Increases in the Levels of *Coxiella burnetii*-Specific Immunoglobulin G1 and G3 Antibodies in Acute Q Fever and Chronic Q Fever

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A detailed analysis of the humoral response to *Coxiella burnetii* may provide insight into the pathogenesis of Q fever, a zoonosis caused by *C. burnetii*. The subclasses of *C. burnetii*-specific antibodies were determined by immunofluorescence in 20 patients with acute Q fever and 20 patients with chronic Q fever. Although immunoglobulin G1 (IgG1) and IgG3 antibodies were found in acute and chronic Q fever, neither IgG2 nor IgG4 was detected. The detection of IgG1 and IgG3 antibodies was not due to an increase of the IgG1 and IgG3 subclasses. Moreover, IgG1 and IgG3 antibodies were not correlated, suggesting that they may play different roles in Q fever.

Q fever is a zoonosis with worldwide distribution caused by *Coxiella burnetii*, an obligate intracellular bacterium (1). Q fever is commonly divided into acute and chronic forms. Acute Q fever manifestations consist of self-limited febrile illness, pneumonia, and granulomatous hepatitis as well as neurological disorders and miscellaneous manifestations (25). Chronic manifestations of Q fever are endocarditis and, less frequently, vascular aneurysm and prosthesis infections. These usually occur in patients with previous vascular or valvular disease or in a context of immunosuppression (17). A doxycycline regimen is recommended to treat acute Q fever, although clinical evaluation is difficult because acute Q fever is often retrospectively diagnosed. Treatment evaluation for chronic Q fever requires prolonged follow-up because of the possibility of late relapse (21). Recently, the combination of doxycycline plus chloroquine has been used with success (22).

The mechanisms of Q fever pathophysiology are still poorly understood, although it appears that specific manifestations are not determined by the genotype of the infecting strain of *C. burnetii* (24). Rather, they seem to be determined by the nature of the host immune response (18). Although the alterations in cell-mediated immune response are critical for the development of Q fever, the role of humoral response has been poorly assessed. The study of specific immunoglobulins in *C. burnetii* infections has been essentially restricted to diagnosis (17). Specific antibodies in the immunoglobulin M (IgM) and IgG classes directed against *C. burnetii* in phase II persist for several months after the onset of the acute illness (27). Specific IgA and IgG antibodies against *C. burnetii* in phase I appear to be characteristic of chronic Q fever (19, 28). In addition, IgA and IgG titers have been considered as markers of disease activity. Their decline is correlated to a definitive recovery from Q fever endocarditis and thus as an indicator that antibiotic treatment can be stopped (23). However, the roles of IgG antibodies and their subclasses have been largely ignored, except in early papers, which showed their phagocytosis-promoting role.

Human IgG consists of four subclasses, which differ in their structural and functional properties. Their roles in combating infectious diseases are highlighted by the occurrence of frequent and/or chronic infections in patients with selective deficiencies in serum IgG subclasses (20). The particular isotypes and/or IgG subclasses involved in antimicrobial responses may affect the outcome of infection. For example, disease progression in leprosy is correlated with selective increases in IgG1 and IgG3 antibodies (14). The asymptomatic state of filarial infection and lymphatic filariasis is associated with elevated levels of IgG4 and IgG3, respectively (13). In Q fever, the roles of IgG subclasses are still ignored. In this report, we investigate the subclass specificity of IgG antibodies against *C. burnetii* in patients with acute Q fever and in patients with Q fever endocarditis.

**MATERIALS AND METHODS**

**Patients.** A total of 60 individuals, from whom informed consent had been obtained, were included in this study. They comprised 20 patients, 12 men and 8 women (mean age, 35 years; range, 25 to 65 years), with acute Q fever and 20 patients, 13 men and 7 women (mean age, 45 years; range, 34 to 71 years), with Q fever endocarditis. Twenty healthy subjects were included as controls, 11 men and 9 women with a mean age of 34 years (range, 26 to 46 years). Acute Q fever was diagnosed by detection of specific antibodies (see below). The diagnosis of Q fever endocarditis was based on the criteria previously described (8), i.e., pathological evidence of endocarditis, a positive echocardiogram, circulating antibody titers, and isolation of *C. burnetii* in the valve or in leukocyte-rich plasma and culture on HEL cells.

