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Serological Evaluation of Q Fever in Humans: Enhanced Phase I Titers of Immunoglobulins G and A Are Diagnostic for Q Fever Endocarditis

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Serological parameters were compared in 15 cases of *Coxiella burnetii* infection comprising 5 cases each of primary Q fever, chronic granulomatous hepatitis, and endocarditis. The diagnosis was made on the basis of clinical history and serology and on the isolation of *C. burnetii* phase I from biopsy specimens of liver and bone marrow from two patients with granulomatous hepatitis and from the aortic valve vegetations of five patients with endocarditis. The temporal sequences of immunoglobulin levels, rheumatoid factor, and specific antibody responses to phase II and phase I antigens of *C. burnetii* were evaluated as predictive correlates of the three Q fever entities. Serum levels of immunoglobulin classes G, M, and A were variable in all the entities of Q fever. Increased mean levels (in milligrams per deciliter) of immunoglobulin G (IgG) and IgA were noted with chronic disease in the sera of some patients, whereas IgM levels were not significantly different from normal values. Rheumatoid factor was significantly elevated in chronic disease but not in primary Q fever. The temporal sequence of *C. burnetii* phase II and phase I antibodies were compared by microagglutination, complement fixation, and indirect microimmunofluorescence tests. All of these serological tests were useful in distinguishing primary from chronic disease. Thus, the ratio of anti-phase II to anti-phase I antibodies was >1, ≥ 1, and ≤ 1 for primary Q fever, granulomatous hepatitis, and Q fever endocarditis, respectively. Moreover, the high phase-specific IgA antibody titers in the indirect microimmunofluorescence test were diagnostic for endocarditis.

Coxiella burnetii causes infections in humans that may be subclinical or acute (classical Q fever) or lead to chronic disease (endocarditis or granulomatous hepatitis) (3, 5, 18, 19, 20, 21, 26, 31, 33, 38, 40, 42, 45, 46). Recent reports of human infection acquired from sheep have renewed concern about the occurrence of Q fever, particularly in its chronic form, in the United States (4, 11, 23, 37).

Of diagnostic importance is the production of antibodies against *C. burnetii* antigens in a temporal manner, with phase II antibodies appearing early and phase I antibodies appearing somewhat later in the course of infection (7, 14, 19, 25, 34). Presence of phase I antibodies correlates well in older age groups with the duration of convalescence after the acute illness (34). Because the phase I surface component antigen is probably less immunogenic than the masked phase II antigen, the antibody response to phase I antigen in patients convalescing from primary disease subsides to low levels detectable by complement fixation (CF) and microagglutination (MA) tests (7). Patients in whom persistent or recrudescent infection leads to chronic disease have greatly increased anti-phase I titers (14, 18, 19, 40, 42, 45), probably because of constant antigenic stimulation. In fact, previous studies have shown that a rising CF antibody titer to phase I antigen reflects a state of chronic disease that may develop into endocarditis (18, 19). Patients with chronic Q fever endocarditis have increased serum levels of gamma globulin (13, 42, 45), including immunoglobulin M (IgM) and IgA (13, 42). CF phase I antibody titers of greater than 1:128 (45) or 1:200 (42) are considered diagnostic for chronic Q fever.

In the present study, we evaluated the serological profiles of the three well-characterized clinical entities of Q fever in humans, namely, primary disease, granulomatous hepatitis, and endocarditis. Our results showed that persistently elevated IgG and IgM antibody titers to phase II antigens were characteristics of granulomatous hepatic disease, whereas increased IgG antibody titers to both phase II and phase I

antigens characterized endocarditis. More important, the presence of specific IgA anti-phase II-I was diagnostic for Q fever endocarditis. Specific diagnostic titers of IgA antibodies were not found in sera from patients with primary or hepatic disease. IgM rheumatoid factor (RF) was present in sera from patients with chronic Q fever but not in sera from patients with primary Q fever infections.

MATERIALS AND METHODS

Antigens and serological procedures. The following purified antigens (28, 44) were each used at a concentration of 200 μg/ml. Antigen 1 (phase I) was from *C. burnetii* cells of strain Nine Mile that had been passaged 306 times in guinea pigs, cloned from primary chicken embryo cell culture plaques (27), and passaged twice in chicken embryos. Antigen 2 (phase II) was from *C. burnetii* cells of strain Nine Mile that had been passaged 94 times in chicken embryos, cloned from primary chicken embryo cell culture plaques, and passaged three times in chicken embryos. Antigen 3 was from *C. burnetii* cells of strain Nine Mile that had been passaged 306 times in guinea pigs, four times in chicken embryos, once in a guinea pig, and three times in chicken embryos and extracted with trichloroacetic acid to remove any trace of phase I antigen and render the antigen reactive only with phase II antibodies (8). Antigens 1 and 2 were used in indirect microimmunofluorescence (IFA) (32) and CF (1) tests, whereas antigens 1 and 3 were used in the MA test (8).

