INFECTION AND IMMUNITY, Feb. 1983, p. 977-981
0019-9567/83/020977-05$02.00/0

Vol. 39, No. 2

Immunoglobulin Responses to *Coxiella burnetii* (Q Fever): Single-Serum Diagnosis of Acute Infection, Using an Immunofluorescence Technique

JON G. HUNT, PETER R. FIELD, AND ALAN M. MURPHY*

Virology Department, Institute of Clinical Pathology and Medical Research, Westmead, New South Wales 2145, Australia

Received 11 August 1982/Accepted 22 October 1982

Blood specimens were collected over various periods of time from 30 abattoir workers with a clinical diagnosis of Q fever. All specimens were tested for complement-fixing antibodies and for specific immunoglobulin M (IgM) globulins to phase 1 and 2 *Coxiella burnetii* organisms by an immunofluorescence technique. All 22 patients with increasing levels of complement-fixing antibodies were shown to have generated specific IgM globulins, as did 4 patients with high convalescent titers but from whom "acute" specimens were not collected. Four individuals who did not show increasing levels of complement-fixing antibodies did not produce measurable levels of specific IgM. All patients with Q fever gave positive specific IgM results by 2 weeks after the onset of symptoms. IgM to phase 1 antigen persisted for 27 weeks in one patient, but IgM to phase 2 antigen was not detectable beyond 17 weeks. The estimation of Q fever-specific IgM has proved useful in confirming infection when only a "convalescent" blood specimen is available.

Q fever is an occupational disease of worldwide significance, having been identified in at least 50 countries. Infection is widespread in domestic animals, who are essentially asymptomatic, but when transferred to humans, severe clinical disease may result. Livestock handlers and abattoir workers of all types are the groups most commonly infected; textile workers and butchers are less commonly infected. Unfortunately, the working conditions of abattoir employees and the manner in which animals are handled make it almost impossible to prevent infection, and, since a satisfactory vaccine for general use has not been produced to date, human cases will continue to occur for the foreseeable future.

Q fever usually presents as an acute febrile systemic disease with few characteristic signs or symptoms to distinguish it from any other febrile illness, such as influenza, brucellosis, or leptospirosis; thus, clinicians rely heavily on laboratory assistance for diagnosis. Cultivation of the organism in the laboratory is a major hazard to personnel, and serological techniques are usually employed for diagnosis, a positive result being arbitrarily determined by a fourfold or greater increase in antibody levels between "acute" and "convalescent" blood specimens. Past experience has shown, however, that the acute blood is frequently not obtained, and, because of the long persistence of immunoglobulin G (IgG) antibodies at various levels after infection, it is virtually impossible to determine with accuracy when antibodies present in a convalescent specimen were produced (5). Only a presumptive diagnosis can be given when the titer is very high. During the years 1979 and 1980, approximately 2,000 cases of Q fever were diagnosed in laboratories throughout Australia, and this is almost certainly only a proportion of the total number. However, it is important to the individual worker that a correct diagnosis be made, as Q fever is recognized as an occupational hazard in this country, and proven cases receive workers' compensation. A more rapid diagnosis will also aid in the commencement of appropriate treatment.

In most primary infections, specific IgM globulins appear in the circulation but remain detectable (by present-day methods) for only relatively short periods of time, varying with the infection, the individual host, and the technique of estimation, for about 4 weeks to 6 months (1). In a previous publication (6) from this laboratory, it was shown that Q fever-specific IgM globulin could be detected for an average period of about 10 weeks after the onset of symptoms and that this test was a useful addition to those already in use. However, the technique required the separation of IgM globulins from IgG by ultracentri-

TABLE 1. Development and persistence of Q fever complement-fixing antibodies and specific IgM globulin

Patient no.	Time (days) after onset of symptoms	Total complement-fixing antibody titer	Specific Q fever IgM by indirect immunofluorescence to	
			Phase 1 antigen	Phase 2 antigen
Primary infections with seroconversions				
1	7	<4	<10	<10
	14	512	160	640
	70, 98	>2,048	40	40
	154	512	<10	<10
	238, 280, 336	128	<10	<10
2	3	<4	<10	<10
	28	256	640	40
	56	256	10	<10
3	<7	<4	<10	<10
	21	64	160	160
	35	64	160	10
	98	64	10	<10
4	<7	<4	NT[a]	NT
	14	<4	<10	<10
	35	64	40	160
	189	32	<10	<10
5	<7	<4	<10	<10
	63	16	160	160
6	<7	<4	<10	<10
	21	64	160	40
7	<7	<4	<10	<10
	14	128	2,560	640
8	<7	<4	NT	NT
	21	512	640	40
	70	512	160	40
	105	512	160	<10
9	4	<4	NT	NT
	14	>2,048	640	640
	203	>2,048	<10	<10
10	<7	<4	<10	<10
	21	512	2,560	160
	56	512	2,560	10
11	7	<4	640	160
	14	1,024	640	40
	350	64	<10	<10
12	5	<4	<10	<10
	21	256	160	160
	189	64	40	<10
13	<7	<4	<10	<10
	14	256	640	160
	56	128	40	40
	791	4	<10	<10
14	3	<4	640	160
	11	64	640	640
	203	32	<10	<10
15	9	<4	40	10
	63	256	40	160
16	13	<4	<10	<10
	17	16	40	40
	35	1,024	640	640
17	<7	<4	<10	<10
	28	16	2,560	40
18	<7	<4	10	<10
	35	32	160	40
19	Preinfection[b]	<4	<10	<10
	14	128	2,560	640
20	Preinfection[b]	<4	<10	<10
	14	32	160	40

