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The antibody response in Legionnaires' disease

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SUMMARY

From 22 patients with Legionnaires' disease, 86 sera were examined for specific serotype 1 IgM and IgG antibodies by the indirect immunofluorescence technique.

No antibody was detectable until 8 days or more from the onset of symptoms. When produced the amount was widely variable and remained detectable for periods from less than 34 days to more than 1 year.

Initially IgM antibody predominated, ten patients produced only IgM in the first 21 days, six produced only IgM in the first 28 days and three did not produce IgG at any time. One patient, and possibly a second, produced only IgG antibody.

Since IgM antibody was still present in one patient after a year it is important not to accept the presence of this as evidence of very recent infection.

It is advisable that any type of serological test for *L. pneumophila* infection should detect the production of both IgM and IgG antibodies.

INTRODUCTION

The isolation of the causative organism (McDade *et al.* 1977) of Legionnaires' disease (Fraser *et al.* 1977) enabled the introduction of serological diagnosis by means of indirect immunofluorescence. McDade *et al.* (1977) established the broad outline of the serological response with a method in which rabbit anti-human conjugate was used to detect both IgM and IgG.

Since the same immunofluorescence technique was introduced into this country early in 1978 the Public Health Laboratory Service records show that over 100 infections have now been diagnosed. There is an obvious need to apply the method with care to take into account variation in the time of appearance of antibody, the type of antibody and the length of time during which it remains detectable.

To satisfy these aspects we have examined sera from a series of Legionnaires' patients from Cambridge, Oxford and Nottingham.

For the purposes of the study a confirmed case was taken to be a patient with a four-fold or greater rise in titre, in conjunction with the clinical features which

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fulfilled the diagnostic criteria (Jenkins *et al.* 1979) and a suspected case was one with a single titre of 1/256 or more. All but one of the patients in this series are regarded as confirmed cases.

MATERIALS AND METHODS

The 86 sera examined were from 11 Cambridge, 6 Nottingham and 5 Oxford patients.

All were considered to be serotype 1 *Legionella pneumophila* infection except one Oxford patient infected with serotype 3.

The antigen used for the serotype 1 infections was formalinized yolk sac suspension prepared from the Pontiac strain and supplied by Dr A. G. Taylor of the Standards Laboratory, Colindale. For the serotype 3, a heat killed homologous antigen was prepared at Oxford.

Indirect immunofluorescence was performed on 5 μl samples of antigen fixed with acetone in the wells of Teflon coated slides. Serum dilutions were added for 30 min at 37 °C and, after washing, sheep anti-human FITC conjugate (Wellcome) was applied for 30 min at 37 °C. The Oxford sera were incubated for 2 h to obtain the maximum IgM staining but the results were sufficiently compatible to be plotted with the others.

The end point was that agreed by two observers as the last dilution to give readily distinguishable specific green fluorescence of the majority of organisms in the antigen.

RESULTS

The titres of specific serotype 1 IgM and IgG antibody in 71 sera of the 86 examined are plotted in Fig. 1. The remaining results are not shown because the samples taken were too close to those plotted.

In no patient was serotype 1 antibody detectable by this method earlier than 8 days from the onset of symptoms and in one instance it was found only after 14 days. The Oxford case was post renal transplant and had unique features since homologous serotype 3 antibody was found on the second day of acute pneumonic illness although heterologous serotype 1 antibody was not detected until the second week of illness. One patient died on the 8th day and diagnosis was obtained only by isolation of the organism (Nagington, Smith & Wreghitt, 1978).

The type of antibody produced was either IgM (3/22 patients), IgG (1 and possibly 2/22 but serum from the second patient was not available for 10 weeks) or both together (17/22) although the titres were then not usually parallel.

The majority of patients (9/13 with sera available) produced only IgM at first and only a few (3/13) produced both IgM and IgG during the first 14 days. Even as late as 28 days, half of those tested (7/14) were only producing IgM and three patients continued to produce only IgM. They were observed for 53, 53 and 100 days respectively.

The maximum geometric mean titres of IgM and IgG were obtained about or shortly after the 28th day.