The IFA test was carried out by the procedure of Philip et al. (32) with rabbit or goat antisera labeled with fluorescein isothiocyanate. IFA(G) tests were performed with anti-human IgG prepared in rabbits against chromatographically purified IgG rendered Fc specific (30). IFA(M) and IFA(A) tests were performed with μ-chain-specific goat anti-human IgM (Behring Diagnostics, Somerville, N.J.) and α-chain-specific rabbit anti-human IgA (Miles Laboratories, Inc., Elkhart, Ind.), respectively.

Quantitation of IgG, IgM, and IgA in human sera was determined in a radial immunodiffusion assay (Accra Assay; Miles Laboratories) as described by Fahey and McKelvey (6).

RF interference. Removal of the RF of the IgM class which could produce false evidence for the presence of an IgM antibody to *C. burnetii* in the IFA assay with fluorescein isothiocyanate-labeled IgM antibody was accomplished by the method of Johnson and Libby (17). Representative sera from patients with primary Q fever, granulomatous hepatitis, or endocarditis (see Tables 1, 2, and 3, respectively) were chromatographed on Quick-sep columns (QAE-Sephadex A50; Pharmacia Fine Chemicals, Inc., Piscataway, N.J.; and IsoLab, Inc., Akron, Ohio) to separate and assay the eluted IgM for *C. burnetii* by the IFA assay without interference from IgG or RF.

RF titers of sera in this study were determined with the Rheumatoid Titration test kit (Difco Laboratories, Detroit, Mich.).

Isolation and confirmation of strains. Portions of aortic valve, liver punch, or bone marrow biopsy suspensions in brain heart infusion broth were inoculated intraperitoneally into guinea pigs weighing 400 to 500 g each and into the yolk sacs of 5-day-old embryonated chicken eggs. Guinea pigs that responded with fever (rectal temperature, ≧40°C) were killed on the second or third day of fever. Suspensions of spleen, liver, and kidney were inoculated into nonimmune guinea pigs and embryonated eggs. Yolk sac suspensions from inoculated eggs were subpassaged until growth of rickettsiae was obtained to prepare purified antigens for serological confirmations with standard *C. burnetii* antiserum. Growth of *C. burnetii* in tissues was monitored by direct staining of smear preparations with fluorescein isothiocyanate-conjugated guinea pig anti-*C. burnetii* serum (29).

RESULTS

Brief clinical history. The patients in this study were diagnosed as having primary Q fever (patients 1 through 5), granulomatous Q fever hepatitis (patients 6 through 10), and Q fever endocarditis (patients 11 through 15) (see Tables 1, 2, and 3, respectively). No clinical data was available for the patients with primary Q fever (see Table 1).

Patients 6 through 10 (see Table 2) were diagnosed as having granulomatous hepatitis after hospital admission.

Patient 9 was admitted with a diagnosis of endocarditis. Follow-up clinical investigation, however, revealed hepatomegaly, splenomegaly, and negative blood cultures. The major specific antibody response in all patients was phase II IgG. In addition, serum collected early in the study from patient 7 showed a highly elevated IgM phase II antibody response that suggested recent infection (cf. Tables 2 and 5). The antibody profile of patient 9 showed high phase I IgG titers by IFA and levels of phase I antibody in the MA and CF tests that were consistent with a diagnosis of endocarditis. Three patients had low phase II IgA antibody titers ranging from 1:8 to 1:64. Also, some patients showed transient anticomplementary CF reactions. Phase I *C. burnetii* was isolated from bone marrow punch biopsy from patient 10 and from a liver punch biopsy from patient 9.

Percutaneous liver biopsy, paraffin-embedded, serial thin sections from patients 6, 8, and 9 showed characteristic fibrin-ringed, doughnut-shaped granulomatous lesions (not shown). The wall of the doughnut consisted primarily of fibrinogen, epithelioid cells, lymphocytes, and polymorphonuclear leucocytes and an occasional multinucleated giant cell (31). The center of the granuloma showed no cellular detail and may have been devoid of tissue.

