Dysregulation of Cytokines in Acute Q Fever: Role of Interleukin-10 and Tumor Necrosis Factor in Chronic Evolution of Q Fever

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Q fever manifests as primary infection or acute Q fever and may become chronic in patients with underlying valvulopathy. Because *Coxiella burnetii* infection depends on host response, we measured tumor necrosis factor (TNF), interleukin (IL)–6, IL-12, and IL-10 in patients with different clinical presentations of acute Q fever. Compared with control subjects, patients with uncomplicated acute Q fever exhibited increased release of the 4 cytokines. Their amounts were higher in patients with hepatitis than in patients with fever or pneumonia. In patients with valvulopathy, who exhibited the highest risk of chronic evolution, the amounts of TNF and IL-10 were higher than in patients without valvulopathy. TNF production was specifically enhanced in patients who developed Q fever endocarditis. These results show that acute Q fever is associated with cytokine overproduction. Persistent TNF amounts were associated with the occurrence of endocarditis in patients with valvulopathy, and that may be a marker of chronic evolution of Q fever.

Q fever is a zoonosis caused by *Coxiella burnetii*, an obligate intracellular microorganism. Its natural history reveals that 60% of patients seroconvert without clinical manifestations after primary infection, and only 2% are hospitalized [1]. The main symptoms reported in acute Q fever consist of isolated fever, hepatitis, or pneumonia [2]. They are associated with specific host conditions. Indeed, patients with isolated fever are more likely male; those with hepatitis are young; and those with pneumonia are much older and more likely immunocompromised [1]. The risk of chronic evolution of Q fever is high in patients with valve lesions, arterial aneurysm, or arterial prosthesis [3]. Hence, 38% of patients with acute infection and a valve lesion develop endocarditis within 2 years [4]. The prevention of endocarditis is based on 1 year of treatment with a combination of doxycycline and hydroxychloroquine [4].

The control of Q fever depends on host immune response, as manifested by granuloma formation [5]. The granulomatous lesions consist of macrophages with epithelioid morphology and multinucleated giant cells; these lesions are paucibacillary and reflect efficient cell-mediated immunity [3, 6]. The cell-mediated immune response is also systemic, and it manifests as a marked proliferative response to *C. burnetii* antigen and as antigen-stimulated interferon (IFN)–γ production, in patients who have convalesced from acute Q fever, in patients with acute Q fever hepatitis [7], or in vaccinated patients [8, 9]. However, immune response does not lead to *C. burnetii* eradication, because bacterial DNA is found in circulating peripheral blood mononuclear cells (PBMCs) and bone marrow several
months to years after acute Q fever [10]. Hence, relapses may occur in circumstances that depress cell-mediated immunity, such as corticosteroid treatment [11], whole body irradiation [11], cyclophosphamide treatment [12], and pregnancy [13]. In humans, previous valvulopathy, pregnancy, and acquired immunodeficiencies favor the chronic evolution of Q fever [14]. Once established, chronic Q fever is characterized by defective cell-mediated immunity [1], thus underscoring the main role of cell-mediated immunity in the protection against *C. burnetii*.

We hypothesized that the production of tumor necrosis factor (TNF), interleukin (IL)–10, IL-6, and IL-12, by PBMCs, is dysregulated in acute Q fever. Hence, we compared cytokine production in acute patients without valve lesions with cytokine production in acute patients with valve lesions. We found that patients with acute Q fever and valve lesions exhibited high production of TNF and IL-10, and persistent TNF amounts may be a marker of chronic evolution of Q fever.

**SUBJECTS, MATERIALS, AND METHODS**

**Patients.** Seventy patients with acute Q fever were included in the study. The diagnosis of Q fever was based on standardized questionnaires that included epidemiological and clinical features, and on determination of antibodies directed against *C. burnetii* in phase 1 and phase 2, by indirect immunofluorescence. Patients with acute Q fever were characterized by titers of IgG antibody to phase 2 *C. burnetii* of >200 and IgM antibody to phase 2 *C. burnetii* of >50 [15]. The chronic evolution of Q fever was diagnosed with use of modified Duke’s criteria, including titers of IgG antibody to phase 1 *C. burnetii* of >800 [16]. The 70 patients were monitored by 1 of the study doctors and were divided into 2 groups, according to clinical findings: (1) patients with acute Q fever, consisting of 25 men and 16 women, with a mean age of 43.5 years (range, 15–80 years); and (2) patients with acute Q fever and valvulopathy, consisting of 20 men and 9 women, with a mean age of 52.5 years (range, 28–78 years). In the latter group, patients were monitored for 3 years, and some of them (*n* = 12) developed Q fever endocarditis. Follow-up of the patients consisted of clinical and serological investigation and cytokine determination. Ten patients (7 men, 3 women) with established Q fever endocarditis were included in the study. Thirty-one healthy persons, consisting of 22 men and 9 women, with a mean age of 41 years (range, 25–57 years), were included as control subjects.

