A combination of interferon-gamma and interleukin-2 production by Coxiella burnetii-stimulated circulating cells discriminates between chronic Q fever and past Q fever

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Abstract

Infection with Coxiella burnetii may lead to life-threatening chronic Q fever endocarditis or vascular infections, which are often difficult to diagnose. The present study aims to investigate whether measurement of in-vitro interferon-gamma (IFN-γ) production, a key cytokine in the immune response against C. burnetii, differentiates chronic from a past cleared infection, and whether measurement of other cytokines would improve the discriminative power. First, C. burnetii-specific IFN-γ production was measured in whole blood of 28 definite chronic Q fever patients and compared with 135 individuals with past Q fever (seropositive controls) and 908 seronegative controls. IFN-γ production was significantly higher in chronic Q fever patients than in controls, but with overlapping values between patients and seropositives. Secondly, the production of a series of other cytokines was measured in a subset of patients and controls, which showed that interleukin (IL)-2 production was significantly lower in patients than in seropositive controls. Subsequently, measuring IL-2 in all patients and all controls with substantial IFN-γ production showed that an IFN-γ/IL-2 ratio >11 had a sensitivity and specificity of 79% and 96%, respectively, to diagnose chronic Q fever. This indicates that a high IFN-γ/IL-2 ratio is highly suggestive for chronic Q fever. In an additional group of 25 individuals with persistent high anti-Coxiella phase I IgG titres without definite chronic infection, all but six showed an IFN-γ/IL-2 ratio <11. In conclusion, these findings hold promise for the often difficult diagnostic work-up of Q fever and the IFN-γ/IL-2 ratio may be used as an additional diagnostic marker.

Keywords: Chronic Q fever, Coxiella burnetii, cytokines, diagnosis, interferon-gamma, interleukin-2

Introduction

Q fever is caused by the intracellular bacterium Coxiella burnetii. Some 1–5% of the infected individuals present with Q fever endocarditis or infection of an aneurysm or vascular prosthesis
If left untreated, these conditions are often fatal. Timely diagnosis is therefore warranted, and treatment should be started before irreversible damage has occurred. However, diagnosis of Q fever endocarditis or vascular infection is not easy, as culture is cumbersome and specific polymerase-chain reaction (PCR) lacks sensitivity [3,4]. To date, serology is a key factor in early detection of persistent infection, based on measurement of antibodies against the two antigenic forms of C. burnetii, phase I and II organisms, with high phase I anti-C. burnetii IgG titres (in the absence of acute Q fever) indicative for chronic Q fever [5]. The appropriate cut-off titre that differentiates it from a past cleared infection is debated; the currently proposed cut-offs 1:1024 or 1:1600 have low specificity [5–7].

Appropriate cytokine production is pivotal for the cellular defence against the intracellular bacterium C. burnetii [8,9], with interferon-γ (IFN-γ), derived from T-cells and NK-cells, stimulating macrophage microbial activity [10]. IFN-γ is under the control of type I interferons and interleukin-12 (IL-12). IL-18 and IL-23 [11]. Other cytokines also play a role: in Q fever endocarditis, up-regulation of monocyte-derived tumour necrosis factor-α (TNF-α), IL-1β and IL-6 from patients has been reported [12], as well as overproduction of IL-10 [13,14]. Benoit et al. showed that C. burnetii induces M2 polarization of macrophages in vitro with up-regulation of IL-1 receptor antagonist (IL-1Ra) and IL-6, and down-regulation of TNF-α [15]. In the adaptive immune response to C. burnetii, cytokines such as IL-2, stimulating lymphocyte proliferation and development of memory responses [16], could also be important.

We recently showed that measurement of the cellular immune response (i.e. in-vitro C. burnetii-specific IFN-γ production in whole blood) identifies individuals who have been previously exposed to C. burnetii [17]. In the present study, we assessed the production of IFN-γ, in combination with other cytokines mentioned above, in patients with chronic Q fever, with the aim of identifying a cytokine profile that may aid in the timely diagnosis of Q fever endocarditis or vascular infection.

