The specific IgM response to *Mycoplasma pneumoniae* infection: interpretation and application to early diagnosis

By J. H. MOULE, E. O. CAUL

Bristol Public Health Laboratory, Myrtle Road, Kingsdown, Bristol BS2 8EL

AND T. G. WREGHITT

*Clinical Microbiology and Public Health Laboratory, Level 6, Addenbrooke's
Hospital, Hills Road, Cambridge CB2 2QW*

(Accepted 28 July 1987)

SUMMARY

Specific IgM antibody production in patients with serologically proven *Mycoplasma pneumoniae* infection by the complement fixation (CF) test was studied using a μ -capture ELISA. Sera from 79% of patients were found to be IgM positive. Patients could be divided into two groups relating to the amount of specific antibody produced. High levels of specific IgM (≥ 10 units) were more commonly found in younger patients. Seventy-six per cent of patients under the age of 20 produced relatively high levels of IgM compared to 35% of patients over the age of 20. In contrast, the number of patients who produced low or undetectable levels of IgM (< 10 units) was found to increase with age. This trend was found to be significant which suggests that low or undetectable levels of IgM may be due to reinfection with *M. pneumoniae*. Specific IgM was found to appear in the serum at approximately 7 days after the onset of symptoms, peaking at between 10 and 30 days, and then falling to undetectable levels at an estimated 12-26 weeks post onset of symptoms. Twenty-eight per cent of acute-phase sera (CF titres < 256) from patients whose sera subsequently showed a fourfold or greater rise in *M. pneumoniae* CF antibody titre were IgM positive. Thus using μ -capture ELISA a diagnosis of *M. pneumoniae* infection may often be made more rapidly than by the complement fixation test.

INTRODUCTION

Mycoplasma pneumoniae causes a wide spectrum of respiratory infection ranging from inapparent or mild upper respiratory tract infection to pneumonia. Although only 3-10% of people infected with *M. pneumoniae* actually develop pneumonia (Forsyth & Chanock, 1966), the organism is the most common cause of primary atypical pneumonia, and in a study in Seattle, Washington it accounted for 20% of all pneumonia cases in the general population (Foy *et al.* 1970).

Specific diagnosis of infection is based upon isolation of the organism or by serological methods. *M. pneumoniae* is difficult to culture, requiring specialized

media (Chanock, Hayflick & Barile, 1962), and therefore laboratory diagnosis of infection is most commonly achieved by serological means. The complement fixation test (CFT) is the most widely used method of serological diagnosis. A high titre of antibody (≥ 256) in a single sample of serum, or a significant rise ($\geq$ fourfold) in antibody titre in paired serum samples obtained during the acute and convalescent phases of the illness is generally regarded as diagnostic of recent infection. However, three major difficulties are encountered with the CFT. First, a time interval of at least a week between acute and convalescent sera is usually required to detect a significant rise in titre. Secondly, because complement-fixing (CF) antibody levels may be detected at relatively high levels for up to several years after infection (Nakamura *et al.* 1970) the significance of CF antibody titres is often difficult to assess, especially if only a single serum sample is received. Thirdly, the CFT is not a highly specific assay, and false positive results have been recorded in patients with extrapulmonary manifestations (Leinikki, Panzar & Tykka, 1978).

A test designed to detect the presence of specific IgM has two main advantages over the CFT. First, because the initial antibody response after primary *M. pneumoniae* infection is of the IgM type, diagnosis of infection may be made earlier than by CFT, and this can be achieved with a single serum specimen. Since many antimicrobial agents used in the treatment of bacterial infections are ineffective in the treatment of *M. pneumoniae* infections (Watson, 1977), a serological test enabling the rapid diagnosis of the disease is advantageous in the selection of early and appropriate chemotherapy. Secondly because IgM, in contrast to IgG, is usually only detectable for a short time after infection, an IgM test may be used to confirm recent infection with *M. pneumoniae*. A number of assays have been developed for the detection of *M. pneumoniae* IgM, and these include CFT (Chamberlain & Saeed, 1983), indirect immunofluorescence (IFA) (Sillis & Andrews, 1978), radioimmunoassay (RIA) (Hu *et al.* 1983; Price, 1980) and indirect ELISA (Dussaix, Slim & Tournier, 1983; Raisanen, Suni & Leinikki, 1980). Recently, a μ -capture ELISA has been developed for the detection of *M. pneumoniae* IgM which is both sensitive and specific (Wreghitt & Sillis, 1985). We have developed this assay to study the nature of the IgM response in serologically proven *M. pneumoniae* infection in order to establish its usefulness in routine diagnosis.