TABLE 1—*Continued*

Patient no.	Time (days) after onset of symptoms	Total complement-fixing antibody titer	Specific Q fever IgM by indirect immunofluorescence to	
			Phase 1 antigen	Phase 2 antigen
Without acute specimen collection				
21	56	512	40	10
	119	512	40	40
	259	128	<10	<10
	315	512	<10	<10
	567	64	<10	<10
22	70	64	160	160
	91	64	160	40
	623	4	<10	<10
23	21, 70	512	640	640
	245, 343, 399, 420	64	<10	<10
24	28, 42	256	640	640
	63	256	160	160
25	14	32	160	160
	63	>2,048	40	40
26	10	8	10	10
	21	1,024	2,560	640
With evidence of previous Q fever				
27	3, 14	16	<10	<10
28	<7, 21	64	<10	<10
29	5, 14	8	<10	<10
30	<7, 21	128	<10	<10

[a] NT, Not tested.
[b] Specimens collected 2 years previously.

fugation in a sucrose density gradient. This is a time-consuming and expensive method, and the number of tests which can be carried out is strictly limited. We have therefore developed an immunofluorescence method using whole serum which is simple, rapid, and relatively inexpensive.

All patients studied were abattoir workers with symptoms consistent with a Q fever infection. In some cases, the diagnosis had already been confirmed by rising titers of serum complement-fixing antibodies. Follow-up blood specimens were solicited to determine changes in the specific IgM globulin levels with time.

Phase 1 and 2 antigens ("Nine Mile" strain) prepared for use in complement fixation tests (Commonwealth Serum Laboratories) were used as antigens in the immunofluorescence tests. Electron microscopy confirmed that the antigens consisted of morphologically intact rickettsial bodies. A drop of diluted antigen suspension was spread on a small glass cover slip, allowed to dry, fixed with acetone, and stored at −70°C. The optimum concentration of approximately 1,000 organisms per field (100× oil immersion objective) was determined by titration.

Fourfold dilutions of sera (1/10 to 1/2,560) were made in phosphate-buffered saline. A 0.1-ml amount of each serum dilution was then placed on a sample of dried antigen and allowed to react for 4 h. After washing with phosphate-buffered saline, 0.1 ml of rabbit antihuman IgM globulin labeled with fluorescein isothiocyanate (DAKO) was then added and again allowed to react for 30 min. After washing, the cover slips were mounted on slides in buffered glycerol and examined in a Universal Zeiss microscope with an incident light source (CSI 250W). The fluorescein isothiocyanate-coupled rabbit antisera were regularly checked for nonspecific reaction with the antigens, and all sera giving a positive reaction were checked for rheumatoid factor. In the initial development stages of the test, the specificity of the reaction was checked by blocking tests with rabbit serum containing antibodies to Q fever.

The results of the complement fixation tests and immunofluorescent IgM tests are shown in Table 1. The patients involved can be divided

into three groups. The first group consisted of patients from whom acute and convalescent sera were collected and who showed seroconversion of complement-fixing antibodies (i.e., rising titers from <4). These cases were presumably acute primary infections. All 22 individuals developed detectable levels of specific IgM to both phase 1 and phase 2 organisms. The "preinfection" sera of two patients were collected 2 years before their illnesses during a survey of abattoir workers.

The second group consisted of six abattoir workers, all of whom had clinical symptoms consistent with Q fever but from whom an acute blood specimen was not available, the first specimen having been collected between 1 and 10 weeks after the onset of symptoms. Complement-fixing antibodies were detectable in all these specimens, but four did not show any increase in titer. The other two had low initial titers which increased significantly in a subsequent specimen. All gave positive specific IgM tests.

The third group was comprised of four abattoir workers who developed pyrexia but who had complement-fixing antibodies in their acute sera which did not subsequently increase. None developed Q fever-specific IgM. One individual was shown to have brucellosis, another *Mycoplasma pneumoniae* infection, and a third influenza B. No diagnosis was obtained for the fourth case.

The minimum period of time after which a positive IgM result was obtained varied. One individual was positive 7 days after the onset of symptoms, but others were negative at 11 and 13 days. However, all sera collected 2 weeks after the onset of symptoms were positive. At 8 weeks, one patient was negative for IgM to phase 2 but positive to phase 1. The longest periods at which positive results were obtained were 17 weeks for phase 2 and 27 weeks for phase 1.