Materials and Methods

Ethics statement
The study was approved by the Medical Ethical Committee Arnhem-Nijmegen and written informed consent was obtained from all subjects.

Study population
Twenty-eight Q fever endocarditis or vascular patients were recruited from participating hospitals. At the time of diagnosis, all patients had phase I IgG titres ≥1:1024 (in the absence of acute Q fever), with either a positive C. burnetii PCR in serum (n = 9) or tissue (n = 8) or both (n = 3) and/or signs of endocarditis as defined by the modified Duke criteria, or undisputable evidence of vascular (prosthetic) infection on positron emission tomography/computed tomography (PET/CT)-scan or ultrasound (n = 8). Nine were diagnosed with Q fever endocarditis and 19 had a vascular (prosthesis) Q fever infection. They fulfilled the criteria for ‘proven chronic Q fever’ of the Dutch consensus group on chronic Q fever diagnostics [18]. Individuals screened in the Dutch Q fever vaccination campaign from January to April 2011, as previously described, were used as controls [17]. They all had pre-existing valvular or vascular risk factors for Q fever endocarditis or vascular infection [19]. Control individuals were classified as seronegative if both serological testing and the Q-vax™ skin test (CSL, Parkville, Australia) were negative (n = 908). Controls were classified as seropositive when serological tests showed anti-C. burnetii antibodies (phase I or II IgG ≥1:32) without signs or symptoms of persistent Q fever infection, and without a serological profile suggesting chronic Q fever infection (phase I IgG ≤1:512), more than 1 year after the Q fever epidemic (n = 135).

In a second stage of our study, an additional group of 25 patients was included, in whom the diagnosis of chronic C. burnetii infection was suspected based on serology, all having persistent phase I IgG ≥1:024, but could not be confirmed with PCR, or definite valvular or vascular focus of infection on echocardiography or PET/CT. Fourteen of them had pre-existing valvular or vascular risk factors.

Measurement of C. burnetii-specific antibodies and detection of C. burnetii DNA
IgG-antibodies against C. burnetii phase I and phase II were measured by a commercially available immunofluorescence assay (IFA; Focus Diagnostics, Cypress, CA, USA).

Coxiella burnetii DNA in blood (serum/plasma) and tissue was obtained using real-time PCR targeting the IS1111a insertion element [20].

In-vitro whole blood stimulation
Cytokine production was measured in whole blood stimulation, based on previous findings [17]. Venous blood was drawn into 5-mL endotoxin-free lithium-heparin tubes (Vacutainer, BD Biosciences) and samples were processed within 12 h. Incubation was performed as previously described [17]. Coxiella burnetii Nine Mile (NM) RSA 493 phase I [21] was used as well as Q-vax vaccine, containing formaldehyde-inactivated C. burnetii Henzerling strain phase I. The mitogen
phytohaemagglutinin (PHA, Sigma-Aldrich, St Louis, MO, USA) was used as positive control. One aliquot was incubated with only culture medium as negative control. After incubation, blood cultures were centrifuged at 4,656 g for 10 min and supernatants were stored at −20°C until assayed.

Cytokine measurements
IFN-γ concentration was measured in all samples, using a commercial enzyme-linked immunosorbent assay (ELISA; Pelikine compact, Sanquin, Amsterdam, the Netherlands) as previously described [17].

To find out if other cytokines would improve the discrimination between patients and controls, a series of other cytokines was measured in a subset of patients and samples: TNF-α, IL-1β, IL-1Ra, IL-2, IL-4, IL-5, IL-6, IL-10 were measured using a multiplex beads assay (Merck Millipore, Billerica, MA, USA). IL-12p70, IL-23 and IL-18 were measured simultaneously in a magnetic beads multiplex assay (Merck Millipore) according to the manufacturer’s instructions.

Statistical analysis
Graphpad Prism (Graphpad Software Inc., version 5) was used to analyze the data. Cytokine results were displayed as individual values or expressed as medians with interquartile range. The Mann–Whitney U-test was used to determine differences between groups. Spearman’s rho correlation coefficient was used to calculate correlation. Receiver operator characteristic (ROC) curves were constructed, and the area under the curve (AUC) was assessed for the accuracy of measuring (a ratio of) cytokine production. p < 0.05 was considered significant.

