Enzyme-Linked Immunosorbent Assay for Diagnosis of Chronic Q Fever

O. PÉTER,^{1*} G. DUPUIS,¹ D. BEE,¹ R. LÜTHY,² J. NICOLET,³ AND W. BURGDORFER⁴

Division of Clinical Microbiology and Infectious Diseases, Valais Central Institute, CH-1951 Sion 3,¹ Department of Medicine, University Hospital, CH-8091 Zurich,² and Department of Veterinary Bacteriology, University of Bern, CH-3000 Bern,³ Switzerland, and Laboratory of Pathobiology, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, Hamilton, Montana 59840⁴

Received 11 March 1988/Accepted 30 June 1988

From 1982 through 1987 we diagnosed 13 chronic Q fever cases. Clinically these patients presented a culture-negative endocarditis, and all but two had high complement-fixing antibody titers to *Coxiella burnetii* phase I (reciprocal titer above 200). With the enzyme-linked immunosorbent assay (ELISA), titers of immunoglobulin G (IgG) to phases I and II of *C. burnetii* averaged 158,000 and 69,900, respectively, whereas they reached 300 and 3,200 in acute Q fever cases. Similarly, IgA to both phases of *C. burnetii* and IgM to phase I were consistently higher during chronic than acute Q fever. The serological follow-up of one patient with chronic Q fever over a 4-year period showed a good correlation between the titers of IgG and IgM antibody titers detected by ELISA and indirect fluorescent-antibody test (IFA) to both phases of *C. burnetii*. Few discrepancies appeared with IgA. Shortly after initiation of antibiotic treatment, a slow and steady decrease of the antibody titers to *C. burnetii* phases I and II was observed. The complement fixation, IFA, and ELISA tests showed the same type of antibody response. The ELISA proved to be an excellent diagnostic test for chronic Q fever. It distinguished negative from positive reactions clearly, and results were highly reproducible. The reading is objective, and the test is simple to perform and more sensitive than the IFA and complement fixation tests. The ELISA is recommended for serologic evaluation of patients with chronic Q fever.

Q fever caused by the obligate intracellular bacterium *Coxiella burnetii* is a worldwide zoonosis that affects a large variety of animals. In humans, most infections occur after inhalation of contaminated dust or aerosols generated by infected domestic animals, mainly sheep, goats, and cows (2). Patients with acute Q fever experience an influenzalike illness of fever, headache, myalgia, and pneumonia, sometimes complicated with hepatitis or myopericarditis or both (4, 6). Many years after the primary episode, a chronic form of the disease, endocarditis, may develop and present a life-threatening complication (9, 15).

C. burnetii is unique among the rickettsiae in that it undergoes a host-dependent phase variation. The virulent phase I is isolated from nature, whereas the avirulent phase II occurs during serial passages in immunologically incompetent hosts such as eggs and cell cultures. This phase variation parallels the smooth-rough lipopolysaccharide variation of gram-negative members of the family *Enterobacteriaceae* (1). In most acute infections, only antibodies to phase II are produced. Detection of high antibody titers to both phases suggests a chronic infection. Classically, a reciprocal titer of equal to or greater than 200 to phase I of *C. burnetii* with the complement fixation (CF) test is considered diagnostic of chronic Q fever (15).

The diagnosis of Q fever is based principally on serology; manifestations of both the primary and chronic illness do not allow a clinical diagnosis and isolation and identification of the causative agent are not routine laboratory procedures. Therefore, the use of accurate and dependable serologic tests for the early diagnosis of Q fever (5, 11), and possibly for the chronic form of this disease, are essential and may help monitor antibiotic treatment.

This paper describes the development of an enzyme-

linked immunosorbent assay (ELISA) for the detection of specific immunoglobulin G (IgG), IgM, and IgA class antibodies to phase I and phase II *C. burnetii* in 13 Swiss patients suffering from chronic Q fever. The results are compared with those obtained by indirect fluorescent-antibody (IFA) and CF tests.