Little work has been published on the identity of the immunoglobulins generated by *Coxiella burnetii* infection, although as long ago as 1967 Hobbs et al (3) showed that total serum IgM globulin levels were slightly increased in normal acute respiratory infection and markedly so in Q fever endocarditis. However, the measurement of antibody responses in this disease is complicated by the "phase phenomenon." Initial isolates of the organism are in so-called phase 1, but after several passages in the yolk sacs of fertile eggs, they undergo minor antigenic change to phase 2. The standard method used for the diagnosis of acute infection is to demonstrate increasing levels of complement-fixing antibodies to phase 2 organisms. For some unknown reason it is difficult to demonstrate an early antibody response by complement fixation with phase 1 organisms in acute infection, but high levels can be shown to be present in chronic Q fever endocarditis.

Agglutinating antibodies, however, can be demonstrated in early convalescence with phase 1 organisms. Phase 2 organisms frequently exhibit autoagglutination, so that it is necessary to use phase 1 organisms in this technique (2). It is not known whether these agglutinating antibodies are IgG or IgM, but in the first few weeks after onset of symptoms, they are probably a mixture of both types. Agglutinating antibodies appear more rapidly than those measured by complement fixation; 50% of patients have demonstrable levels in the first week of illness, and 92% of patients have demonstrable levels in the second week. In contrast, complement-fixing antibody is rarely found in the first week, but approximately 65% of patients are positive in the second week (4). However, the complement fixation test is more widely used, chiefly because the antigen is easily prepared. Antibodies remain detectable by both methods for several years, so that the demonstration of increasing titers is obligatory for diagnosis. It is not clear why specific IgM globulin could not be identified in the first week of illness and before the appearance of complement-fixing antibodies. IgM antibodies could be expected to be detectable by immunofluorescence in the first week, as agglutinating antibodies are known to be present and IgM-type globulins usually appear slightly before those of the IgG class. The technique may be too insensitive to demonstrate small amounts of IgM, or this globulin may be slow to appear in the circulation in Q fever infections.

As measured by the complement fixation technique, phase 2 Q fever-specific IgM was shown to be detectable for about 10 weeks, but with the more sensitive immunofluorescence method, we have found persisting detectable levels up to 17 weeks. The disadvantage of this somewhat longer period is more than offset by the much simpler technique which can be used. In two cases, phase 1-specific IgM was demonstrated 27 weeks after the onset of illness, but this was the longest period of time positive results were obtained. In general, phase 1-specific IgM occurred in higher titer and persisted for slightly longer periods. For diagnostic purposes, however, the estimation of phase 2-specific IgM alone is the method of choice and can be readily carried out by using the standard commercial complement-fixing antigen which contains morphologically intact organisms that react satisfactorily in the fluorescence test. Eighteen months of experience with this test has shown that in acute cases of Q fever, when complement-fixing antibodies are detectable,

specific IgM will be present. A diagnosis can thus be made with a single early convalescent serum.

Many acute blood specimens received from abattoir workers have a complement-fixing antibody titer which does not increase in the convalescent specimen. These results would be interpreted as indicative of a past infection with Q fever. In such cases, we have not been able to demonstrate IgM in either serum. Thus, the demonstration of a positive complement fixation test and negative specific IgM in the acute blood is sufficient to exclude Q fever from the differential diagnosis.

The type of immunoglobulin response in second attacks of Q fever is unknown, but Peacock et al. (7) have shown that individuals with previous experience of Q fever, when stimulated with 0.1 ml of vaccine intradermally, respond with IgM antibodies. Secondary infection with Q fever appears to be very uncommon, and whether specific IgM is generated with either symptomatic or subclinical secondary infection has yet to be determined. Certainly, heterologous lower respiratory tract infections do not appear to stimulate a Q fever-specific IgM response. In addition to patients with influenza B, *M. pneumoniae*, and brucellosis who had evidence of previous Q fever infection, we have tested a number of paired sera from other patients with evidence of influenza A, influenza B, *M. pneumoniae*, psittacosis, and adenovirus. All gave negative results.

Likewise, there is little information on the presence of specific IgM in chronic Q fever endocarditis, although, as mentioned above, Hobbs et al. (3) showed high levels of serum total (presumably specific) IgM. Two patients with suspected aortic valve endocarditis recently referred to this laboratory had high levels of both phase 1- and phase 2-specific IgM in addition to phase 1 and phase 2 complement-fixing antibodies. The diagnoses were confirmed after surgical removal of the valve leaflets by demonstrating *C. burnetii* organisms in the valve tissue. It is likely that high levels of specific IgM are common in Q fever endocarditis, for, as Hobbs et al. have pointed out, "continued intravascular stimulation with particulate antigens appears to maintain IgM globulin levels."

It is possible, therefore, in virtually all acute cases, to diagnose Q fever by testing a single convalescent serum collected between 2 and 8 weeks after the onset of symptoms. In practice, we have found that the best approach is to test for Q fever complement-fixing antibodies in the usual way and if this test is positive to then test for phase 2-specific IgM. If this is positive, "a current (or recent) infection" is reported; if negative, we report "evidence of a previous infection."

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