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Antibody to *Mycoplasma pneumoniae* in normal subjects and in patients with chronic bronchitis

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Mycoplasma pneumoniae, formerly known as Eaton agent, causes respiratory illness which varies greatly in severity and type, and, much less commonly, a variety of non-respiratory clinical syndromes (Lambert, to be published). Infection may also be clinically silent, and serological surveys have shown that infection is common in many parts of the world (Hayflick & Chanock, 1965).

The sera of 1150 people mainly domiciled in the south of England were tested for antibody against *M. pneumoniae*. The sera of 140 people with chronic bronchitis were also examined in the same way.

METHODS

Complement-fixing antibody was measured by a standard macro-method, using 2 units of complement, 4 units of antigen and over-night fixation at 4° C. The antigen was at first prepared as described by Chanock *et al.* (1962). In this method, phenol is added to the crude culture grown in liquid medium as a means of decreasing the anticomplementary effect of the preparation. We obtained more satisfactory reduction of anticomplementary effect by washing the cultures, grown to counts of 10^6–10^7 viable units per ml., three times in veronal buffer, and then placing the final suspension in a boiling-water bath for 10 min. Satisfactory antigen was prepared by this method, but batches varied somewhat in titre and specificity. The problem of preparing a constant specific antigen of high titre was solved by the technique developed by Kenny & Grayston (1965). In this method the washed concentrated liquid culture is extracted by chloroform and methanol and then partitioned against 0·1 M–KCl. The final product is a soluble lipid antigen which is specific and of high titre. Antigen titres of 320, using extracts representing 100-fold concentration of the original culture, were commonly achieved. The latter part of the work was done with these extracted antigens and most of the earlier sera were also retested using these antigens. Sera were tested at a dilution of 1/10 and positive sera were titrated in a second test.

Series A consists of 623 sera sent to St George's Hospital Medical School for routine Kahn testing of applicants for visas to the U.S. Embassy. The range was wide, with a predominance of young people, and the only data available were age and sex. These sera may well have included some from people with chronic or recent acute chest illness. Series B consists of 527 unselected sera from in-patients of St George's Hospital whose blood was grouped for any reason. Full clinical

details were available and patients with chronic or recent acute chest illness were excluded. Series C consists of 140 sera from patients with chronic bronchitis attending St George's Hospital, Brompton Hospital, or chest clinics in the London area. All had been diagnosed by chest physicians as suffering from chronic bronchitis, and none of them had experienced a recent acute chest illness.

RESULTS

The proportion of positive sera was similar in series A and B (Table 1), and there was no significant difference between males and females in the prevalence of antibody to *M. pneumoniae* (Table 2). The pooled results of the two series of normal sera show an over-all prevalence of antibody of 18·6 %, rising to a peak of 27 % in the fourth decade, and then declining slowly to 16·4 % in all patients over 40, and 15·5 % in all patients over 50.

Table 1. Mycoplasma pneumoniae *antibody in normal subjects*

	Series A		Series B		Total A and B		
Age (years)	No. tested	No. positive	No. tested	No. positive	No. tested	No. positive	% positive
0–9	—	—	47	5	47	5	10·6
10–19	32	5	27	4	59	9	15·3
20–29	194	30	54	15	248	45	18·1
30–39	160	47	70	15	230	62	27·0
40–49	104	18	100	19	204	37	18·1
50–59	73	13	84	13	157	26	16·6
60 and over	60	9	145	21	205	30	14·6
Total	623	122	527	92	1150	214	18·6

Table 2. Mycoplasma pneumoniae *antibody in normal subjects*

	Male			Female		
Age in (years)	No. tested	No. positive	% positive	No. tested	No. positive	% positive
0–19	45	5	11	61	9	15
20–29	89	17	19	159	28	18
30–39	102	25	24	128	37	29
40–49	71	14	20	133	23	17
50–59	68	11	16	89	15	17
60 and over	84	15	18	121	15	12
Total	459	87	19	691	127	18·4

Of the 140 patients with chronic bronchitis in series C, 50 (36 %) showed antibody to *M. pneumoniae* (Table 3). Their age distribution was, of course, different from that of the normal controls. Table 4 shows a matched group of patients over 40, including 137 of those with bronchitis, and 566 controls. Antibody to *M. pneumoniae* is much more commonly found in patients with chronic bronchitis than in normal people ($P < 0{\cdot}0001$).