MATERIALS AND METHODS

Test sera

Patients whose sera showed a fourfold or greater rise in *M. pneumoniae* CF antibody, or single high titres (≥ 256) were included in this study. These criteria, in conjunction with a compatible clinical picture, were considered evidence of current or recent infection with the organism. Acute and convalescent sera from 110 patients and single serum samples from a further 50 patients were tested for specific IgM antibody by μ -capture ELISA. In addition, serum samples were collected from 100 control patients who presented with atypical pneumonia but who had no evidence of recent *M. pneumoniae* infection by CFT. Sera were received for routine testing between 1976 and 1984 and were subsequently stored frozen at -20°C . Further specimens were obtained up to 20 weeks after onset of

illness from 13 patients whose sera were initially positive for *M. pneumoniae*-specific IgM.

Control sera for specificity of assay

In order to evaluate the specificity of the μ -capture ELISA, serum samples positive for rheumatoid factor and sera from serologically proven cases of recent infections with *Chlamydia psittaci*, *Coxiella burneti*, *Legionella pneumophila* and Epstein-Barr virus were also tested in the assay. A total of 10 sera in each of these groups were tested.

μ -capture ELISA

The standardization of the ELISA used in this study including details of the test procedure and methods of antigen and conjugate preparation has been described elsewhere (Wreghitt & Sillis, 1985). In this study a different positive control serum was used to that described previously, so that the cut-off value was established at 1 unit rather than 0.33 unit (Wreghitt & Sillis, 1985).

Complement fixation test

All complement fixation tests were carried out according to the method of Bradstreet & Taylor (1962) using standardized reagents. *M. pneumoniae* lipid antigen was supplied by the Division of Microbiological Reagents and Quality Control, Central Public Health Laboratory, Colindale, London. Guinea-pig complement in 20% Richardson's preservative was obtained freeze-dried and was reconstituted in sterile distilled water.

RESULTS

IgM response in relation to age

Sera from 126 (79%) patients with serological evidence of *M. pneumoniae* infection were IgM-positive and 34 (21%) were IgM-negative. Of the 110 patients from whom both acute and convalescent phase sera had been obtained 89 (81%) were IgM-positive, compared to 37 (74%) patients from whom only single serum samples were obtained. It was found that those patients who were IgM-positive could be divided into two groups: (1) those producing relatively high levels of specific IgM (≥ 10 units) in convalescent phase sera and (2) those in whom specific IgM was detectable only at a low level (≥ 1 unit but < 10 units) in convalescent phase sera. It was found that there was quite a marked degree of polarization between these two groups - no patient was found who produced between 6 and 10 units of IgM (Fig. 1). Of the 110 patients from whom paired sera had been obtained, the patient's age was known in 98 cases. It was found that with increasing age there was a relative decrease in the number of high-level IgM-positive patients, with a corresponding increase in the number of low-level IgM-positive and IgM-negative patients (Table 1). A chi-square test for trend (Peto *et al.* 1978) yielded 7.08, showing that this trend is significant ($P < 0.01$).

Fig. 1. Levels of specific IgM in convalescent sera from patients with *M. pneumoniae* in relation to age. ●, Strong positive sera (≥ 10 units IgM); ○, weak positive sera (≥ 1 unit but < 10 units IgM); □, negative sera (< 1 unit IgM).