MATERIALS AND METHODS

Serum samples. Blood samples were received from 13 patients hospitalized in Zurich, Bern, Lucerne, and Geneva with chronic Q fever. These 13 cases, for whom detailed clinical descriptions have been presented elsewhere (3, 7), showed a clinical picture compatible with culture-negative endocarditis. All but two met the classic serologic criteria, i.e., CF titers above 1:200 to *C. burnetii* phase I. Heart valves or blood samples from five of these patients elicited antibodies to *C. burnetii* when inoculated into guinea pigs.

Also evaluated for the presence of IgG, IgM, and IgA to both phases of *C. burnetii* were the sera drawn during the acute phase of illness from 40 Q fever patients.

Negative control sera were obtained from blood donors who showed no antibodies to *C. burnetii* by either the IFA or CF test.

ELISA. The ELISA was performed in 96-well flat-bottomed polystyrene microtiter plates (Virion International, Cham, Switzerland), as described previously (12), with sonicated purified antigens: *C. burnetii* phase I, strain Herzberg (Veterinaria AG, Zurich, Switzerland), and *C. burnetii* phase II, strain Nine Mile (Commonwealth Serum Laboratories, Melbourne, Australia), diluted at optimal concentration in 0.1 M carbonate buffer (pH 9.6). Briefly, each well of the microtiter plates was coated with 0.1 ml of diluted antigen. Before use, the plates were washed and the serum samples were tested in serial twofold dilutions from 1:200 to 1:819,200 in phosphate-buffered saline-Tween buffer (pH

* Corresponding author.

FIG. 1. Antibody titers detected by ELISA in samples from patients with acute and chronic Q fever. Reciprocal antibody titers to *C. burnetii* phases I and II obtained with ELISA (IgG, IgM, IgA) in patients with chronic Q fever (each bar represents one individual) and in acute Q fever (each bar represents average titer of the 40 cases) and minimum-maximum observed titers (vertical lines). Dotted horizontal lines indicate the average titers for the chronic cases.

7.2). Positive and negative control sera were included in each test series. After incubation for 1 h at 37°C, the plates were washed and incubated for 3 h at room temperature after the addition of 0.1 ml of alkaline phosphatase-conjugated rabbit anti-human IgG (γ -chain specific), IgM (μ -chain specific), or IgA (α -chain specific) (Orion, Finland) at the optimal dilution. The plates were washed again, and the enzyme-bound conjugate was made to react with *para*-nitrophenylphosphate (Virion International). After 1 to 2 h of incubation at 37°C, the A_{405} was determined with the Virion reader (Virion International). The last positive dilution of the positive control serum was calibrated by computer to an optical density of 0.35 for each plate. This value served as the cutoff point.

IFA test. The IFA test was performed by the method of Philip et al. (13) and adapted to *C. burnetii* (purified phases I and II, strain Nine Mile; Rocky Mountain Laboratories, Hamilton, Mont.) as described elsewhere (11). Serial two-fold dilutions of sera were prepared in phosphate-buffered saline containing 1% normal yolk sac and fluorescein isothiocyanate-conjugated goat anti-human IgG (γ -chain specific), IgM (μ -chain specific) (Biomérieux, France), and IgA (α -chain specific) (Dakopatts, Denmark) were used as conjugates.

CF test. The micromethod was performed as described by the Centers for Disease Control, Atlanta, Ga. (8), except that the starting serum dilution was 1:10 and the sensitized erythrocyte concentration was 2.5%. The antigen *C. burnetii* phase I was strain Herzberg (Veterinaria AG); the antigen *C. burnetii* phase II was strain Nine Mile (Virion International). Serum, antigen, complement (Virion International), and hemolytic system (Boehringer, Marburg, Germany) were prepared in Veronal buffer (Oxoid BR 16). The endpoint was defined as the highest dilution giving $\geq 75\%$ fixation.

Rheumatoid factors. IgM-reactive sera were tested for rheumatoid factor with the tube test (Roche Diagnostica, Basel, Switzerland). IgM was separated from whole serum

on a minicolumn filled with Bio-Gel A-5M (200-400 mesh) (Bio-Rad Laboratories GmbH, Glattbrugg, Switzerland) in accordance with the method described by Pyndiah et al. (14).