Specific antibody profiles were obtained from patients with Q fever endocarditis (see Table 3). Patient 14 underwent a porcine aortic valve replacement in September 1981 shortly after the convalescent serum was collected. Patient 12

TABLE 1. Reciprocal rheumatoid factor and *C. burnetii* titers in patients with primary Q fever infections

Patient no.	Bleeding date (mo/day/yr)	Reciprocal titer of indicated antibody by following test:											
		RF	MA		CF			IFA					
								IgG		IgM		IgA	
			Phase II	Phase I	Phase II	AC[a]	Phase I	Phase II	Phase I	Phase II	Phase I	Phase II	Phase I
1	4/29/77	<8	<4	<4	<8	<8	<8	64	<8	16	<8	<8	<8
	5/27/77	<8	2,048	32	128	<8	8	4,096	2,048	256	512	<8	<8
2	3/01/78	<8	4	<4	<8	<8	<8	32	<8	64	<8	<8	<8
	3/31/78	<8	512	4	64	<8	<8	512	<8	2,048	8	<8	<8
3[b]	9/24/76	<8	<4	<4	<8	<8	<8	<8	<8	<8	<8	<8	<8
	9/27/76	<8	8,192	16	64	<8	<8	1,024	<8	128	8	<8	<8
	10/21/76	<8	16,384	64	256	<8	<8	8,192	256	8	128	<8	<8
	2/17/77	<8	16,384	32	256	<8	32	8,192	256	8	32	<8	<8
	3/25/77	<8	2,048	32	128	<8	16	16,384	256	<8	<8	<8	<8
	5/23/77	<8	256	8	32	<8	8	1,024	256	<8	<8	<8	<8
4[c]	1/31/79	<8	<4	<4	<8	<8	<8	<8	<8	<8	<8	<8	<8
	5/24/79	<8	4,096	32	128	<8	32	128	<8	256	32	<8	<8
	6/06/79	<8	4,096	32	128	<8	16	512	<8	1,024	32	<8	<8
	6/29/79	<8	4,096	32	128	<8	16	1,024	64	1,024	16	<8	<8
	4/17/80	<8	1,024	8	64	<8	<8	2,048	32	16	16	<8	<8
5	2/14/79	<8	<4	<4	<8	<8	<8	16	<8	16	<8	<8	<8
	3/07/79	<8	256	<4	32	<8	<8	256	<8	128	<8	<8	<8
	3/30/79	<8	256	<4	128	<8	<8	1,024	<8	1,024	16	<8	<8

[a] AC, Anticomplementary.
[b] Onset of disease, 21 September 1976.
[c] Onset of disease, 2 May 1979.

underwent a porcine aortic valve replacement in 1977, and another porcine valve was inserted because of destruction due to endocarditis in July 1981. The aortic valve of patient 11 was replaced in September 1981. Patient 13 had an aortic prosthetic valve replacement in August 1976, and patient 15 underwent similar surgery in December 1981.

Isolates of phase *C. burnetii*. Guinea pigs inoculated with aortic valve tissues from all of the patients with endocarditis responded with fever and *C. burnetii* antibodies. Subpassage of tissue suspensions from inoculated animals into embryonated eggs produced abundant growth of *C. burnetii* identified by direct immunofluorescence and MA, CF, and IFA tests with standard guinea pig antiserum. Growth of two isolates (from patients 13 and 15) in embryonated eggs approached 3 to 7 mg of purified antigen per g of yolk sac tissue, a yield two to seven times greater than that obtained with the laboratory-adapted phase I Nine Mile tick strain (44).

C. burnetii isolates from heart valves also showed variability in their staining with the rickettsial stain of Giménez (9). Yolk sac smears containing numerous rickettsiae identified by direct fluorescent staining showed only minimal staining with carbol-basic fuchsin, whereas Nine Mile and other *C. burnetii* strains grown in embryonated eggs stained vividly.

Serological profiles for primary Q fever, granulomatous hepatitis, and endocarditis. A comparison was made of the specific *C. burnetii* phase II and phase I antibody titers in the three clinical entities of Q fever as determined by MA, CF, and IFA tests (see Tables 1, 2, and 3). All three tests adequately diagnosed the entities and distinguished primary from chronic disease.

In patients with primary Q fever (Table 1), the MA and CF tests showed characteristic rises in antibody titers to phase II and also usually to phase I antigens, but titers to the latter were low. Anticomplementary activity was <8 in the CF test. The IFA test usually showed rises in IgG and IgM titers to both phase II and phase I antigens. The strongest IgG antibody response was against phase II antigen; however, in some individuals, the IgM phase I titer approached or was greater than the phase II titer. This activity was not due to RF, since RF levels were <8 for all primary Q fever cases. No IgA antibodies to phase II and phase I antigens were detected. The ratios of phase II to phase I titers by all tests were generally much greater than 1.