Results

Patients and controls
From January 2011 until January 2012, blood samples were obtained from 28 patients with Q fever endocarditis or vascular infection. Their mean age (± standard deviation) was 66.2 (±11.8) years; 78.6% were male. At the time of blood sampling, ten patients did not yet receive antibiotics. The median duration of antibiotic therapy among patients on treatment (n = 18) was 7.5 months (range 1–26 months). Patient characteristics at the time of blood sampling are shown in Table 1.

Of the control individuals, 908 individuals (aged 63.6 ± 14.0, 61.6% male) were seronegative; 135 individuals (aged 60.8 ± 15.1, 77.8% male) were seropositive. None of the seropositive controls had detectable IgM antibodies without IgG, suggesting the absence of acute Q fever.

Interferon-gamma production in 24 h-stimulated whole blood
We measured the IFN-γ production (stimulated–unstimulated) in undiluted whole blood incubated for 24 h with PHA, Q-vax

<table>
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<tr>
<th>Number</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Focus of infection</th>
<th>Phase I IgG titre (inverse)</th>
<th>Phase II IgG titre (inverse)</th>
<th>PCR serum/ plasma</th>
<th>Duration of antibiotic treatment (months)</th>
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n.a., not available.

*At the moment of blood sampling.

As measured with immunofluorescence assay (IFA).

Not available, complement fixation test (CFT) IgG anti-phase I > 160 and anti-phase II > 640.
or *C. burnetii*-NM in all patients and all controls (Fig. 1). Aspecific PHA-induced IFN-γ production did not differ between groups. In contrast, both *C. burnetii*-antigens induced significantly more specific IFN-γ in patients (median 151 pg/mL and 2486 pg/mL by Q-vax and *C. burnetii*-NM, respectively) than in seropositive controls (3.0 pg/mL and 120 pg/mL) (p <0.001) and seronegative controls (0.0 and 0.0 pg/mL) (p <0.001). Of interest, longer incubation shows higher mitogen-induced IFN-γ production than *C. burnetii*-specific production (data not shown). Apparently, the specific response is more rapid than the mitogen response.

ROC curves for *C. burnetii*-induced IFN-γ for patients vs. seropositive controls showed an accuracy (AUC) of 0.8664 (95% CI, 0.7933–0.9395; p <0.0001) (Fig. 2a); Q-vax-stimulated IFN-γ production showed lower accuracy (0.8484; 95% CI, 0.7639–0.9330; p <0.0001) (Figure S1). Therefore, further analyses were performed only with *C. burnetii*-NM-stimulated samples. There was a considerable overlap between IFN-γ production in seropositive controls and *Q* fever patients (Fig. 2b). Choosing 500 pg/mL as the optimal cut-off, 75.0% (21/28) of the patients and 17.8% (24/135) of the seropositive individuals had a value above this cut-off.

Interestingly, the height of IgG anti-phase I antibody titre was significantly correlated with the amount of IFN-γ produced. Spearman’s rho in the total of seropositive controls and *Q* fever patients was 0.3069 (p <0.001).

Further analyses of IFN-γ production did not show significant differences between untreated (n = 10) and treated (n = 18) *Q* fever patients (p 0.11); duration of treatment (in months) did not correlate with IFN-γ production (p 0.37).

**Cytokine profiles in 48 h *C. burnetii* Nine Mile-stimulated diluted blood in a subset of patients and controls**

To find out whether measurement of one or more additional cytokines would help to distinguish chronic *Q* fever from past infection, both conditions showing high IFN-γ production, we measured the production of TNF-α, IL-1β, IL-1Ra, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-23, IL-18 and IL-2. Because not all cytokines were expected to be detectable in the first 24 h, 48-h *C. burnetii*-NM-stimulated diluted blood samples were used. Cytokine production in samples of 16 patients (obtained in the first 10 months of the study), 18 seropositive controls with substantial IFN-γ production (data not shown). The results for IFN-γ, IL-2, IL-1β, TNF-α, IL-10 and IL-1Ra are shown as Figure S2 in the supporting information. As expected, the median IFN-γ production did not differ significantly between patients and the selected seropositive individuals. Most interestingly, production of IL-2 was significantly lower in patients (median 5.0 pg/mL) than in these seropositive controls (median 39.5 pg/mL) (p <0.001).