**Cytokine determination.** PBMCs were separated by Ficoll gradient (MSL; Eurobio), as previously described [17], and were suspended in RPMI 1640 containing 25 mM HEPES, 10% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin (Invitrogen). All media were checked for the absence of endotoxins with the Limulus amebocyte assay (Boehringer Ingelheim). PBMCs (10⁶/mL) were incubated for 18 h at 37°C in flat-bottom 24-well culture plates (Nunc; PolyLabo), with or without heat-inactivated *C. burnetii* (bacterium-to-cell ratio of 20:1), as previously described [18]. Once collected, cell supernatants were stored at −80°C, until cytokine determination. TNF (detection limit, 10 pg/mL), IL-6 (detection limit, 3 pg/mL), and IL-10 (detection limit, 5 pg/mL) assays were provided by Beckman Coulter. IL-12 p40 (detection limit, 15 pg/mL) assays were provided by R&D Systems. The intra- and interspecific coefficients of variation ranged from 5% to 10%.

**Statistical analysis.** Results were expressed as individual values and as medians with 25th–75th percentiles. Qualitative data were compared by Pearson’s χ² test. Quantitative data were compared by the nonparametric Mann-Whitney U test. Differences were considered significant at *P* < .05.

**RESULTS**

**Cytokine profile of patients with acute Q fever.** We investigated production of TNF, IL-6, and IL-12 (proinflammatory cytokines) and IL-10 (an anti-inflammatory cytokine), in patients with acute Q fever without valvulopathy and in healthy control subjects. In the absence of stimulation, TNF production by PBMCs was detected in 33 of 39 patients and in 16 of 31 control subjects (*P* < .004). The amounts of TNF were significantly (*P* < .02) higher in patients than in control subjects (median, 50 pg/mL [range, 25–98 pg/mL] and 24 pg/mL [range, 9–35 pg/mL], respectively; figure 1A, groups 1 and 2). IL-6 was detected in 26 of 33 patients and in 13 of 26 control subjects. The amounts of IL-6 were significantly (*P* < .0001) higher in patients than in control subjects (median, 5360 pg/mL [range, 1685–9360 pg/mL] and 230 pg/mL [range, 100–655 pg/mL], respectively; figure 1B, groups 1 and 2). IL-2p40 was detected in 25 of 35 patients and in 3 of 12 control subjects (*P* < .02). The amounts of IL-12p40 did not differ significantly between patients and control subjects who produced IL-12p40 (figure 1C, groups 1 and 2). IL-10 was detected in 26 of 37 patients and in 9 of 27 control subjects (*P* < .003). The amounts of IL-10 were significantly (*P* < .05) higher in patients than in control subjects (median, 50 pg/mL [range, 18–95 pg/mL] and 36 pg/mL [range, 26–57 pg/mL], respectively; figure 1D, groups 1 and 2). We then investigated cytokine production by PBMCs stimulated by *C. burnetii*. *C. burnetii* elicited the release of TNF, IL-6, IL-12p40, and IL-10, in all patients and control subjects. The production of TNF, IL-6, IL-12p40, and IL-10 was similar in control subjects and in patients, but high IL-12p40 release was more frequently observed in patients than in control subjects (figure 2, groups 1 and 2). Taken together, these results show that the production of TNF, IL-6, IL-12p40, and IL-10, by unstimulated PBMCs, was up-regulated in patients with acute Q fever, whereas the *C. burnetii*-stimulated production of cytokines was not affected.
Figure 1. Production of cytokines in acute Q fever. Peripheral blood mononuclear cells (10⁶/mL) from control subjects (1), patients with acute Q fever (2), and patients with acute Q fever and valvulopathy (3) were cultured for 18 h at 37°C. Supernatants were collected and assayed for the presence of tumor necrosis factor (TNF [A]), interleukin (IL)–6 (B), IL-12 (C), and IL-10 (D). Individual results are presented for each cytokine, and horizontal lines represent medians.