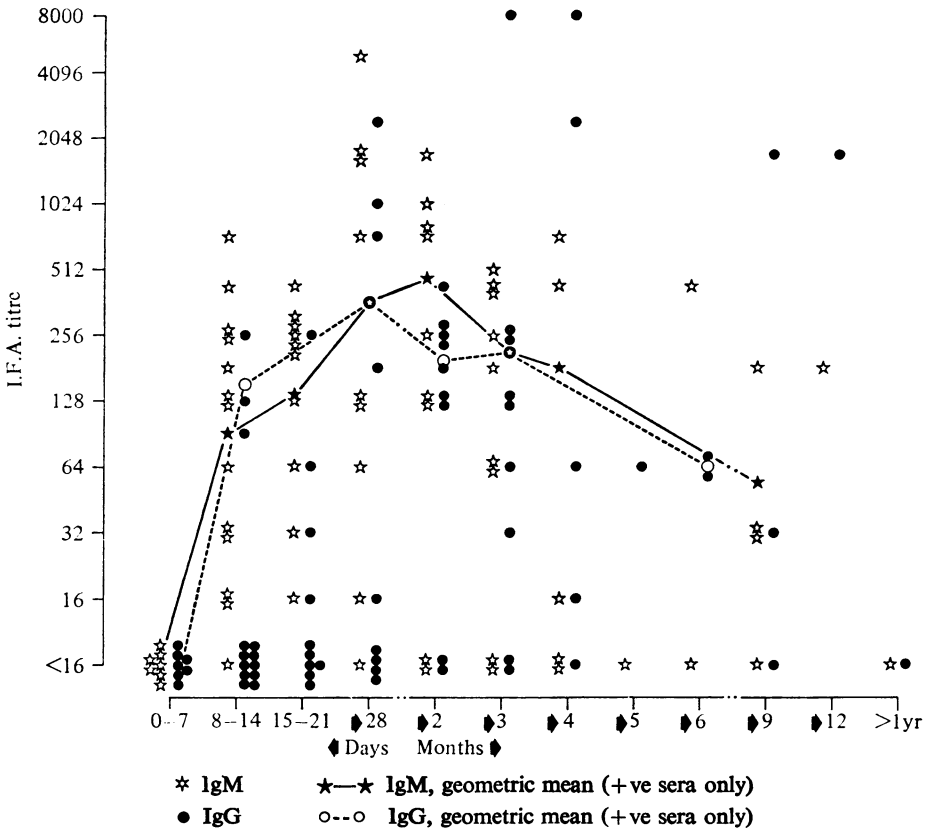

Fig. 1. Specific Type 1 IgM and IgG antibody titres in 71 sera from 22 patients.

The amount of antibody produced was very variable, depending on the individual patient. For this reason, and also because of the sometimes late appearance of antibody, a standard deviation of the mean has no value and is not shown in Fig. 1.

The persistence of detectable antibody was also widely variable, the shortest duration of specific IgM being 34 days and the shortest duration of specific IgG was 9 months. The shortest duration of both antibodies together was 4 months. At the other extreme, one of two patients tested had appreciable IgM and IgG after 12 months.

Some examples of the differing forms of response in individual patients are shown in Figs. 2, 3 and 4.

The results from individual patients, according to the sera available, are shown in Table 1.

DISCUSSION

From the results obtained it is clear that to avoid false negative tests any diagnostic serological method should be capable of detecting both IgM and IgG. Since antibody may be slow to appear a late serum should be obtained from any suspected case at least 28 days after the onset.

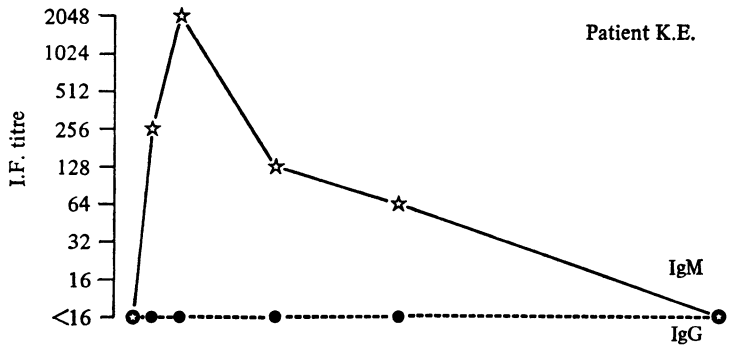

Fig. 2. Antibody response in patient who produced only specific IgM and for short duration.