The production of the monocyte-derived cytokines IL-1β and TNF-α was high in both chronic *Q* fever patients and in seropositive individuals, but not significantly different (p 0.24 and p 0.16 for IL-1β and TNF-α, respectively). However, both were significantly higher than in seronegative controls. The same pattern was seen for IL-6 (not shown).

The anti-inflammatory cytokine IL-10 production also did not differ between patients and seropositive controls. However, patients had significantly higher IL-10 production than seronegative individuals (p <0.01). IL-1Ra did not differ between groups.

The *C. burnetii*-NM-stimulated production of IL-4, IL-5, IL-18 and IL-23 remained below detection limit. IL-12p70 levels were very low and did not discriminate.

**IFN-γ/IL-2 ratio for diagnosis of chronic *Q* fever**

In previous subset, patients with chronic *Q* fever showed high IFN-γ and low IL-2 production, whereas seropositive controls with high IFN-γ production showed high IL-2.

Next, we assessed IL-2 production in all patients and all 102 seropositive controls with substantial IFN-γ production ≥32 pg/mL. The IFN-γ/IL-2 ratio was calculated for each individual (Fig. 2d). The AUC of the ROC-curve for patients vs. seropositive controls was 0.8873 (95% CI, 0.7983–0.9762; p <0.0001) (Fig. 2c). In 22/28 (78.6%) patients, the ratio was very low and did not discriminate.
The IFN-γ/IL-2 ratio did not correlate with the duration of antibiotic treatment in patients (p = 0.44), neither was there a significant difference between treated and untreated patients (p = 0.55). However, patients with positive C. burnetii PCR in serum or plasma at the moment of blood sampling (n = 10), had a significantly higher IFN-γ/IL-2 ratio than those with negative PCR (n = 18) (median IFN-γ/IL-2 ratio of 92.9 and 15.9, respectively, p < 0.01).

**IFN-γ/IL-2 ratio in individuals with high phase I IgG without definite chronic Q fever**

Subsequently, the IFN-γ/IL-2 ratio was determined in the group of 25 individuals (aged 63.7 ± 13.9, 72.0% male) in whom chronic Q fever was suspected based on persistent high IgG anti-C. burnetii phase I ≥1:1024, but without definite diagnosis of chronic C. burnetii infection based on PCR, echocardiography or PET/CT-scan. The results are shown separately for those with and those without pre-existing cardiovascular risk factors for chronic infection (Fig. 3). All but six had an IFN-γ/IL-2 ratio below the cut-off. A high IFN-γ/IL-2 ratio was found in 2/11 (18%) in the group without and 4/14 (29%) in the group with risk factors. The details of these six patients are shown in Table 2. Interestingly, patient 1, without pre-existing risk factors but persistent high phase I IgG and a high IFN-γ/IL-2 ratio of 39, was a veterinarian with non-specific complaints and a newly diagnosed cardiac murmur. Echocardiography and PET/CT-scan were normal and PCR on serum negative. After discontinuation of 1.5 years treatment with doxycycline and hydroxychloroquine without decreasing antibody titres, titres rose again. It cannot be excluded that this veterinarian experienced ongoing exposure to C. burnetii. Patient 2, having no valvular or vascular risk factors for chronic Q fever but persistent high phase I IgG and IFN-γ/IL-2 above the cut-off, was immunocompromised (prednisone use after kidney transplantation). He was started on antibiotics because of suspected mitral valve involvement based on PET/CT, which was considered inconclusive for endocarditis by the nuclear medicine physician and could not be confirmed by echocardiography. Likewise, patient 3 was treated for suspected Q fever vascular infection; however, the PET/CT was interpreted to be inconclusive because of diffuse uptake in the recently inserted vascular prosthesis. Patients 5 and 6 had cardiac predisposition but no evidence of persistent infection based on PCR, echocardiography or PET/CT-scan. Both, however, were started on long-term antibiotics because of the high risk of Q fever endocarditis. Only patient 4 did not receive antibiotics and had spontaneously decreasing phase I IgG titre, which persisted at 1:512 without signs or symptoms of chronic active infection.