Cytokine profile and clinical manifestations of acute Q fever. Because patients with acute Q fever exhibited different clinical presentations, time courses, and serological patterns, we investigated the relation between these variables and the production of cytokines by PBMCs. First, the overproduction of TNF, IL-6, IL-12, and IL-10 was not affected by the interval between disease onset and cytokine measurement: 23 of 41 patients were investigated 1–3 months after the onset of the disease and 10 patients were investigated 4–12 months after onset of the disease. Second, the overproduction of cytokines was not related to circulating levels of specific antibodies: patients with acute Q fever were characterized by the presence of IgG (median titer, 800 [range, 400–1600]) and IgM (median titer, 200 [range, 100–400]) directed against Coxiella burnetii in phase 2. Third, the clinical presentation of acute Q fever was associated with changes in cytokine production. Patients with acute

Figure 2. Coxiella burnetii–stimulated production of cytokines in acute Q fever. Peripheral blood mononuclear cells (10⁶/mL) from control subjects (1), patients with acute Q fever (2), and patients with acute Q fever and valvulopathy (3) were incubated with heat-inactivated C. burnetii (bacterium: cell ratio, 20:1) for 18 h at 37°C. Supernatants were collected and assayed for presence of tumor necrosis factor (TNF [A]), interleukin (IL)–6 (B), IL-12p40 (C), and IL-10 (D). Individual results are presented for each cytokine, and horizontal lines represent medians.
Q fever were divided into 3 subgroups, consisting of isolated fever, hepatitis, and pneumonia. The amounts of TNF released by PBMCs were significantly ($P < .002$) higher in patients with hepatitis than in patients with isolated fever (median, 65 pg/mL [range, 32–113 pg/mL] vs. median, 10 pg/mL [range, 6–36 pg/mL]; figure 3A, groups 1 and 2). Similarly, IL-10 production was higher in patients with hepatitis ($P < .02$) than in patients with isolated fever (median, 68 pg/mL [range, 40–195 pg/mL] vs. median, 8 pg/mL [range, 6–30 pg/mL]; figure 3B, groups 1 and 2). Although the production of TNF and IL-10 was high in patients with pneumonia (figure 3, group 3), the size of the sample (7 patients) was not sufficient for statistical analysis. The amounts of IL-6 and IL-12p40 were higher in patients with hepatitis ($P < .02$) than in patients with isolated fever (IL-6: median, 12,400 pg/mL [range, 8360–17,800 pg/mL] vs. median, 183 pg/mL [range, 108–383 pg/mL]; IL-12p40: median, 53 pg/mL [range, 21–99 pg/mL] vs. median, 870 pg/mL [range, 7305–11,780 pg/mL]). Nevertheless, there was a greater heterogeneity for IL-6 and IL-12p40 data than for TNF and IL-10 data. Taken together, these results show that TNF and IL-10 were increased in patients with hepatitis.

**Cytokine profile of patients with acute Q fever and valvulopathy.** Because the occurrence of Q fever in patients with valvulopathy increases the risk of chronic evolution of Q fever [4], we wondered if these patients exhibit specific patterns of cytokine production. The unstimulated production of TNF, IL-6, and IL-12 was significantly higher in patients with valvulopathy than in control subjects (TNF: median, 72 pg/mL [range, 28–146 pg/mL], $P < .02$; IL-6: median, 5800 pg/mL [range, 2025–9520 pg/mL], $P < .0001$; IL-12p40: median, 53 pg/mL [range, 21–99 pg/mL], $P < .04$). Nevertheless, they were not different from those of patients with acute Q fever without valvulopathy (figure 1, cf. group 3 with groups 1 and 2). In contrast, the production of IL-10 was significantly higher in patients with valvulopathy (median, 183 pg/mL [range, 108–383 pg/mL]) than in control subjects ($P < .05$) and patients without valvulopathy ($P < .03$) (figure 1D). On the other hand, *C. burnetii*-stimulated production of TNF was higher in patients with valvulopathy (median, 1951 pg/mL [range, 1016–2830 pg/mL]) than in control subjects (median, 805 pg/mL [range, 496–1639 pg/mL], $P < .001$), but it was not significantly different from that in patients without valvulopathy (figure 2A). The production of IL-6 (median, 12,400 pg/mL [range, 8360–17,800 pg/mL]), IL-12p40 (median, 339 pg/mL [range, 250–548 pg/mL]), and IL-10 (median, 353 pg/mL [range, 170–705 pg/mL]) was slightly higher in patients with valvulopathy than in control subjects, but the amounts of cytokines were similar to those of patients with acute Q fever without valvulopathy (figure 2B, figure 2C, and figure 2D, respectively). Hence, acute Q fever is specifically associated with the up-regulation of IL-10 production in the context of valvulopathy.