**Immunofluorescence test.** Blood was collected by venipuncture, allowed to clot at room temperature, and centrifuged at 700 × g for 10 min. The resulting serum was stored at −20°C until it was analyzed. *C. burnetii* organisms in phase I or phase II (Nine Mile strain; ATCC VR-615) were obtained as previously described (26). Slides with smears of formaldehyde-inactivated bacteria in phase I or phase II were incubated with serial dilutions of patient serum for 30 min. After washing in phosphate-buffered saline, the bacteria were labeled with fluorescein-conjugated (F(ab′)2) goat antibodies directed against human IgG, IgM, or IgA (Immunotech, Marseille, France) at a 1:50 dilution for 30 min. The slides were then washed in phosphate-buffered saline and examined by fluorescence microscopy (Axioskop microscope; Zeiss, Jena, Germany). The levels of IgG, IgM, and IgA antibodies in the two groups of patients were determined. The cutoff titers in immunofluorescence have previously been determined as 1/50, 1/25, and 1/25 for IgG, IgM, and IgA, respectively (26). To determine the IgG subclass of specific antibodies, the second incubation was carried out with monoclonal antibodies to IgG1, IgG2, IgG3, or IgG4 (Immunotech) at a 1/10 dilution.

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After being washed, the slides were incubated with fluorescein-conjugated F(ab\textsuperscript{2}) antibody against mouse IgG (Immunotech) at 1:50 dilution and examined with the fluorescence microscope.

**RESULTS AND DISCUSSION**

In acute Q fever, elevated titers of specific IgG antibodies were detected in 20 of 20 patients (Table 1). Significant IgM titers (in 18 of 20 patients) accompanied specific IgG antibodies, but to a lesser extent. Specific IgA antibodies were detected at only very low levels in 10 of 20 patients. Our data confirm that a titer of the IgG antibodies directed against phase I C. burnetii of more than 1:200 is diagnostic of acute Q fever (26). Moreover, the increase in IgM was not a sufficient criterion for the diagnosis of recent infection. As the IgM avidity for C. burnetii increases with time postinfection (9), we tested the avidity of specific antibodies. Immunofluorescence was performed in the presence of 0.5 M guanidine chloride, a mild detergent (15). We found that the IgM titers did not change (data not shown), suggesting that the patients were at a late stage of the illness. It is also known that C. burnetii-specific IgM antibodies are still present several months after infection (6, 7). We then investigated the IgG subclasses of the specific antibodies (Table 2). Significant levels of IgG1 and IgG3 specific to C. burnetii were found in 20 of 20 patients and 13 of 20 patients, respectively. Specific IgG2 and IgG4 antibodies were not detected. These results reinforce those of another study done on a limited number of patients with acute Q fever, which indicated that IgG1 is the major subclass of IgG antibodies active against C. burnetii (9). Our results also show that specific IgG3 antibodies are synthesized in response to C. burnetii.

Specific antibodies, as detected by immunofluorescence performed on C. burnetii in phase I, were then studied in patients with chronic Q fever (Table 1). Specific IgG antibodies were elevated in all tested patients. Titers of specific IgA antibodies were also high in 17 of 20 patients, but they always remained lower than those of IgG. Specific IgM antibodies were found at only low levels in 6 of 20 patients. These results are in accordance with previous data, which show that IgG and IgA titers against phase I C. burnetii of more than 1:800 and 1:25, respectively, can be considered major criteria in the diagnosis of chronic Q fever endocarditis (26). The subclass distribution of IgG antibodies was then studied (Table 2). High levels of C. burnetii-specific IgG1 were detected in all the patients tested, and specific IgG3 antibodies were found in 12 of 20 patients. Specific IgG2 and IgG4 were not found. Our results clearly show that IgG1 and IgG3 specific to C. burnetii were found as often in patients with acute Q fever as in those with chronic Q fever. Moreover, their titers were higher in chronic Q fever than in acute Q fever, as was found with specific IgG. It is noteworthy that in other infectious diseases, such as human leprosy (14) or filariasis (13), IgG1 and IgG3 antibodies are markers of progressive disease. Our results suggest that the progression of pathologic outcome of C. burnetii infection is not related to specific IgG subclasses.