The dilution factor was calculated as the IgM concentration ratio in the eluate and in whole serum. Immunoglobulin concentrations were determined by nephelometry on a Beckman ICS Analyzer II (Beckman Instruments International, Geneva, Switzerland).

RESULTS

The ELISA of the first serum specimen from the 13 chronic Q fever patients revealed high IgG antibody titers to both phase I and II *C. burnetii* (Fig. 1). Titers (reciprocal values) averaged 158,000 (range, 6,400 to 819,200) and 69,900 (range 12,800 to 204,800) to phase I and II, respectively. IgM antibodies and rheumatoid factors were also present in all chronically ill patients. Average IgM titers of the original sera to phase I reached 5,200 and to phase II reached 3,200. Nine of the 13 chronic Q fever patients revealed IgA antibody to phase I or II or both with titers averaging 5,700 and 9,000 to phase I and II, respectively. The remaining four patients were negative for IgA at the time that the first serum sample was evaluated.

In the acute cases (Fig. 1), the average IgG antibody titers were 300 (range, ≤ 100 to 3,200) to phase I and 3,200 (range ≤ 100 to 12,800) to phase II. The IgM titers to phase I averaged 130 and those to phase II averaged 3,200. IgA antibodies to phase I were never observed but were present in low titers to phase II.

The serological follow-up of a single patient over a period of 4 years and 4 months permitted evaluation by ELISA, IFA, and CF tests of 30 serum samples. During this period, the patient underwent two heart valve replacements. One week prior to the first operation, a slight increase was recorded for IgG, IgM, and IgA to both phases of *C. burnetii* by ELISA (Fig. 2C). With IFA (Fig. 2B), all three immuno-

RECIPROCAL
TITERS RECIPROCAL
TITERS

globulins increased to phase I only, whereas by the CF test (Fig. 2A), there was no increase at all. Following surgery, the patient received antibiotherapy (trimethoprim [160 mg] plus sulfamethoxazole [800 mg] twice a day, rifampin [300 mg] three times a day). His titers dropped until October 1982, when they stabilized or slightly increased by the ELISA, IFA, and CF tests until January 1983, when a second heart valve replacement was performed. The patient was under continuous antibiotherapy until September 1985. During this period, two significant rises of antibody titers were detected by ELISA, the first one from February through May 1984 and the second one from March through May 1985. In September 1985, low IgA titers to the phase II and considerably lowered IgG titers to both phases were detectable by ELISA and IFA. The CF test revealed antibody titers of 40 to phase I and 20 to phase II. At that time, the physicians decided to stop the antibiotherapy. In the serum sample taken in October 1986, the ELISA detected a slight increase of IgG and IgA titers to phase II. The last serum sample received in September 1987 (not shown here)

had low stable antibody titers when evaluated by each of the three serological tests.

DISCUSSION

Chronic Q fever (Q fever endocarditis) in Switzerland is still underdiagnosed because few physicians are aware of this ailment, clinical signs are nonspecific, routine laboratory techniques do not allow culturing and identification of the causative agent, and the serologic diagnosis of this disease is usually performed against phase II *C. burnetii*—a test inadequate for the diagnosis of the chronic form of Q fever. With the development of more sensitive serologic tools such as ELISA and IFA, increased numbers of chronic Q fever cases have come to our attention.

In our present study, 2 of 13 chronic Q fever cases would not have met the classic criteria of Turck et al. (15), who identified the chronic form of the disease, based on antibody titers to the phase I being equal or superior to 200 in the CF test. The recently developed IFA and ELISA tests, on the

FIG. 2. Serological follow-up of a patient with chronic Q fever (*C. burnetii* phase I [—] and phase II [---]). (A) Reciprocal antibody titers obtained with the CF test. (B) Reciprocal antibody titers obtained with the IFA test. (C) Reciprocal antibody titers obtained with the ELISA. Symbols: ■, IgG; ▲, IgM; ○, IgA. Clinical signs and treatment of the patient with chronic Q fever are shown below. TM + SMX, trimethoprim plus sulfamethoxazole. Long vertical ticks on the ordinates that are labeled J correspond to January; other months are shown consecutively by first letter.

