A study of the specific IgM antibody response in *Mycoplasma pneumoniae* infection in man

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(Received 8 April 1982; accepted 9 October 1982)

SUMMARY

Sera from 96 patients with *Mycoplasma pneumoniae* infection were examined for specific IgM antibody. The complement fixation test was used to measure the IgM after separation by ultracentrifugation through sucrose density gradients. Of patients under the age of 20 years 78% displayed positive specific IgM titres. In older patients only 34% were IgM positive. The specific IgM remained detectable, but at decreasing levels, during the follow-up period of 5 months. This diagnostic technique appears to be of value in children and young adults experiencing primary infection with *M. pneumoniae*.

INTRODUCTION

Mycoplasma pneumoniae is an important pathogen causing a variety of syndromes in man. Those associated with the respiratory tract often reach epidemic proportions, mainly during the winter months. The organism is difficult to culture, and diagnosis is usually made by serological methods based on the demonstration of a significant rise in titre of specific antibody in paired serum samples obtained during the acute and convalescent stages of the disease. The time interval between the sera should be at least 1 week, which results in a delay in diagnosis. Since many of the antimicrobial agents most commonly used in the treatment of bacterial respiratory infections are ineffective in the treatment of *M. pneumoniae* infections (Watson, 1977), a rapid serological test yielding a presumptive diagnosis would be advantageous to the clinician in the early selection of appropriate chemotherapy (Emmons, Schluederberg & Cordero, 1969; Caruntu *et al.* 1976; Skaug *et al.* 1976).

As in other microbial infections, the initial antibody response after *M. pneumoniae* infection is of the IgM type, with later predominance of IgG. This has led to the development of tests for the detection of *M. pneumoniae*-specific IgM antibody in the patient's serum. These include an indirect staphylococcal radioimmunoassay (Brunner *et al.* 1978), an indirect immunofluorescent antibody test (IFA) (Biberfeld, 1971; Skaug *et al.* 1976) and an enzyme immunoassay (Raisanen, Suni & Leinikki, 1980). The complement fixation test (CFT), which is routinely used for the serological diagnosis of many viral infections, has also been adapted for rapid diagnosis of *M. pneumoniae* infection. Serum may be treated with 2-mercaptoethanol to disrupt the IgM molecule, which results in a decrease in the

specific CF antibody titre compared with untreated sera (Emmons *et al.* 1969). Alternatively, the immunoglobulins may be initially separated by ultracentrifugation in a sucrose density gradient before performing the CFT (Schmidt *et al.* 1966).

The aim of our study was to assess the value of specific IgM antibody detection in single serum samples in the laboratory diagnosis of recent *M. pneumoniae* infection. Investigations were also performed concerning the prevalence of *M. pneumoniae*-specific CF antibody in healthy individuals in the general population.

MATERIALS AND METHODS

Serum specimens

Sera were obtained from patients in whom a fourfold or greater rise in *M. pneumoniae*-specific CF antibody, or single high stationary titres (≥ 256) were demonstrated. These criteria were considered evidence of current or recent infection with the organism. Ninety-six patients were investigated for specific IgM antibody, and further specimens were obtained from 18 IgM positive patients up to 5 months after onset of illness. Sera received for screening for rubella antibody or endocrinological disorders from 382 individuals were included as controls. All sera were stored at -20°C until required for testing.

Sucrose density gradient centrifugation

Fractionation of selected sera was performed by centrifugation on sucrose density gradients. A 0.5 ml volume of a 1:2 dilution of serum was inactivated at 56°C for 30 min, cooled to 4°C , and layered onto a linear sucrose gradient (12.5–37.5% (w/v) sucrose in Oxoid CFT diluent) (Best, Banatvala & Watson, 1969). The gradients were centrifuged at 130000 *g* for 18 h at 4°C . Eight fractions were collected from the base of the tube. Gel diffusion was used to confirm the presence of IgM and IgG in the fractions, using commercially available IgM and IgG antisera prepared in sheep (Wellcome Reagents Ltd., Beckenham, England).

Complement fixation tests

Complement fixation tests were performed on whole sera and sucrose fractions in microtitre plates (Bradstreet & Taylor, 1962). All sera and fractions were tested using *M. pneumoniae* CF antigen supplied by the Division of Microbiological Reagents and Quality Control, Central Public Health Laboratory, Colindale, England. Sera from each patient were tested in parallel. The quantity of *M. pneumoniae*-specific IgM present in the sucrose gradient fractions was expressed as a percentage of the total antibody detected.

RESULTS

Clinical features

The majority (57%) of the 96 patients with serological evidence of recent *M. pneumoniae* infection presented with respiratory tract infection. Other recorded clinical features were as follows: fever (18%), myalgia or arthralgia (15%), CNS symptoms (7%), rash (7%), lymphadenopathy (6%), cardiac involvement (4%), gastrointestinal symptoms (3%) and no data was available for 7%.