Table 1. *Specific IgM production in the sera of 98 patients in relation to age*

Age (years)	Total patients	IgM-positive			IgM-negative	
		Total (%)	≥ 10 units	$\geq 1, < 10$ units	< 1 unit	
1-10	7	6 (86%)	4	2	1	
11-20	26	24 (92%)	21	3	2	
21-30	20	13 (65%)	10	3	7	
31-40	21	14 (67%)	5	9	7	
41-50	12	8 (67%)	7	1	4	
> 50	12	4 (33%)	1	3	8	
Total	98	69 (70%)	48	21	29	

IgM response in relation to onset of symptoms

The date of onset of symptoms was known for 44 of the 89 *M. pneumoniae* IgM-positive patients from whom both acute and convalescent sera had been obtained. It can be seen (Fig. 2) that IgM was only detected at very low levels (< 3.3 units specific IgM) up to 7 days after the onset of symptoms. On or after the seventh day after onset, IgM could be demonstrated at high levels (≥ 10 units specific IgM) in most sera. However, some patients did not have detectable IgM in their sera until up to 15 days after the onset of symptoms. The peak of IgM production was found to be between 10 and 30 days after the onset of symptoms.

When sera obtained from 13 patients with recently diagnosed *M. pneumoniae* infection were tested for specific IgM by μ -capture ELISA at various times after the onset of symptoms, the level of *M. pneumoniae* IgM decreased with time. One patient was found to have become IgM-negative 20 weeks after the onset of symptoms. For the remaining 12 patients it was not possible to say when they became IgM-negative, as insufficient follow-up samples were collected. However, the rate of decline in IgM in the sera from these patients would suggest that negative values would be reached between 12 and 26 weeks after onset.

Fig. 2. Specific IgM production in patients with *M. pneumoniae* in relation to time after onset of symptoms.

Early diagnosis of infection

In order to assess the clinical value of the μ -capture ELISA in the early diagnosis of *M. pneumoniae* infection, acute-phase sera (from patients whose sera subsequently showed a fourfold or greater rise in *M. pneumoniae* CF antibody titre) were tested for the presence of specific IgM. Of 101 acute-phase sera with less than significant levels of CF antibody (CF titre < 256), 38 (38%) were found to be IgM-positive. Of these 101 sera 43 had undetectable levels of CF antibody (CF titre < 16), of which 12 (28%) were found to be IgM-positive.

Specificity of the assay

Control sera used to investigate the specificity of the μ -capture ELISA were all negative for *M. pneumoniae* IgM by μ -capture ELISA (< 1 unit). A further 100 sera from patients presenting with primary atypical pneumonia, but with no evidence of recent infection with *M. pneumoniae* when tested by CFT, were included as controls and were all *M. pneumoniae* IgM-negative.

DISCUSSION

In this study we have shown that the μ -capture ELISA described here may be successfully used in the diagnosis of recent infection with *M. pneumoniae*. However, the main limitation of this assay lies in the fact that not all patients with *M. pneumoniae* infection produce specific IgM, there being a significant decrease in specific IgM production in association with increasing age. This difference in specific IgM response may be explained by an increasing incidence of reinfection in the older age groups. Second attacks of pneumonia due to *M. pneumoniae* have been documented following the waning of immunity with time (Foy *et al.* 1977). The decreasing attack rate of infection with *M. pneumoniae* in patients over the

age of 20 years is probably due to acquired immunity, although more than one episode of infection may be necessary to build up resistance (Foy *et al.* 1983). It is possible that reinfections with *M. pneumoniae* are characterized by the production of low or undetectable levels of specific IgM.

Chamberlain & Saeed (1983) have investigated the production of *M. pneumoniae* IgM in relation to age by CFT. They found that 78% of patients under the age of 20 years were specific IgM-positive, compared with only 34% older patients. By μ -capture ELISA we found that 91% of patients under the age of 20 were specific IgM-positive, compared with 50% of older patients. This would therefore suggest that the CFT is insufficiently sensitive to detect low levels of specific IgM. Biberfeld (1971), using CFT and tetrazolium reduction inhibition, found that specific IgM antibody 'predominated' over IgG antibody in 77% of patients aged 5–19 years, compared with 34% of patients aged 20 years or older. However, Biberfeld gives no information regarding the numbers of patients in whom low levels of IgM were detected compared with the numbers of patients in whom specific IgM was undetectable.