Granulomatous hepatic Q fever (Table 2) was characterized by extremely high MA and CF

TABLE 2. Reciprocal RF and *C. burnetii* titers in patients with granulomatous Q fever hepatitis

Patient no.	Bleeding date (mo/day/yr)	Reciprocal titer of indicated antibody by following test:											
		RF	MA		CF			IFA					
								IgG		IgM		IgA	
			Phase II	Phase I	Phase II	AC[a]	Phase I	Phase II	Phase I	Phase II	Phase I	Phase II	Phase I
6[b]	4/21/81	512	16,384	256	4,096	16	64	524,288	2,048	8,192	512	<8	32
	6/18/81	128	4,096	128	2,048	8	128	2,097,152	512	512	128	<8	<8
	7/20/81	256	32,768	256	512	<8	128	≧4,194,304	256	128	64	<8	<8
	9/18/81	128	131,072	512	2,048	<8	32	≧4,194,304	1,024	512	128	<8	<8
7	8/18/81	—[c]	512	8	64	32	32	256	8	256	64	8	<8
	8/26/81	—	131,072	128	1,024	1,024	1,024	32,768	64	262,144	1,024	64	<8
	9/02/81	—	131,072	128	512	64	64	65,536	128	131,072	512	32	<8
	9/18/81	128	131,072	2,048	2,048	<8	32	262,144	128	131,072	1,024	32	<8
	10/21/81	—	262,144	2,048	4,096	<8	32	262,144	512	131,072	2,048	32	<8
	11/13/81	64	32,768	256	128	<8	<8	≧4,194,304	256	4,096	1,024	16	<8
	12/11/81	64	256	64	256	<8	<8	16,384	64	256	32	<8	<8
	1/08/82	64	128	64	128	<8	8	16,384	128	256	64	<8	<8
8[d]	8/07/80	—	4,096	64	4,096	16	64	32,768	128	1,024	128	32	16
	8/18/80	256	4,096	32	≧4,096	8	32	65,536	64	1,024	64	16	<8
	8/25/80	128	32,768	64	16,384	16	64	262,148	512	1,024	128	<8	<8
	9/03/80	32	16,384	64	4,096	<8	32	131,072	1,024	1,024	512	16	<8
	9/18/80	128	8,192	32	2,048	<8	32	65,536	256	256	64	16	<8
	12/11/80	32	16,384	64	4,096	<8	64	131,072	1,024	256	64	8	<8
	1/31/83	128	128	<4	64	<8	<8	262,148	32	32	16	<8	<8
9[e]	9/19/81	64	2,048	512	128	<8	512	65,536	16,384	512	256	<8	<8
	9/25/81	128	2,048	512	256	<8	512	262,144	8,192	512	512	<8	<8
	10/11/81	256	65,536	512	4,096	<8	1,024	262,144	32,768	4,096	1,024	<8	<8
	11/23/81	256	65,536	4,096	2,048	<8	512	≧4,194,304	131,072	4,096	512	<8	<8
10[f]	4/01/81	128	4,096	512	64	32	32	4,096	16	512	128	16	<8
	4/30/81	128	4,096	256	1,024	64	64	131,072	512	256	32	16	<8
	5/28/81	64	1,024	32	1,024	32	64	65,536	256	128	16	16	<8
	6/04/81	64	1,024	32	1,024	32	64	65,536	256	128	16	<8	<8
	6/11/81	128	1,024	32	1,024	32	64	65,536	128	64	16	16	<8
	8/06/81	64	1,024	32	1,024	32	64	65,536	128	64	16	<8	<8

[a] AC, Anticomplementary.
[b] Liver punch biopsy; granuloma present 21 April 1981.
[c] —, Not done.
[d] Liver punch biopsy; granuloma present 18 July 1980.
[e] Liver punch biopsy; granuloma present 29 September 1981. Phase I *C. burnetii* isolated.
[f] Bone marrow biopsy; ph I *C. burnetii* isolated 1 April 1981.

titers against phase II antigens and moderately high titers against phase I antigens. All but one of the patients (patient 9) had anticomplementary activity, particularly in sera collected early. The IFA test often showed rises in IgG, IgM, and IFA titers to phase II and phase I antigens. The extremely high antibody response was against phase II antigen, whereas phase I titers were significantly higher than in primary Q fever. All of these patients' sera had significant levels of IgM activity against phase II and phase I antigens. Most of the activity was determined to be RF in the standard assay and fractionation experiments (see Table 5). Transitory but low (≦64) levels of IgA antibody were noted in some of these patients. The ratio of phase II to phase I titers was ≧1.