The specific IgG1 and IgG3 antibodies we found may be related to increases in these subclasses, as compared to the IgG2 and IgG4 subclasses. Similarly, altered IgG subclasses are found in bacterial infections (12). No significant subclass differences between healthy controls and patients with acute or chronic Q fever were observed (Table 3). Camacho et al. (3) reported an increase in IgG1 and IgG3 levels in sera of patients with chronic Q fever, but not in those of patients with acute Q fever, compared to the levels in sera of controls. This apparent discrepancy may be due to the methods for measurement of IgG subclasses: Camacho et al. used a radial immunodiffusion test, whereas we used an enzyme immunoassay. The relatively high percentage of IgG1 compared to those of the three other subclasses may explain the presence of the specific IgG1 antibodies we found. Hence, levels of IgG and IgG1 antibodies were correlated in acute Q fever (r = 0.638; P = 0.0025) and in chronic Q fever (r = 0.505; P = 0.023). However, the relative percentage of IgG3 in patient sera did not explain the detection of specific IgG3 antibodies in patients with acute Q fever and in those with chronic Q fever (Table 3). In addition, levels of specific IgG1 and IgG3 antibodies were not correlated in acute Q fever (r = 0.074; P = 0.755) and in chronic Q fever (r = 0.157; P = 0.509). Again, IgG1 and IgG3 antibodies may play different roles in Q fever.

### Table 1. Antibody titers in Q fever

<table>
<thead>
<tr>
<th>Form of disease</th>
<th>Titer (range)</th>
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<tbody>
<tr>
<td></td>
<td>IgG</td>
</tr>
<tr>
<td>Acute</td>
<td>1,600 ± 227 (400–3,200)</td>
</tr>
<tr>
<td>Chronic</td>
<td>4,800 ± 720 (1,600–12,800)</td>
</tr>
</tbody>
</table>

* Classes of specific antibodies against C. burnetii in phase II and phase I were determined by indirect immunofluorescence. Titers are shown as mean (± standard deviation [SD]) dilution factors.

### Table 2. IgG subclasses of specific antibodies

<table>
<thead>
<tr>
<th>Form of disease</th>
<th>Titer (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgG1</td>
</tr>
<tr>
<td>Acute</td>
<td>337 ± 60 (50–800)</td>
</tr>
<tr>
<td>Chronic</td>
<td>1,677 ± 630 (50–12,800)</td>
</tr>
</tbody>
</table>

* IgG subclasses of specific antibodies against C. burnetii in phase II and phase I were determined by indirect immunofluorescence. Titers are shown as mean (± SD) dilution factors.

### Table 3. IgG subclasses in Q fever

<table>
<thead>
<tr>
<th>Form of disease</th>
<th>Level of Ig (g/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgG1</td>
</tr>
<tr>
<td>Acute</td>
<td>9.24 ± 2.78</td>
</tr>
<tr>
<td>Chronic</td>
<td>8.92 ± 3.24</td>
</tr>
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</table>

* IgG subclasses in sera from controls (n = 20) and patients with acute Q fever (n = 20) or chronic Q fever (n = 20) were determined by enzyme immunoassay. The levels of IgG subclasses shown are means ± SDs.
The mechanisms that control the production of IgG subclasses in humans still need to be explained. The stage of B-cell differentiation governs the expression of IgG1 and IgG2, since their relative proportions are distinct in serum and IgG-secret-}

ing B cells. Different subsets of T-helper cells are responsible for this differentiation of B cells (5). The switching of the antibody response to one or the other IgG subclass may require cytokines secreted by these different subsets of T-helper cells (16). However, no relationship between interleukin-2, interleukin-4, interleukin-6, or interferon gamma secreted by T-helper clones and IgG subclasses has been observed (5). The preferential generation of one IgG subclass is of pathophysiological importance, since the functional activities of IgG subclasses are clearly distinct. Human IgG2 antibodies recognize carbohydrate epitopes, whereas IgG1 and IgG3 bind protein antigens (20). Since the main epitopes of C. burnetii are shared by lipopolysaccharide (10), it would be expected that most specific antibodies would be IgG2. The high level of IgG1 and IgG3 specific to C. burnetii found in Q fever suggests a response to protein antigens and immunoblotting has revealed a variety of protein antigens in acute or chronic Q fever (2). However, polysaccharide antigens of bacteria, such as Haemophilus influenzae, induce IgG1 antibodies (11). Another property common to human IgG1 and IgG3 is their ability to enhance the uptake of C. burnetii by macrophages. It has recently been demonstrated with murine macrophages that internalization of Cryptococcus neoformans mediated by IgG1 or IgG2 antibodies inhibits fungal growth, whereas opsonization by IgG3 antibodies leads to intracellular replication of C. neoformans (29). We hypothesize that IgG1 and IgG3 play different roles in C. burnetii infections by affecting processes such as phagocytosis and intracellular survival in different ways.

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REFERENCES