Results obtained using the μ -capture ELISA would suggest that during *M. pneumoniae* infection, specific IgM antibody rises sharply on or after the seventh day after the onset of symptoms. However, a few patients did not start to produce IgM until 2 weeks after the onset of the illness. This could be due to the fact that *M. pneumoniae* infections are often characterized by an insidious onset, which may make it difficult to determine the date of onset of symptoms accurately. The peak specific IgM response was shown to be between 10 and 30 days after the onset of symptoms, which is in agreement with the findings of Wreghitt & Sillis (1985).

Sera obtained from patients in the months following proven *M. pneumoniae* infection showed a gradual decline in specific IgM level. However, it was not possible to determine exactly when patients became specific IgM-negative, although the results obtained in this study would suggest that specific IgM reaches undetectable levels at between 12 and 26 weeks after the onset of illness. Obviously further work is required here in order to confirm this. Chamberlain & Saeed (1983) have estimated that specific IgM detected by CF would become negative 6–12 months after onset. Similarly, Skaug *et al.* (1976) found that nine patients remained IFA-specific IgM-positive for 6 months, but all patients were specific IgM-negative 8–10 months post-infection. In contrast, Biberfeld (1971) found that 11 out of 20 sera were positive for specific IgM by IFA 2–4 years post-infection. Wreghitt & Sillis (1985) have demonstrated that the time taken for specific IgM levels to decline depends upon the test employed. When detected by indirect ELISA, specific IgM was found to decline at a much slower rate than by μ -capture ELISA or IFA. This decline in the level of *M. pneumoniae*-specific IgM is most important in assessing the significance of results obtained with single sera. A test that detects specific IgM 2 years after infection will be of little diagnostic use. The μ -capture ELISA described here has the advantage that it may be used in the diagnosis of recent infection with *M. pneumoniae*. A strongly positive result by μ -capture ELISA may be regarded as evidence of recent *M. pneumoniae* infection. However, low levels are more difficult to interpret, as they could be due to a primary infection several months before or could also be due to a current reinfection.

Twenty-eight per cent of acute-phase sera with *M. pneumoniae* CF titres < 16 from patients with rising CF titres to *M. pneumoniae* were specific IgM-positive by μ -capture ELISA. This is partly due to the fact that IgM to *M. pneumoniae* rises shortly before IgG (Skaug *et al.* 1976), and partly due to the greater sensitivity of ELISA over CFT. Of greater significance is the fact that 38 % of acute-phase sera with titres that were not in themselves significant (< 256) were specific IgM-positive. These results would therefore suggest that the μ -capture ELISA might serve a useful role in the early diagnosis of *M. pneumoniae* infection, especially during epidemic years.