other hand, are far superior to the CF test in that they are capable of evaluating class-specific IgG, IgM, and IgA. Indeed, Peacock et al. (10) pointed out that the presence of high IgG and IgA titers to phase I of *C. burnetii* by IFA is diagnostic of Q fever endocarditis. In our hands, the presence of specific IgA to phase I or II or both, associated with elevated IgG titers to *C. burnetii* phase I and II, is highly suggestive of a chronic infection. However, the absence of specific IgA does not exclude such a diagnosis when high IgG titers are found.

Acute and chronic Q fever cases are clearly distinguished by the ELISA, as they are by the IFA and CF tests when *C. burnetii* phase I antigen is used (3). All three immunoglobulins, G, M, and A, with the phase I antigen were always more elevated in chronic than in acute cases. In some chronic cases, reactions to phase II are higher but are of no differential diagnostic value, since similar antibody titers may be found in both clinical entities. Anti-phase I IgG levels appear to be the most important parameter for the diagnosis of chronic Q fever. The specificity of IgM in chronic Q fever might be a subject of controversy due to the presence of rheumatoid factors. However, the complete absorption of specific IgG, as checked in our ELISA at the lowest dilutions, did not significantly reduce the IgM response. So it must be admitted that there are some specific IgM antibodies, but their clinical significance is not known. IgA antibodies were detected in 9 of our 13 chronic Q fever

cases in the first serum sample tested by ELISA. Comparatively, we detected IgA antibodies in all the chronic cases by IFA. In the four negative ELISA cases, a very weak fluorescent pattern was observed by IFA from the first to the last dilution. Such reactions were very difficult to interpret but could not be considered negative. This discrepancy between the ELISA and the IFA test may be related to the antigens used (strains, antigen purification), to the specificity of the conjugates, to the techniques (antigenic site presentation), or to the presence of high IgG titers, which would block more antigenic sites with ELISA than with IFA.

In general, the three tests have demonstrated similar antibody responses in the serological follow-up of a patient. Antibody titers to both phases of *C. burnetii* slowly decreased year by year. However, the ELISA exhibited a higher sensitivity than the IFA and CF tests. Three significant rises of antibody titers were detected. The first one appeared 6 months after the first heart valve replacement and the second in the period from February through May 1984. At this particular time, the patient complained of digestive trouble (diarrhea, vomiting) and asked for termination of antibiotherapy. The physicians changed antibiotics (from trimethoprim plus sulfamethoxazole and rifampin to doxycycline) for 1 month and then returned to the previous regimen. The third peak appeared from March through May 1985 without known clinical implication. These three events were objectively revealed by ELISA but not by IFA or CF.

Indeed, a onefold dilution increase of the antibody titers is not considered a significant rise with any serological test.

The ELISA proves to be an excellent diagnostic test for chronic Q fever. It distinguishes clearly between the acute and chronic stages of the disease. The ELISA, as well as the IFA, is more sensitive than CF, evaluating class-specific immunoglobulins. Even though the ELISA is more time and antigen-consuming, it has several advantages over IFA. First, the reading is objective, which means greater precision, with a very high reproducibility of the antibody titer determination; second, it shows higher sensitivity, which leads to better serological follow-up of patients under treatment. It may prove extremely useful in detecting any recurrence of the disease when antibiotherapy is temporarily halted or terminated. Therefore, strict serological follow-up is highly recommended and should be applied to all patients suffering from chronic Q fever.

The ELISA is also applicable to the early diagnosis of acute Q fever. It demonstrates a sensitivity and specificity similar to those of the IFA test with the advantages and disadvantages cited above.