Because patients with acute Q fever and valvulopathy exhibited different prognoses, they were divided into 2 groups: patients who did not develop chronic endocarditis and patients who developed chronic endocarditis. The distribution of valvulopathies was similar in each group. Indeed, most of the patients had mitral disease occurring on native valves (table 1). The production of TNF and IL-10, by unstimulated PBMCs, was significantly higher in patients who went on to develop Q fever endocarditis than in patients with an uncomplicated course (TNF: median, 269 pg/mL [range, 71–509 pg/mL] vs. median, 35 pg/mL [range, 20–73 pg/mL], $P < .02$; IL-10: median, 183 pg/mL [range, 10–431 pg/mL] vs. median, 10 pg/mL [range, 4–14 pg/mL], $P < .003$; figure 4, groups 1 and 2). The

**Table 1. Valvular parameters in acute Q fever and Q fever endocarditis.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Acute Q fever Without chronic evolution (n = 13)</th>
<th>Acute Q fever With chronic evolution (n = 12)</th>
<th>Q fever endocarditis (n = 10)</th>
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<td>10</td>
<td>8</td>
</tr>
<tr>
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<td>2</td>
<td>2</td>
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<tr>
<td>Ejection fraction, %</td>
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<td>30–78</td>
<td>43–68</td>
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Figure 4. Tumor necrosis factor (TNF) and interleukin-10 (IL-10) and chronic evolution of Q fever. Peripheral blood mononuclear cells (10^6/mL) from patients with acute Q fever and valvulopathy without (1) and with (2) chronic evolution and from patients with established Q fever endocarditis (3) were cultured for 18 h at 37°C. Supernatants were collected and assayed for presence of TNF (A) and IL-10 (B). Individual results are presented for each cytokine, and horizontal lines represent medians.

Figure 5. Time course of tumor necrosis factor (TNF) and interleukin-10 (IL-10) production in Q fever. Patients with acute Q fever and valvulopathy who did not exhibit chronic evolution (A and B) and patients with acute Q fever and valvulopathy who exhibited chronic evolution (C and D) were monitored for 3 years. Peripheral blood mononuclear cells were isolated at the beginning of infection history and after 1 and 2 years. They were cultured for 18 h at 37°C. Supernatants were collected and assayed for presence of TNF (A and C) and IL-10 (B and D). Individual results are presented for each cytokine.

amounts of TNF and IL-10 were similar in patients who went on to develop endocarditis and in those with established Q fever endocarditis (figure 4, cf. group 2 with group 3). Hence, the overproduction of TNF and IL-10 is associated with the risk of developing chronic Q fever in patients with valvulopathy. To assess the prognosis value of TNF and IL-10 amounts, we serially measured their production in the 2 groups of patients during 3 years. In patients with an uncomplicated time course, the amounts of TNF (figure 5A) and IL-10 (figure 5B) decreased steadily to the levels of control subjects. In patients who developed Q fever endocarditis, the amounts of TNF remained constant or increased markedly in most of the patients who developed endocarditis (figure 5C). In contrast, the amounts of IL-10 decreased in most patients (figure 5D). Taken together, these results show that persistent amounts of TNF may be a marker of chronic evolution of Q fever.

DISCUSSION

In this report, we have shown that acute Q fever is associated with an overproduction of cytokines. The patients with uncomplicated acute Q fever exhibited increased production of TNF, IL-6, IL-12, and IL-10, by unstimulated PBMCs, an increased production that results from their in vivo activation. This finding is consistent with previously reported high circulating levels of TNF and IL-6 in acute Q fever [19]. The overproduction of these cytokines is nevertheless distinct from that observed in Q fever endocarditis, because the amounts of TNF, IL-6, and IL-10 were markedly lower in acute Q fever than in chronic Q fever [17, 20]. The mechanisms leading to the overproduction of TNF and IL-6 are unknown. The increase in IL-10 production likely results from the overproduction of
TNF, which has been reported to stimulate IL-10 production in monocytes [21]. We suggest that the overproduction of IL-10 controls the inflammatory response and limits its pathogenic effects. Indeed, high levels of cytokine antagonists, such as IL-1 receptor antagonist, are found in acute Q fever but not in chronic Q fever, in which the inflammatory response is no longer controlled [19].