REFERENCES

- BIBERFELD, G. (1971). Antibody responses in *Mycoplasma pneumoniae* infection in relation to serum immunoglobulins, especially IgM. *Acta Pathologica et Microbiologica Scandinavica*, section B **79**, 620-634.
- BRADSTREET, C. M. P. & TAYLOR, C. E. D. (1962). Technique of complement-fixation test applicable to the diagnosis of virus diseases. *Monthly Bulletin of the Ministry of Health and the Public Health Laboratory Service* **21**, 96-104.
- CHAMBERLAIN, P. & SAEED, A. A. (1983). A study of the specific IgM antibody response in *Mycoplasma pneumoniae* infection in man. *Journal of Hygiene* **90**, 207-211.
- CHANOCK, R. M., HAYFLICK, L. & BARILE, M. D. (1962). Growth on artificial medium of an agent associated with atypical pneumonia and its identification as a PPLO. *Proceedings of the National Academy of Sciences of the United States of America* **48**, 41-49.
- DUSSAIX, E., SLIM, A. & TOURNIER, P. (1983). Comparison of enzyme-linked immunosorbent assay (ELISA) and complement fixation test for detection of *Mycoplasma pneumoniae* antibodies. *Journal of Clinical Pathology* **36**, 228-232.
- FORSYTH, B. R. & CHANOCK, R. M. (1966). *Mycoplasma pneumoniae*. *Annual Review of Medicine* **17**, 371-382.
- FOY, H. M., KENNY, G. E., McMAHAN, R., MANSY, A. M. & GRAYSTON, J. T. (1970). *Mycoplasma pneumoniae* pneumonia in an urban area. *Journal of the American Medical Association* **214**, 1666-1672.
- FOY, H. M., KENNY, G. E., SEFI, R., OCHS, H. D. & ALLAN, I. D. (1977). Second attacks of pneumonia due to *Mycoplasma pneumoniae*. *Journal of Infectious Diseases* **135**, 673-677.
- FOY, H. M., KENNY, G. E., COONEY, M. K., ALLAN, I. D. & VAN BELLE, G. (1983). Naturally acquired immunity to pneumonia due to *Mycoplasma pneumoniae*. *Journal of Infectious Diseases* **147**, 967-973.
- HU, P. C., POWELL, D. A., ALBRIGHT, F., GARDNER, D. E., COLLIER, A. M. & CLYDE, W. A. (1983). A solid-phase radioimmunoassay for detection of antibodies against *Mycoplasma pneumoniae*. *Journal of Clinical and Laboratory Immunology* **11**, 209-213.
- LEINIKKI, P. O., PANZAR, P. & TYKKA, H. (1978). Immunoglobulin M antibody response against *Mycoplasma pneumoniae* lipid antigen in patients with acute pancreatitis. *Journal of Clinical Microbiology* **8**, 113-118.
- NAKAMURA, S., EBISAWA, I., KITAMOTO, O. & SATO, T. (1970). Persistence of serum antibody following *Mycoplasma pneumoniae* infection. *American Review of Respiratory Diseases* **101**, 620-622.
- PETO, R., PIKE, M. C., ARMITAGE, P., BRESLOW, N. E., COX, D. R., HOWARD S. V., MANTEL, N., MCPHERSON, K., PETO, J. & SMITH, P. G. (1977). Design and analysis of randomized clinical trials which require prolonged observations of each patient. 2. Analysis and examples. *British Journal of Cancer* **35**, 1-39.
- PRICE, P. C. (1980). Direct radioimmunoassay for the detection of IgM antibodies against *Mycoplasma pneumoniae*. *Journal of Immunological Methods* **32**, 261-273.
- RAISANEN, S. M., SUNI, J. & LEINIKKI, P. (1980). Serological diagnosis of *Mycoplasma pneumoniae* infection by enzyme immunoassay. *Journal of Clinical Pathology* **33**, 836-840.
- SILLIS, M. & ANDREWS, B. (1978). A simple test for *Mycoplasma pneumoniae* IgM. *Zentralblatt für Bakteriologie, Mikrobiologie, Infektionskrankheiten und Hygiene, Abteilung 1: Originale, Reihe A: Medizinische Mikrobiologie und Parasitologie* **241**, 239-240.

- SKAUG, K., ENG, J., ORSTAVIK, I. & HAUG, K. W. (1976). The diagnostic value of determination of IgM antibodies against *Mycoplasma pneumoniae* infections by the indirect immunofluorescent antibody test. *Acta Pathologica et Microbiologica Scandinavica*, section B **84**, 170-176.
- WATSON, G. I. (1977). The treatment of *Mycoplasma pneumoniae* infections. *Scottish Medical Journal* **22**, 331-335.
- WREGHITT, T. G. & SILLIS, M. (1985). A μ -capture ELISA for detecting *Mycoplasma pneumoniae* IgM: comparison with indirect immunofluorescence and indirect ELISA. *Journal of Hygiene* **94**, 217-227.