⁶

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