The overproduction of cytokines is related to the clinical presentation of acute Q fever. Indeed, patients with Q fever hepatitis exhibited higher amounts of cytokines than did patients with isolated fever. This finding is not related to the interval between disease onset and cytokine measurement, because, in each group, 60% of the patients were studied before the third month of disease evolution. Our results agree with the exacerbated inflammatory pattern of Q fever hepatitis, which associates elevated sedimentation rate, inflammatory granulomas, and presence of autoantibodies, including anti–smooth muscle antibodies, anti-nuclear antibodies, and anti-phospholipid antibodies [3, 22]. In these patients, corticosteroids in association with antibiotics were proposed to obtain efficient cure [1]. Our results provide the pathophysiological basis for this therapeutic strategy.

To our knowledge, this is the first study of IL-12 in Q fever. IL-12 was detected as released IL-12p40, by unstimulated and \textit{C. burnetii}–stimulated PBMCs from patients and control subjects. The p70 heterodimer, which is considered biologically active IL-12 [23], was not detected under our conditions. IL-12 drives the development of Th1 type 1 immune responses and is a potent inducer of IFN-\(\gamma\) production [24]. It plays a crucial role in the development of specific immunity against a number of intracellular pathogens [23]. The expression of granulomas and the increase in lymphoproliferation and IFN-\(\gamma\) production suggest that IL-12 drives the Th1 protective immune response in acute Q fever [1]. The increased release of IL-12p40 in acute Q fever supports the hypothesis of IL-12–mediated development of the protective Th1 response. The trend of reduced IL-12 release in complicated acute Q fever may be linked with a less efficient Th1 immune response. Nevertheless, we cannot rule out that the production of IL-12p40 partly reflects the bioactivity of IL-12 in Q fever, because IL-12p40 and IL-12p70 display distinct roles in the establishment of protective responses [25].

The last finding was the clear relation between the overproduction of TNF and IL-10 and the risk of developing chronic Q fever. Indeed, patients with acute Q fever and valvulopathy exhibited higher production of both cytokines than did patients with acute Q fever in the absence of valvulopathy. The overproduction was more pronounced in patients who went on to become chronic than in those who had a favorable outcome, and it was similar to that found in patients who had established Q fever endocarditis. These results are not related to the type of valve involved, the presence of a prosthetic valve, or the hemodynamic parameters. This is consistent with the report of Fenollar et al. [4], which found that the type of preexisting valvular disease did not influence the progression to endocarditis. The follow-up of patients with acute Q fever and valvulopathy revealed that the pattern of TNF production was predictive of the chronic evolution of Q fever. Indeed, persistent amounts of TNF were associated with the occurrence of chronic Q fever. Such patterns did not result from differences in therapeutic regimen, because the combination of doxycycline and hydroxychloroquine was similarly administered in each group of patients. More surprisingly, IL-10 production decreased in patients with chronic evolution and in patients without chronic evolution. Indeed, IL-10 overproduction has been associated with microbicidal impairment of chronic Q fever [18] and with the occurrence of relapses in Q fever endocarditis [20]. That TNF production was a more sensitive marker of prognosis than IL-10 in acute Q fever remains unexplained. We suggest that the dysfunction of IL-10 characterizes chronic Q fever when the lesions are stabilized, whereas TNF is dominant when the lesions are developing.

In conclusion, cytokines were overproduced in acute Q fever, which is associated with the most inflammatory clinical form of Q fever. The occurrence of acute Q fever in patients with underlying valvulopathy increases the risk of their developing chronic Q fever. We provide evidence that the production of TNF and IL-10 was markedly increased in these patients and was associated with prognosis. Hence, the follow-up of TNF and IL-10 amounts may be useful for the prognosis of patients with acute Q fever and valvulopathy and may allow case-by-case therapeutic management.

References

9. Izzo AA, Marmion BP. Variation in interferon-\(\gamma\) responses to \textit{Coxiella}