Q fever endocarditis (Table 3) was characterized by extremely high MA and CF titers against phase II and phase I antigens. Anticomplementary activity was not detectable in the CF test. The IFA test gave high undulating levels of IgG, IgM, and IgA titers to phase II and phase I antigens. Most sera had moderate to high levels of IgM reactivity against phase II and phase I antigens. This activity was determined to be RF in the standard assay and the fractionation experiments (see Table 5). In contrast to titers seen in patients with primary disease and granulomatous hepatitis, high levels of IgA titers against

TABLE 3. Reciprocal RF and *C. burnetii* titers in patients with Q fever endocarditis

Patient no.	Bleeding date (mo/day/yr)	Reciprocal titer of indicated antibody by following test:											
		RF	MA		CF			IFA					
								IgG		IgM		IgA	
			Phase II	Phase I	Phase II	AC[a]	Phase I	Phase II	Phase I	Phase II	Phase I	Phase II	Phase I
11[b]	9/17/81	1,024	2,048	16,384	256	<8	2,048	1,048,576	2,097,152	2,048	16,384	8,192	≧16,384
	9/30/81	1,024	2,048	2,048	256	<8	2,048	524,288	1,048,576	2,048	16,384	8,192	16,384
	10/81	512	16,384	262,144	256	<8	2,048	≧4,194,304	≧4,194,304	16,384	1,048,576	≧16,384	≧16,384
12[c]	3/24/81	2,048	4,096	512	256	<8	256	16,384	8,192	1,024	512	4,096	8,192
	5/21/81	1,024	≧4,096	1,024	512	<8	256	16,384	8,192	1,024	512	2,048	4,096
	7/10/81	1,024	4,096	1,024	512	<8	256	32,768	16,384	1,024	512	4,096	16,384
13[d]	5/07/76	128	1,024	1,024	64	<8	512	2,048	16,384	8	512	64	512
	8/19/76	64	2,048	2,048	128	<8	1,024	1,024	131,072	<8	512	64	512
	11/10/76	256	2,048	1,024	64	<8	2,048	2,048	65,536	<8	256	32	256
	1/18/77	64	2,048	512	128	<8	1,024	65,536	262,273	<8	16	32	256
	3/09/77	64	512	256	64	<8	2,048	4,096	≧262,273	<8	16	8	512
	6/14/77	256	1,024	256	64	<8	512	256	32,768	32	512	<8	256
	10/05/77	256	256	64	128	<8	32	256	16,384	32	1,024	<8	512
	3/30/78	128	128	32	128	<8	32	512	32,768	64	1,024	<8	512
	12/05/78	128	128	64	128	<8	32	16,384	32,768	128	512	<8	256
	7/11/81	128	256	64	128	<8	32	16,384	16,384	1,024	1,024	<8	64
	7/25/81	256	128	64	64	<8	64	256	1,024	128	8	<8	64
14[e]	Acute convalescent	128	512	512	512	<8	512	8,192	4,096	512	512	1,024	4,096
		128	4,096	1,024	512	<8	1,024	32,768	8,192	128	64	1,024	4,096
15[f]	10/10/79	512	8,192	4,096	2,048	<8	64	131,072	32,768	4,096	256	1,024	1,024
	10/19/79	512	16,384	4,096	512	<8	128	131,072	65,536	4,096	512	1,024	4,096
	12/11/79	256	8,192	512	512	<8	128	65,536	32,768	128	256	1,024	2,048
	3/18/80	256	4,096	512	64	<8	8	8,192	16,384	512	512	1,024	4,096
Urine (10X)	10/05/79	<8	512	<4	—[g]	—	—	512	1,024	<8	<8	32	8,192

[a] AC, Anticomplementary.
[b] Aortic valve replacement, September 1981. Isolated *C. burnetii* phase I from valve tissue.
[c] Porcine aortic valve replacement in 1977; aortic prosthesis valve replacement July 1981. Isolated *C. burnetii* phase I from tissue around valve prosthesis.
[d] Aortic valve replacement, August 1976. Isolated *C. burnetii* phase I from valve tissue.
[e] Porcine aortic valve replacement, September 1981. Isolated *C. burnetii* phase I from valve tissue.
[f] Aortic valve replacement, December 1981. Isolated *C. burnetii* phase I from valve tissue.
[g] —, Not done.