ACKNOWLEDGMENTS

We acknowledge the cooperation of D. Laufer (Hôpital Cantonal, Geneva), F. Burkhardt (Universität Bern), M. F. Paccaud (Institut d'Hygiène, Geneva), L. Matter (Mikrobiologie, St Gall), and the technicians of the Department of Serology, particularly M. C. Mottiez and A. F. Rodrigues (Institut Central des Hôpitaux Valaisans, Sion) for their skillful technical assistance.

This work was supported by a grant from the Fonds National Suisse de la Recherche Scientifique (no. 3.967-0.85).

LITERATURE CITED

- Amano, K.-I., and J. C. Williams. 1984. Chemical and immunological characterization of lipopolysaccharides from phase I and phase II *Coxiella burnetii*. *J. Bacteriol.* **160**:994-1002.
- Baca, O. G., and D. Paretsky. 1983. Q fever and *Coxiella burnetii*: a model for host-parasite interactions. *Microbiol. Rev.* **47**:127-149.
- Dupuis, G., O. Péter, R. Lüthy, J. Nicolet, W. Burgdorfer, and M. Peacock. 1986. Serological diagnosis of Q-fever endocarditis. *Eur. Heart J.* **7**:1062-1066.
- Dupuis, G., O. Péter, D. Pedroni, and J. Petite. 1985. Aspects cliniques observés lors d'une épidémie de 415 cas de fièvre Q. *Schweiz. Med. Wochenschr.* **115**:814-818.
- Field, P. R., J. G. Hunt, and A. M. Murphy. 1983. Detection and persistence of specific IgM antibody to *Coxiella burnetii* by enzyme-linked immunosorbent assay. A comparison with immunofluorescence and complement fixation test. *J. Infect. Dis.* **148**:477-487.
- Fiset, P., and T. E. Woodward. 1982. Q fever, p. 435-448. In A. S. Evans and H. A. Feldman (ed.), *Bacterial infections of humans*. Plenum Publishing Corp., New York.
- Laufer, D., P. D. Lew, I. Oberhansli, J. N. Cox, and M. Longson. 1986. Chronic Q fever endocarditis with massive splenomegaly in childhood. *J. Pediatr.* **108**:535-539.
- Palmer, D. F., and S. D. Wahley. 1986. Complement fixation test, p. 57-66. In N. R. Rose, H. Friedman, and J. L. Fahey (ed.), *Manual of clinical laboratory immunology*, 3rd ed. American Society for Microbiology, Washington, D.C.
- Palmer, S. R., and S. E. J. Young. 1982. Q fever endocarditis in England and Wales, 1975-1981. *Lancet* **ii**:1448-1449.
- Peacock, M. G., R. N. Philip, J. C. Williams, and R. S. Faulkner. 1983. Serological evaluation of Q fever in humans: enhanced phase I titers of immunoglobulins G and A are diagnostic for Q fever endocarditis. *Infect. Immun.* **41**:1089-1098.
- Péter, O., G. Dupuis, W. Burgdorfer, and M. G. Peacock. 1985. Evaluation of complement fixation and indirect immunofluorescence tests in the early diagnosis of primary Q fever. *Eur. J. Clin. Microbiol.* **4**:394-396.
- Péter, O., G. Dupuis, M. G. Peacock, and W. Burgdorfer. 1987. Comparison of enzyme-linked immunosorbent assay and complement fixation and indirect fluorescent-antibody tests for detection of *Coxiella burnetii* antibody. *J. Clin. Microbiol.* **25**:1063-1067.
- Philip, R. N., E. A. Casper, R. Ormsbee, M. G. Peacock, and W. Burgdorfer. 1976. Microimmunofluorescence test for the serological study of Rocky Mountain spotted fever and typhus. *J. Clin. Microbiol.* **3**:51-61.
- Pyndiah, N., U. Krech, P. Price, and J. Wilhelm. 1979. Simplified chromatographic separation of immunoglobulin M from G and its application to toxoplasma indirect immunofluorescence. *J. Clin. Microbiol.* **9**:170-174.
- Turck, W. G., G. Howitt, L. A. Turnberg, H. Fox, M. Longson, M. B. Matthews, and R. Das Gupta. 1976. Chronic Q fever. *Q. J. Med.* **45**:193-217.