

Antibody to *Mycoplasma hominis* in patients with respiratory disease

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SYNOPSIS An attempt was made to evaluate the role of *Mycoplasma hominis* in respiratory infection in adults. Sera collected from patients admitted to hospital with respiratory infections were examined for antibody to *M. hominis*. The incidence of antibody was no higher than that found previously in patients without respiratory disease.

A number of different species of mycoplasmas can be isolated from the upper respiratory tract of man but little evidence has been produced that they behave as pathogens. The exception is *Mycoplasma pneumoniae* which is well recognized as a respiratory pathogen and is rarely isolated from the oropharynx of healthy individuals. *M. hominis* is a frequent commensal in the genital tract but can also be isolated from the oropharynx in a small percentage of people. A strain of this organism, *M. hominis* DC 63, was used in volunteer studies (Mufson, Ludwig, Purcell, Cate, Taylor-Robinson, and Chanock, 1965) and produced pharyngitis under experimental conditions. These studies raised the question whether *M. hominis* was ever a natural respiratory pathogen.

We report here the results of examining for antibodies to *M. hominis* a collection of acute and convalescent specimens of serum from patients admitted to hospital with acute respiratory disease. In these patients the most frequent diagnosis was pneumonia, with exacerbation of chronic bronchitis as the next most frequent condition. The antigenic heterogeneity of *M. hominis* strains has been demonstrated (Nicol and Edward, 1953; Purcell, Wong, Chanock, Taylor-Robinson, Canchola, and Valdesuso, 1967) and for this reason more than one strain was used in our investigation. The incidence of antibodies to

M. hominis in this group of patients with respiratory disease is compared with that found previously in others without this condition (Jones and Sequeira, 1966).

Materials and Methods

Three hundred and fifty-two specimens of serum that had been collected from 335 patients admitted to the Brompton Hospital, London, during the years 1966, 1967, and 1968, were kindly made available to us for testing by Dr B. E. Andrews and Dr J. Inglis. Three hundred and fifty-two sera were tested by the metabolic inhibition test. One hundred and eighty-one sera were tested by the complement-fixation test as the remainder of the specimens were found to be anti-complementary. The techniques used have been described previously (Jones and Sequeira, 1966). In addition sera that gave positive results in the screening metabolic inhibition test at a dilution of 1/5 were tested for the presence of antibody using glucose broth containing indicator and the Oxford staphylococcus as the test organism. These tests and the titrations of the metabolic inhibition antibody were performed in microtitre plates to conserve serum.

Complement-fixing antigens were prepared

with *M. hominis* DC 63, a strain kindly provided by Dr D. Taylor-Robinson, Salisbury, and with *M. hominis* M 130, a strain isolated by Dr B. E. Andrews, Colindale, from the respiratory tract of an adult. Also complement-fixing antigen prepared with *M. hominis* PG 21 was obtained from Baltimore Biological Laboratory. These three strains and *M. hominis* H 27, a strain of genital origin, were used for the metabolic inhibition tests.

Results

The age and sex of 198 patients and the sex of a further 105 patients were known. The sera were classified into those collected within 14 days of the onset of symptoms (98 acute specimens) and those collected later in the illness (194 convalescent specimens). Sixty sera were unclassifiable as the date of onset of disease was not known, but as most of the patients had been ill for some time before admission to hospital, it is likely that these sera were mainly in the convalescent category.

All the sera were examined by the metabolic inhibition technique to the four strains of *M. hominis* and 181 sera were examined by the complement-fixation technique using the three antigens. Twenty-four sera (12 acute, 5 convalescent, 7 unclassified) were found to inhibit the Oxford staphylococcus as well as the strains of *M. hominis*; this inhibition was presumed to be due to antibiotic in the serum and these results were deleted from the analysis of the metabolic inhibition test results.