**Discussion**

In the present study, we show that Q fever endocarditis or vascular patients exhibit high in-vitro IFN-γ production and low IL-2 production, while individuals with a past infection showed both high IFN-γ and high IL-2 production. The production of monocyte-derived pro-inflammatory cytokines IL-1β, TNF-α and IL-6 was high in patients as well as in individuals with past infection and did not discriminate. Similarly, anti-inflammatory cytokines IL-10 and IL-1Ra were not significantly different in Q fever patients and individuals with a past infection. In this population, the IFN-γ/IL-2 production ratio >11 had a sensitivity and specificity of 79% and 96%, respectively, to diagnose chronic Q fever.
Schoffelen et al. IFN-γ/IL-2 ratio to diagnose chronic Q fever

(a) IFN-γ

(b) 10,000

(c) IFN-γ/IL-2

(d) 700

(e) 10,000

IFN-γ (pg/mL)

IL-2 (pg/mL)
IFN-γ The horizontal dotted line indicates the previous established cut-off fever, while the other 14 had pre-existing valvular or vascular defects. Eleven had no pre-existing cardiovascular risk factors for chronic Q fever based on PCR, echocardiography or PET/CT-scan.

vascular patients display high \( C. burnetii \) these patients is even higher than in individuals with past \( C. burnetii \) infection. We show here that unresponsiveness and impaired IFN-γ production \([22,23]\).

We found that chronicity of Q fever infection is due to T-lymphocyte production in whole blood, while it is commonly assumed that unresponsiveness and impaired IFN-γ production \([22,23]\). We show here that \( C. burnetii \)-specific IFN-γ production in these patients is even higher than in individuals with past \( C. burnetii \) infection. We obtained the same results when using isolated peripheral blood mononuclear cells from chronic patients and controls (unpublished data). This is in accordance with the findings of Limonard et al. \([24]\) reporting higher numbers of IFN-γ-positive cells in three chronic Q fever patients compared with nine convalescent controls, using a Coxella ELISPOT assay. Apparently, this high IFN-γ production by peripheral blood cells is not sufficient to activate \( C. burnetii \)-infected monocytes/macrophages at the site of infection, to such an extent that the infection is cleared. We hypothesize that downstream of IFN-γ, the response to this cytokine is aberrant in patients with persistent \( C. burnetii \) infection.

The measurement of the production of monocyte-derived cytokines did not aid in differentiation between chronic active Q fever patients and individuals previously exposed to \( C. burnetii \). Nevertheless, both groups had higher production than naive controls. From this it can be concluded that restimulation with \( C. burnetii \) in primed individuals leads to enhanced production of cytokines by monocytes. With regard to the production of anti-inflammatory cytokines in chronic Q fever patients, only that of IL-10 was higher than in seronegative controls. This is in line with previous reports on patients with Q fever endocarditis \([13,14]\). The latter studies also reported low IL-10 in individuals with Q fever without chronic evolution. IL-10 appeared to induce \( C. burnetii \) replication and its neutralization inhibits bacterial replication in monocytes from patients with Q fever endocarditis \([25]\). Thus, IL-10 may be important in the development of chronic Q fever, but we found that measurement of \( C. burnetii \)-specific IL-10 production is not a useful diagnostic marker.

Our finding that IL-2 production was significantly lower in chronic Q fever patients than in seropositive controls may be to do with the types of T cell involved in the \( C. burnetii \)-specific

![FIG. 3. The ratio of IFN-γ and IL-2 production in individuals with high phase I IgG, without definite chronic Q fever. These 25 individuals were diagnosed with persistent phase I IgG ≥1:1024, but no definite Q fever based on PCR, echocardiography or PET/CT-scan. Eleven had no pre-existing cardiovascular risk factors for chronic Q fever, while the other 14 had pre-existing valvular or vascular defects. The horizontal dotted line indicates the previous established cut-off IFN-γ/IL-2 ratio at 11.](image)

Interestingly, we found that Q fever endocarditis and vascular patients display high \( C. burnetii \)-specific IFN-γ production in whole blood, while it is commonly assumed that chronicity of Q fever infection is due to T-lymphocyte unresponsiveness and impaired IFN-γ production \([22,23]\).