TABLE 4. Levels of immunoglobulins in patients with Q fever by radial immunodiffusion assay

Disease entity and patient no.	Bleeding date (mo/day/yr)	Immunoglobulin level (mg/dl)[a]		
		IgG	IgM	IgA
Primary				
3 (onset, 9/21/76)	9/24/76	713	64	140
	2/17/77	990	150	150
4 (onset, 5/2/79)	5/24/79	1,350	222	125
	6/29/79	1,100	325	137
Granulomatous hepatitis				
7	8/26/81	450	130	380
	10/21/81	670	72	400
8	9/18/81	1,700	200	460
	12/18/81	730	97	550
9	10/11/81	370	72	160
	11/23/81	370	83	190
Endocarditis				
11	9/17/81	5,300	72	>1,000
	9/30/81	6,700	47	>1,000
12	3/13/81	4,500	63	>1,000
	7/10/81	3,800	35	>1,000
13	8/19/76	1,770	190	302
	3/09/77	1,176	116	228
14	Convalescent	1,500	40	770
15	10/10/79	1,600	350	470
	12/11/79	1,400	180	440

[a] The approximate normal range (mean) of immunoglobulins in adults is 770 to 1,500 (1,142) mg/dl for IgG, 60 to 208 (102) mg/dl for IgM, and 134 to 297 (210) mg/dl for IgA (data provided by Accra Assay, Miles Laboratories).

phase II and phase I antigens were noted in patients with endocarditis. The ratio of phase II to phase I titers was ≦1.

Immunoglobulin concentrations. Levels of immunoglobulin in patients with Q fever, as determined by RIA, are shown in Table 4. The levels of immunoglobulin during the course of infection and convalescence in two patients with primary Q fever were within the normal range, except for slightly elevated IgM in the convalescent serum of patient 4. In patients with granulomatous Q fever hepatitis, IgG and IgM levels in serum were usually within the normal range. The IgA levels in the sera of patients 7 and 8 were elevated. Determination of immunoglobulin levels in the sera of five patients with chronic Q fever endocarditis revealed significantly elevated IgG and IgA levels. All IgG levels were elevated or in the high range; IgG levels in serum were 2.5 to 4.5 times the upper limits of the normal range in patients 11 and 12. IgA amounts varied from the high in the normal range to 3.5 times the upper limits of the normal range. IgM levels in the sera of these patients varied from low to high in the normal range.

Evaluation of autoimmune disease during Q fever. IgM in serum is usually present in low concentrations as compared with IgG. Antigen-specific IgG can interfere with serological tests and IgG-IgM interactions, and IgM RF, an autoantibody to the Fc fragment of IgG, can cause false-positive titers.

The data in Table 5 reflect the interfering effects of IgG antibody on detection of the specific IgM response in two of the three clinical entities of Q fever. The IgM titer in the sera of five patients with diagnosed primary Q fever was nearly equivalent in fractionated and unfractionated sera, whereas the IgM titer in patients with granulomatous hepatitis and endocarditis showed interference of IgG antibody on specific IgM titers. The effect was most pro-

TABLE 5. Reciprocal titers of IgA and IgM in unfractionated and QAE-Sephadex-fractionated sera of three clinical entities of Q fever as determined by IFA

Disease entity and patient no.	Bleeding date	Reciprocal titer of indicated immunoglobulin in:							
		Unfractionated serum				Fractionated serum			
		IgA		IgM		IgA		IgM	
		Phase II	Phase I	Phase II	Phase I	Phase II	Phase I	Phase II	Phase I
None (control)[a]	10/11/78	<8	<8	32	<8	<10	<10	40	<10
	2/11/80	<8	<8	16	<8	<10	<10	40	<10
	3/22/80	<8	<8	8	<8	<10	<10	20	<10
Primary									
1	4/29/77	<8	<8	16	<8	<10	<10	20	<10
	5/27/77	<8	<8	256	512	<10	<10	160	160
2	3/01/78	<8	<8	64	<8	<10	<10	40	<10
	3/31/78	<8	<8	2,048	8	<10	<10	640	<10
3	3/25/77	<8	<8	<8	<8	<10	<10	40	10
4	6/29/79	<8	<8	1,024	16	<10	<10	320	10
Granulomatous hepatitis									
6	7/20/81	<8	<8	128	64	<10	<10	40	20
7	9/18/81	32	<8	131,072	1,024	<10	<10	320	40
	10/21/81	32	<8	131,072	2,048	20	<10	160	40
8	9/03/80	16	<8	1,024	512	10	<10	80	20
9	10/11/81	<8	<8	4,096	1,024	10	10	<10	<10
10	4/01/81	16	<8	512	128	20	<10	80	20
Endocarditis									
11	9/17/81	8,192	≧16,384	2,048	16,384	320	640	20	20
	9/30/81	8,192	16,384	2,048	16,384	640	640	20	<10
	10/81	≧16,384	≧16,384	16,384	1,048,576	640	640	20	<10
12	7/10/81	4,096	16,384	1,024	512	40	160	20	<10
13	7/11/81	<8	64	1,024	1,024	<10	80	<10	<10
14	Convalescent	1,024	4,096	128	64	40	160	20	<10
15	10/10/79	1,024	1,024	4,096	256	640	1,280	20	<10
	10/19/79	1,024	4,096	4,096	512	320	640	40	<10
	12/11/79	1,024	2,048	128	256	640	1,280	20	<10