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Table 3. Mycoplasma pneumoniae *antibody in chronic bronchitis*

Age *(years)*	No. *tested*	No. *positive*	% *positive*
20–39	*3*	*2*	—
40–49	*19*	*3*	—
50+	*118*	*45*	*38*
Total	*140*	*50*	*36*

Table 4. Mycoplasma pneumoniae *antibody in subjects over* 40

	Normal		Chronic bronchitis	
	No. tested	% positive	No. tested	% positive
Male	233	18	115	32
Female	343	15	22	50
Total	566	16·4	137	35·0

DISCUSSION

Infection by *M. pneumoniae* is evidently common in southern England. The age distribution of antibody in normal subjects conforms with that of clinical infection by this organism, which is common in children (but not infants) and young adults and less common in the middle-aged and elderly.

The greater than normal incidence of antibody to *M. pneumoniae* in patients with bronchitis suggests that their undue tendency to acquire respiratory infections includes a susceptibility to this particular pathogen. The range of antibody titres found (mostly 40 or less) in bronchitis was, however, no different from those found in sera from normal people. Since complement-fixing antibody tends to decline or disappear in time, the findings suggest that *M. pneumoniae* infection remains more common or more persistent in patients with bronchitis at an age when it has become an uncommon infection in normal people. Thus, one-third of patients with bronchitis had antibody and they were nearly all over 40, whereas only one-sixth of normal people over 40 showed antibody to *M. pneumoniae* in their serum.

The relatively transient nature of complement-fixing antibody proved an advantage in demonstrating this tendency of bronchitic patients to go on acquiring *M. pneumoniae* infection into middle and old age. For the same reason, the results using the complement-fixation method must underestimate the total number of past *M. pneumoniae* infections in the normal population tested. Other more recently developed methods of antibody measurement may prove more suitable than the complement-fixation test in future epidemiological studies. Of these, the tetrazolium reduction test (Taylor-Robinson, Sobeslavsky, Jensen, Senterfit & Chanock 1966) appears to be specific and sensitive. The indirect haemagglutination-inhibition test, utilizing sensitized tanned red cells, is sensitive but suffers from the disadvantage of poor specificity. For example, 60 % of sera from children 2–4 years old gave positive results in 1/10 dilution, a result inconsistent with the known

epidemiology of *M. pneumoniae* infection (Taylor-Robinson, Shirai, Sobeslavsky & Chanock, 1966).

The percentage of positive results found at different ages by these two methods suggests that antibody persists for many years; and antibody measured by another new technique, that of haemagglutination inhibition (Feldman & Suhs, 1966), is known to persist for at least 10 years. The complement-fixation method is, however, a satisfactory one for routine work in the diagnosis of Eaton agent infection, especially if a soluble extracted antigen is used (Kenny & Grayston, 1965).

Patients with chronic bronchitis seem to be unduly susceptible to a variety of respiratory pathogens, and the agents associated with exacerbations are those prevalent in the community. For example, Ross *et al.* (1966) found rising antibody titres to influenza A and to respiratory syncytial virus in bronchitic exacerbations when these agents were prevalent, and Somerville (1963) reported an association between respiratory syncytial virus infection and exacerbations of chronic bronchitis at a time when this virus was prevalent in the community. In normal adults respiratory syncytial virus causes no symptoms or minor respiratory illness.

Vaccines against *M. pneumoniae* have already been prepared and tested (Smith, Friedewald & Chanock, 1967; Metzgar *et al.* 1966). They may prove valuable in military and other establishments in which Eaton agent infection is prevalent, but infection in the general civilian population is not common or severe enough to justify their large-scale use. If a prospective study confirms that patients with chronic bronchitis suffer unduly from *M. pneumoniae* infections, vaccination against this organism may have to be added to the protective measures taken for patients with chronic respiratory disease.

SUMMARY

Mycoplasma pneumoniae is a common respiratory pathogen in the south of England. Of 1150 sera from normal people, 19 % had antibody against this organism. Antibody was found with significantly greater frequency in patients with chronic bronchitis.

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