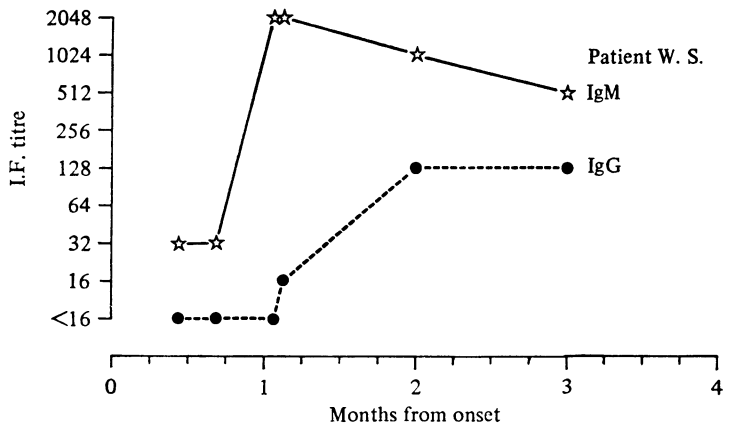

Fig. 3. Antibody response in patient who produced mainly specific IgM and a delayed small IgG titre.

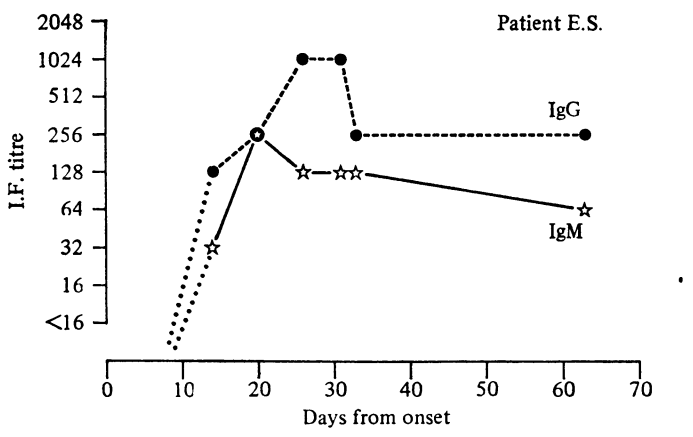

Fig. 4. Antibody response in patient with specific IgG production greater than IgM.

Table 1. *Type of antibody produced by 22 patients*

Day of disease ...	0–14	15–21	22–28	Later
IgM	9	10	7	3
IgG	0	0	1	4
IgM and IgG	3	5	6	8
No antibody	1	0	0	0
No. of conversions of IgM to IgM and IgG (cumulative score)	(0)	(1)	(2)	(6)
Total tested	13	15	14	15

In the case of patients who die within 8 days, antibody is unlikely to be detected and this will also be the case in a proportion of patients who die within 14 days so that lung should be obtained for examination at post-mortem before *L. pneumophila* infection can be excluded.

The persistence of antibody for a year or longer in patients who recover means that caution must be used in the interpretation of serological results. Even specific IgM may persist for more than 1 year. Thus, with such a patient, an attack of influenza might not be distinguishable from Legionnaires' disease if an early serum was not available.

The criteria applied by the P.H.L.S. Working Party on Legionnaires' Disease take account of this by defining a four-fold rise in titre to 1/64 or more associated with severe pneumonia as *diagnostic* of infection and a single titre of 1/256 or more as *presumptive* evidence.

In serological surveys in progress, low titres of antibody are encountered in a small percentage of individuals and it is probable that these represent antibody from infection in the past.

It should be noted that the methods in use for the diagnosis of Legionnaires' disease are under active review and it is thought that the serological pattern described could act as a basis for further development.

We are indebted to our many colleagues who have helped to provide the sera and clinical details of these patients. Also to Mr D. J. Smith and Mrs Elaine Rosenthal for their technical assistance.

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