Strains of <i>M. hominis</i>	No. of Sera Positive	No. of Sera with Titres of			
		>1/5 to 1/5	>1/5 to 1/10	1/5	1/10 1/20
H27	5 (1.5%)	2 ^{ab}	0	3 ^{def}	0 0
PG21	6 (1.8%)	1 ^b	1 ^c	3 ^{def}	1 ^b 0
DC63	5 (1.5%)	1 ^b	0	3 ^{def}	0 1 ^b
M130	4 (1.2%)	2 ^{ab}	0	1 ^f	1 ^d 0

Table I Results of testing 328 sera by the metabolic inhibition test¹

¹Individual patients are denoted by letters a-h.

No. of Sera	No. of Sera Reacting with			
	H 27	DC 63	PG 21	M 130
Acute 85	0	0	0	0
Convalescent 189	3 (1.6%)	2 (1.1%)	2 (1.1%)	3 (1.6%)

Table II Incidence of metabolic inhibition antibody in acute and convalescent sera

ISOLATION OF *M. HOMINIS*

During the study a strain of *M. hominis* was isolated from the respiratory tract of one of the patients. There was no detectable complement-fixing antibody to *M. hominis* PG 21 in paired sera from this patient, and no detectable metabolic inhibition antibody using the homologous strain in the test.

METABOLIC-INHIBITION TEST RESULTS

The number of sera that reacted with each strain of *M. hominis* is shown in Table I. The total of 20 positive results was derived from the sera of eight patients (2.4% of the 335 patients in the study) and the pattern of reaction with different strains is shown. In only three patients were the antibody titres in excess of 1/5. In only one patient (c) was there an unequivocal rise in metabolic inhibition antibody titre. The difference in incidence of antibody classified as acute and convalescent is shown in Table II and is not regarded as significant.

COMPLEMENT-FIXATION TEST RESULTS

The sera from 172 patients were tested by the complement-fixation technique. The number of sera with titres of 1/5 or greater with each antigen is shown in Table III. No titres in excess of 1/10 were detected. The number of sera that reacted with one or more of the antigens was 41 (24%). Twenty-three out of 109 male patients were positive (21%) and 18 out of 63 female patients were positive (29%). The incidence of antibody detected in acute and convalescent sera was not significantly different (Table IV).

More sera reacted with DC 63 and M 130 than

Antigen	No. of Sera Positive	No. of Sera with Titres of			
		>1/5 to 1/5	>1/5 to 1/10	1/5	1/10
PG 21	23 (13%)	0	1 ^j	20	2
DC 63	29 (16%)	2 ^{ij}	0	23	4
M 130	30 (16%)	2 ^{ij}	0	21	7

Table III Results of testing 181 sera by the complement-fixation test¹

¹Individual patients are denoted by letters i-j

No. of Sera	No. of Sera Reacting with		
	PG 21	DC 63	M 130
Acute 54	7 (13%)	10 (18%)	10 (18%)
Convalescent 99	13 (13%)	14 (14%)	14 (14%)

Table IV The incidence of complement-fixation antibody in acute and convalescent sera.

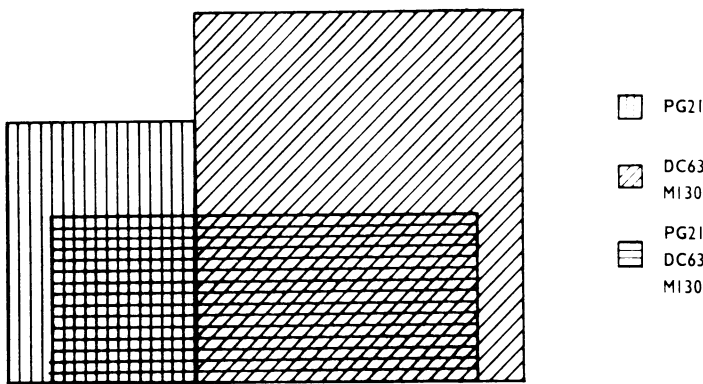


Fig. Representation of the proportions of sera reacting with various *M. hominis* CF antigens.

with PG 21; 10 sera reacted with all three antigens, 19 sera with DC 63 and M 130 only, 11 sera with PG 21 only, and one serum reacted with M 130 only. No reactions were recorded with DC 63 alone or with the combinations of PG 21 and DC 63 or PG 21 and M 130. These relationships are shown in the figure diagrammatically.