[a] The control was vaccinated on 8 August 1978.

nounced in the sera of patients with endocarditis. In these sera, very low (1:10 to 1:40) specific antibody titers to *C. burnetii* phase I or phase II antigens were detected in the isolated IgM fraction, even though titers as high as 1:1,048,576 with the IgM conjugate were noted in the unfractionated sera. The IgG interference with IgM-specific titers in the sera of patients with granulomatous hepatitis was significant. Specific IgM titers (<1:10 to 1:320) were found in the IgM fraction, whereas titers as high as 1:131,072 were noted in the unfractionated sera.

In patients with granulomatous hepatitis and endocarditis, the bulk of the specific antibody was in the IgG antibody fraction. Segregation of IgA antibody in the chromatographic separation was incomplete, as almost equal titers of IgA antibody to *C. burnetii* were found in the IgG and IgM fractions with the IFA test.

Other evidence for autoimmune or immune

TABLE 6. Serological profiles of Q fever[a]

Disease entity	Levels of:			Antibody response								AC activity	RF
	IgG	IgM	IgA	Phase II				Phase I					
				Ig[b]	IgG	IgM	IgA	Ig[b]	IgG	IgM	IgA		
Primary	U	U	U	M	M	L to M	–	L	L	L	–	–	–
Granulomatous hepatitis	U	U	U or E	M to H	H	M to H	– to L	L to M	L to M	L to M	– to L	– to L	+
Endocarditis	E	U	E to HE	H	M to H	L to M	L to M	M to H	M to H	M	M to H	–	+

[a] U, Unchanged; E, elevated; HE, highly elevated; L, low; M, moderate; H, high; –, absent; +, present.
[b] Ig, Total immunoglobulin.

complex disease was noted in attempts to visualize *C. burnetii* organisms with anti-human IgM or IgG in sections of the excised valves. The entire section of tissue fluoresced, owing to enormous amounts of IgG or IgM which could not be removed from the tissue by washing in saline or buffer at pH 2.5. In the washed tissue, rickettsiae could be detected by indirect immunofluorescence only with guinea pig serum from animals hyperimmunized against *C. burnetii* and with fluorescein isothiocyanate-conjugated rabbit anti-guinea pig serum. The picture revealed by staining with anti-human IgG or anti-human IgM suggested reactions of the patient's IgG and IgM with his own tissue or of IgM antibody against homologous IgG.

DISCUSSION

The results of this study indicate that (i) the choice of antibody assay method is critical for proper evaluation of specific antibody levels to *C. burnetii*; (ii) at least three clinical entities of Q fever can be distinguished by IFA of specific IgG, IgM, and IgA; and (iii) autoimmune mechanisms are induced during primary and chronic Q fever as demonstrated by this study and previously by other investigators (18, 20, 26, 31, 40, 42, 45).

Table 6 shows a summary of the serological profiles of the three clinical entities of Q fever in humans, which is meant to provide a range of values for comparison of single and serial samples from patients. Host response to *C. burnetii* infection is characterized by an early rise in antibody titer to phase II antigen and a later rise in antibody titer to phase I antigen. In primary Q fever, phase II antibody usually persists in moderate titers for 3 months to 1 year, whereas phase I antibody titers remain at low to moderate levels (Table 1). The development of chronic Q fever may be expressed as granulomatous hepatitis or endocarditis or both. We have shown that these clinical entities of Q fever may be recognized by serological profiles of phase II and phase I antibodies.