We show here that \( C. burnetii \)-specific IFN-γ production in these patients is even higher than in individuals with past \( C. burnetii \) infection. We obtained the same results when using isolated peripheral blood mononuclear cells from chronic patients and controls (unpublished data). This is in accordance with the findings of Limonard et al. \([24]\) reporting higher numbers of IFN-γ-positive cells in three chronic Q fever patients compared with nine convalescent controls, using a Coxella ELISPOT assay. Apparently, this high IFN-γ production by peripheral blood cells is not sufficient to activate \( C. burnetii \)-infected monocytes/macrophages at the site of infection, to such an extent that the infection is cleared. We hypothesize that downstream of IFN-γ, the response to this cytokine is aberrant in patients with persistent \( C. burnetii \) infection.

The measurement of the production of monocyte-derived cytokines did not aid in differentiation between chronic active Q fever patients and individuals previously exposed to \( C. burnetii \). Nevertheless, both groups had higher production than naive controls. From this it can be concluded that restimulation with \( C. burnetii \) in primed individuals leads to enhanced production of cytokines by monocytes. With regard to the production of anti-inflammatory cytokines in chronic Q fever patients, only that of IL-10 was higher than in seronegative controls. This is in line with previous reports on patients with Q fever endocarditis \([13,14]\). The latter studies also reported low IL-10 in individuals with Q fever without chronic evolution. IL-10 appeared to induce \( C. burnetii \) replication and its neutralization inhibits bacterial replication in monocytes from patients with Q fever endocarditis \([25]\). Thus, IL-10 may be important in the development of chronic Q fever, but we found that measurement of \( C. burnetii \)-specific IL-10 production is not a useful diagnostic marker.

Our finding that IL-2 production was significantly lower in chronic Q fever patients than in seropositive controls may be to do with the types of T cell involved in the \( C. burnetii \)-specific

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### TABLE 2. Characteristics of six individuals with high IFN-γ/IL-2 ratio and persistent high phase I IgG without definite chronic Q fever

<table>
<thead>
<tr>
<th>Case number, sex, age (yr)</th>
<th>Pre-existing valvular or vascular risk factor(s)</th>
<th>Serology at first diagnosis of persistent high phase I IgG (phase I IgG/phase II IgG; inverse)</th>
<th>Results of PET/CT scan and echocardiography</th>
<th>Long-term (1.5-2 years) antibiotic treatment</th>
<th>Serology at moment of blood sampling (phase I IgG/phase II IgG; inverse)</th>
<th>IFN-γ/IL-2 ratio at moment of blood sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, Female, 41</td>
<td>None</td>
<td>1024/1024</td>
<td>PET/CT and echocardiography negative</td>
<td>Yes</td>
<td>1024/1024</td>
<td>39</td>
</tr>
<tr>
<td>2, Male, 67†</td>
<td>None</td>
<td>8192/8192</td>
<td>PET/CT suspect aspect located at mitral valve, not conclusive for endocarditis; TEE negative</td>
<td>Yes</td>
<td>8192/8192</td>
<td>12</td>
</tr>
<tr>
<td>3, Male, 71</td>
<td>Prosthesis aorta; fem-fem crossover bypass</td>
<td>16384/16384</td>
<td>PET/CT possible focus of infection at fem-fem crossover bypass, not conclusive, could be risky to operation; TEE negative</td>
<td>Yes</td>
<td>4096/4096</td>
<td>55</td>
</tr>
<tr>
<td>4, Male, 64</td>
<td>Prosthesis aorta</td>
<td>1024/4096</td>
<td>PET/CT negative</td>
<td>No</td>
<td>512/1024</td>
<td>40</td>
</tr>
<tr>
<td>5, Male, 71</td>
<td>Mitral and tricuspidal valve repair</td>
<td>4096/2048</td>
<td>Echocardiography negative</td>
<td>Yes</td>
<td>4096/4096</td>
<td>19</td>
</tr>
<tr>
<td>6, Male, 37</td>
<td>Mechanical aortic valve prosthesis</td>
<td>32768/16384</td>
<td>PET/CT and echocardiography negative</td>
<td>Yes</td>
<td>4096/4096</td>
<td>63</td>
</tr>
</tbody>
</table>

PET/CT, positron emission tomography/computed tomography; TEE, transoesophageal echocardiography; IFN-γ, interferon-gamma; IL-2, interleukin-2.