Discussion

The frequency with which *M. hominis* has been isolated from the respiratory tract has varied with different workers but has always been of a low order (Organick and Worman, 1966; Kundsinn and Praznik, 1967). Klein, Buckland, and Finland (1969) isolated *M. hominis* from the throat of approximately 3% of neonates and it has been our experience also that this organism may be isolated from a small proportion of newborn babies without any suggestion that it causes symptoms. It may be that those individuals who acquire this organism in the throat in early life continue to carry it in adulthood as a commensal.

Glezen, Thornburg, Chin, and Wenner (1967), studying children during two winter periods, and Stewart and Chowdray (1968), studying a large number of adults and children, failed to isolate *M. hominis* from patients with respiratory disease and from controls without this disease. However, during a prolonged microbiological study in the United States, Mufson, Chang, Gill, Wood, Romansky, and Chanock (1967) isolated mycoplasmas other than *M. pneumoniae* from 53% of 427 patients with pneumonia and from 47% of 1,134 of patients without respiratory disease. These workers identified a random half of the mycoplasmas they isolated; 6.9% of those

from the pneumonia group were *M. hominis* whereas 3.4% of them from the controls were this organism. They demonstrated antibody to *M. hominis* in 3.8% of the pneumonia group and 0.3% of controls and on this evidence suggested that *M. hominis* might be associated with pneumonia. It was during this study that *M. hominis* DC 63 was isolated from a patient with pneumonia who subsequently developed homologous fluorescent-stainable and complement-fixing antibody. Complement-fixing antibody to *M. hominis* DC 63 was not, however, demonstrated in any of the 17 other patients from whom *M. hominis* was isolated. In our one respiratory patient from whom *M. hominis* was isolated, no immunological response to this organism was demonstrable.

Mufson *et al* (1965), by giving a large intranasal dose of *M. hominis*, produced pharyngitis with concomitant antibody response, measurable by indirect haemagglutination, but significantly not by complement fixation.

The form of the immunological responses in puerperal infections associated with *M. hominis* has been recorded (Stokes, 1955; Tully and Smith, 1968; Jones and Tobin, 1969), and in one of the cases studied by Stokes a mycoplasma was isolated from an empyema following lobectomy. By inference, if *M. hominis* played a part, even only a subsidiary role, in respiratory infections, then an increased incidence of antibody would be expected in the patients we have studied compared with others without respiratory disease.

The incidence of metabolic inhibition antibody in these patients with respiratory disorders (2.4%) is of the same order, although lower than that detected in over 600 hospital patients in the earlier study (females 4.5%, males 3.2%). This lower result may have been affected by the detection of 7% of the apparently positive metabolic inhibition sera from the analysis as they contained antibiotic.

The incidence of complement-fixation antibody detected by a single antigen in the present study ranged from 13 to 16% but the three antigens together detected antibody in 24% of the sera. In the earlier study the incidence of complement fixing antibody with one antigen was 16% in 2,265 females and 10% in 560 males. This has demonstrated the value of using more than one antigen in a serological survey for antibody to *M. hominis*.

Allowing for differences in the age and sex distribution of the patients in this study, there was no significant difference in the incidence of antibody in this group as detected by any single antigen compared with that found in patients in the earlier study. No significant differences between sera classified as acute and convalescent were demonstrated. We conclude that in the absence of any detectable increase in incidence of antibody, using techniques known to be capable of detecting such responses, it is unlikely that *M.*

hominis had any part in the illness of the group of patients studied.

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