Table 1. *Specific IgM antibody in the serum of 96 patients with M. pneumoniae infection*

Age group	Number	IgM positive
7 months-4 years	13	9 (69%)
5-9 years	15	12 (80%)
10-14 years	10	8 (80%)
15-19 years	8	7 (88%)
≥ 20 years	50	17 (34%)

Fig. 1. Levels and persistence of *M. pneumoniae*-specific IgM. ●—●, Patients aged 7 months to 19 years. ○—○, Patients aged ≥ 20 years.

Specific IgM determination

Of the 96 patients studied, 31 were initially diagnosed on the basis of a fourfold or greater rise in titre, and 65 on the basis of a titre of ≥ 256 . The occurrence of specific IgM antibody in these two groups was not significantly different and therefore the two groups are merged for further analysis. Table 1 shows the results of the specific IgM antibody tests studied in the 96 patients. Overall 78% of children and young adults up to the age of 20 years had specific IgM antibody detected in their sera. By contrast, over the age of 20 years, only 34% of patients displayed specific IgM antibody.

Follow-up sera were available from 19 patients with detectable IgM antibody in their first serum. The percentage of *M. pneumoniae*-specific IgM antibody peaked during the first month of infection and remained detectable, but at decreasing levels, during the follow-up period of 5 months (Fig. 1). One patient had no detectable specific IgM antibody 5 months after infection.

Variable titres of *M. pneumoniae* CF antibody were detected amongst 309 (79 %) of the 382 controls. Thirty of the group had titres ≥ 256 . Although this titre is considered to be diagnostic of recent infection, none had *M. pneumoniae*-specific IgM. They were all over 20 years of age.

DISCUSSION

The CFT for the diagnosis of recent or current *M. pneumoniae* infection depends on the demonstration of either a significant rise in antibody titre in paired sera or a high antibody titre in a single specimen. For the latter criterion to be valid, it is helpful to know the antibody titres in the general population. In our study we found that titres of ≥ 256 were relatively uncommon in the control group (8 %). We therefore regard such a titre as highly suggestive of recent infection, although lower titres have been considered significant by other workers (Stallman & Allan, 1970). Nakamura *et al.* (1970) studied the persistence of serum antibody following *M. pneumoniae* infection and found that titres of 256 can persist for at least 6 months. It is possible therefore that even individuals with a titre of 256 have had a remote infection and it is notable that none of the 30 controls with high titres in this study had detectable specific IgM antibody.

We have shown that sucrose density gradient centrifugation with CF tests on the fractions detects *M. pneumoniae*-specific IgM. This method may be used to confirm a diagnosis using single sera taken in the early stages of the illness. It is particularly useful in children and young adults, as is evident from the 78 % of these patients who displayed a positive specific IgM titre. Over the age of 20 years, only 34 % of patients had positive IgM titres, which indicates that this technique may be applied to diagnosis in this group with a lower degree of success.

In terms of the frequency of specific IgM detection our results are in agreement with Biberfeld (1971) who investigated the persistence of *M. pneumoniae*-specific IgM by IFA and CFT. He found that the majority (77 %) of children and young adults (aged 5–19 years) produced a specific IgM response, whereas only 34 % of older patients (≥ 20 years) produced this response. His study showed that the specific IgM content of the serum increased during the first few weeks after the onset of illness and fell off gradually. Eleven out of 20 sera were positive for specific IgM by IFA 2–4 years post infection. Ten of these 11 sera had positive CF IgM antibody at this time. These results are in contrast to the present study. We were only able to follow up some of the patients for 5 months and only 1 of 19 had become specific IgM negative at this time. However, the slope of the decline in the others suggests that they would become negative 6–12 months after the infection. These results are more consistent with those of Skaug *et al.* (1976) who determined the diagnostic value of *M. pneumoniae*-specific IgM using IFA. They found that nine patients remained IgM positive for six months, but all patients in their study were IFA IgM negative 8–10 months post infection.

Our study shows that recent infection with *M. pneumoniae* can be confirmed by finding high levels of specific IgM. Low levels are more difficult to interpret. The usefulness of the test is very much related to the age of the patient, being much greater in those under 20 years of age. This is presumably due to the fact that most individuals experience primary *M. pneumoniae* infection in childhood or adolescence

and produce a specific IgM response at that stage. Subsequent exposure results in a secondary response without IgM production.

We are grateful to Mr P. J. Pead, Public Health Laboratory, Portsmouth, and Dr M. S. Shafi, Public Health Laboratory, Central Middlesex Hospital, for their advice in the preparation of this paper. Our thanks are also due to Marjorie Davis for typing the manuscript and our colleagues in the Virus Department, Public Health Laboratory, Portsmouth, for their technical assistance.

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