The host factors involved with the development of chronic Q fever is not understood, but autoimmune pathogenesis may play a role. Expression of autoimmune disease may be recognized as RFs which comprise auto-antibodies of the IgM class that react with the Fc portion of IgG. IgG antibody that is bound specifically to antigen may bind RF, and the bound RF may cause a false-positive reaction for IgM antibody in the IFA assay (17, 35, 36). RF antibodies found in chronic diseases are probably caused by the duration of the infection and the high serum elevations of IgG (24, 36) that first binds to antigen. RF antibody reacts with the Fc portion of the bound IgG antibody, and the class-specific antibody to human IgM recognizes the RF antibody. Turck et al. (42) observed RF in four of five chronic Q fever patients. Salomen et al. (36) documented that RF antibodies interfered with detection of specific IgA antibodies as well as IgM antibodies in rubella and influenza infections. In IgA antibody assays, immunoglobulin clusters composed of specific IgG antibodies and RF molecules may cause steric hindrance to the binding of IgG antibodies (36). The high apparent specific IgM titers in Q fever endocarditis and hepatic disease are due to the auto-antibody to the Fc fragment of IgG.

The same phenomenon was apparently not found in experimentally infected guinea pigs, for Tokarevich and co-workers did not find RF-like antibodies in their animals (41). A soluble component of *C. burnetii* has been reported to form a circulating immune complex in the sera of experimentally infected guinea pigs (43) and in the sera of patients with acute Q fever (20). Kazar et al. (18) suggested that the high levels of phase I antibodies in chronic Q fever occurred in response to immunopathological changes rather than a manifestation of immunity. It is well known that serum antibody levels that vary widely in different patients with the same disease are undoubtedly influenced by specific pathological processes.

It is obvious from the results obtained in this study that high levels of humoral antibody do not always prevent the occurrence of chronic disease. Some people exhibit classical primary infection without complications. Others experience primary infection that progresses to chron-

ic disease characterized by circulating immune complexes, liver dysfunction, splenomegaly, and hepatomegaly. Still others with anomalous or damaged rheumatic heart valves develop valvular disease and endocarditis (3, 5, 18, 19, 20, 26, 31, 33, 38, 40, 42, 45).

Koster et al. have demonstrated specific unresponsiveness to *C. burnetii* antigen in vitro with lymphocytes from patients with Q fever endocarditis (F. T. Koster, J. C. Williams, and J. S. Goodwin, submitted for publication). In contrast, all patients with self-limiting primary Q fever or granulomatous hepatitis had brisk lymphocyte proliferation to *C. burnetii* antigens as well as to control antigens. The cell-mediated immune system in Q fever endocarditis must therefore be compromised by a host defect or a particular characteristic of the pathogenic strain of *C. burnetii*. The participation of a T cell-monocyte suppressor circuit was incriminated for lymphocyte unresponsiveness associated with Q fever endocarditis (Koster et al., submitted for publication).

C. burnetii is an obligately intracellular parasite of eucaryotic cells and is activated to multiply and carry out sporogenic differentiation in the phagolysome (10, 22). These capabilities may allow the organism to coexist with and take advantage of immunological defenses (12, 15; Koster et al., submitted for publication).

The presence of specific humoral *C. burnetii* IgA antibody in patients with Q fever endocarditis is diagnostic. It has been established that precursor IgA B cells are present in gut- and bronchial-associated lymphoreticular tissue (2). After antigen sensitization, these precursor B cells pass from the bronchial tissues through the mesenteric lymph nodes. From the thoracic duct lymph, the B cells reach the blood circulation and are found in the spleen (in the murine model) and later in distant mucosal tissues (2). The mechanisms of synthesis and transport of *C. burnetii* IgA antibody in the sera of patients with Q fever endocarditis are unknown.

It is tempting to speculate that the pathogenicity of Q fever infections in some patients is immunologically potentiated by the antigen-antibody reaction in tissue, resulting in tissue damage. Lumio et al. (20) found 10 of 11 patients tested to have titers of auto-antibody. In this study, all patients with Q fever granulomatous hepatitis or Q fever endocarditis had significant levels of RF. This factor is seldom evident until after 6 to 8 weeks of illness (39). The most pathogenic immune complexes are those that are soluble, either at antigen equivalence or slight antigen excess (16). These same complexes have been found to be effective in activation of the complement system (16). Participation of immune complexes in the clinical pattern of granulomatous hepatitis Q fever may be evidenced by the transient anticomplementary reaction in the sera of these patients. Transient anticomplementary activity was observed in the sera of experimentally infected guinea pigs in which immune complexes were demonstrated (43). Our serological profiles of patients with chronic Q fever strongly suggest that immune complex disease should be considered a feature of the disease.

The serological profiles obtained by MA, CF, and IFA serological tests adequately differentiate the three entities of Q fever disease in humans. We strongly recommend routine use of the IFA, IgG, IgM, and IgA assays in the diagnosis and treatment of Q fever disease.

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