†This patient was immunocompromised (prednisone use after kidney transplantation).
immune responses. In chronic infection, we assume that increased numbers of circulating C. burnetii-specific effector T cells produce IFN-γ and low amounts of IL-2 upon activation. In seropositive controls, it is probably the central memory T cell that dominates and produces mainly IL-2 [26]. We showed that the ratio of IFN-γ/IL-2 production is more specific than IFN-γ production alone (96% vs 82%), and has a slightly higher sensitivity (79% vs 75%) to distinguish patients from seropositive controls. The relative high specificity of the IFN-γ/IL-2 ratio suggests that seropositive individuals with a ratio above the cut-off merit thorough follow-up for the progression to Q fever endocarditis or vascular infection.

In tuberculosis, measurement of specific IL-2 production in addition to IFN-γ also helps to differentiate between active and latent infection. Here too, a high specific IFN-γ and low IL-2 production is found in active infection, and both high IFN-γ and high IL-2 production indicate latent tuberculosis [27,28]. A shift from T cells secreting only IFN-γ and IFN-γ/IL-2 to T cells secreting IFN-γ/IL-2 and only IL-2 is reported during treatment of active tuberculosis [29].

Ten of the 28 patients were not yet on antibiotic treatment at the moment of blood sampling. The absence of a significant difference in cytokine profile between untreated and treated patients suggests that the cytokine profile observed is not affected by the use of antibiotics. Still, the IFN-γ/IL-2 ratio seems to be related to the load of C. burnetii, because patients with positive PCR for C. burnetii DNA in serum or plasma have a significantly higher ratio than patients with negative PCR. Longitudinal studies are needed to assess the applicability of the IFN-γ/IL-2 ratio in follow-up during treatment of patients with chronic Q fever.

All 28 patients included in the first part of the study to evaluate the performance of cytokine(s) production measurements, had definite Q fever endocarditis or vascular infection. We subsequently performed the measurements of IFN-γ/IL-2 in a group of 25 individuals with an uncertain diagnosis (i.e. having high phase I IgG without definite diagnosis of chronic C. burnetii infection based on clinical criteria, PCR or definite focus of infection on diagnostic imaging). It shows the difficulties in assessing the value of a new marker in a group of patients with uncertain diagnosis, some of whom are highly suspected of having chronic Q fever, but not fulfilling the criteria for definite chronic Q fever, neither those of Raoult et al. nor those of the Dutch consensus [18,30]. In the great majority of these difficult cases (19/25), the IFN-γ/IL-2 ratio was low. This may imply absence of chronic C. burnetii infection or a more low-grade infection. In some, the diagnosis remains unclear because PCR and imaging techniques may have lacked sensitivity to make a definite diagnosis before long-term antibiotic treatment was started.

In conclusion, we found that a high IFN-γ/IL-2 ratio is highly suggestive for chronic Q fever. This finding holds promise for the often difficult diagnostic work-up of Q fever patients and may be used as an additional diagnostic marker.

Acknowledgements

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Transparency Declaration

A patent application has been submitted by TSc, TSp, mGN, JWMvdm, LABJ and mvD for this assay to diagnose Q fever status and registered by the number PCT/NL 2013/050167. All other authors declare that they have no conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Receiver operator characteristics (ROC) curve of the Q-vax-stimulated IFN-γ production after 24 hours in whole blood of chronic Q fever patients vs seropositive controls.

Figure S2. C. burnetii-induced cytokine profile in patients with chronic Q fever, compared with seronegative and seropositive controls.

References


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