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Altered interferon-γ response in patients with Q-fever fatigue syndrome

Stephan P. Keijmel [a,b,*], Ruud P.H. Raijmakers [a,b], Chantal P. Bleeker-Rovers [a,b], Jos W.M. van der Meer [b], Mihai G. Netea [b], Teske Schoffelen [a,b], Marcel van Deuren [b]

[a] *Radboud Expertise Centre for Q Fever, Department of Internal Medicine, Division of Infectious Diseases, Radboud university medical center, P.O. Box 9101, 6500 HB, Nijmegen, The Netherlands*
[b] *Department of Internal Medicine, Radboud university medical center, P.O. Box 9101, 6500 HB, Nijmegen, The Netherlands*

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Summary *Objectives:* Whether immunological mechanisms underlie Q-fever fatigue syndrome (QFS) remains unclear. For acute Q-fever, the antigen-specific interferon-γ (IFNγ) response may be a useful tool for diagnosis, and the IFNγ/interleukin(IL)-2 production ratio may be a marker for chronic Q-fever and treatment monitoring. Here we explored the specific IFNγ production and IFNγ/IL-2 ratio in QFS patients.
Methods: IFNγ and IL-2 production were tested in *ex-vivo* stimulated whole blood of QFS patients ($n = 20$), and compared to those previously determined in seropositive controls ($n = 135$), and chronic Q-fever patients ($n = 28$). Also, the correlation between patient characteristics and IFNγ, IL-2, and IFNγ/IL-2 ratio was determined.
Results: QFS patients were younger ($p < 0.001$), but gender distribution was similar to seropositive controls and chronic Q-fever patients. *Coxiella burnetii* Nine Mile stimulation revealed a higher IFNγ production in QFS (median 319.5 pg/ml) than in seropositive controls (120 pg/ml, $p < 0.01$), but comparable to chronic Q-fever (2846 pg/ml). The IFNγ/IL-2 ratio was similar to that in seropositive controls, but lower than in chronic Q-fever patients ($p < 0.01$). Symptom duration was positively correlated with IL-2 production, and negatively correlated with the IFNγ/IL-2 ratio.
Conclusions: These results point to an altered cell-mediated immunity in QFS, and suggest a different immune response than in chronic Q-fever.
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* Corresponding author. Department of Internal Medicine/Division of Infectious Diseases 463, Radboud university medical center, P.O. Box 9101, 6500 HB, Nijmegen, The Netherlands. Tel.: +31 24 3618819; fax: +31 24 3616519.
E-mail addresses: Stephan.Keijmel@radboudumc.nl (S.P. Keijmel), Ruud.Raijmakers@radboudumc.nl (R.P.H. Raijmakers), Chantal.Bleeker-Rovers@radboudumc.nl (C.P. Bleeker-Rovers), Jos.vanderMeer@radboudumc.nl (J.W.M. van der Meer), Mihai.Netea@radboudumc.nl (M.G. Netea), Teske.Schoffelen@radboudumc.nl (T. Schoffelen), Marcel.vanDeuren@radboudumc.nl (M. van Deuren).

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Introduction

At present, the Netherlands is faced with the aftermath of the largest Q-fever outbreak worldwide lasting from 2007 to 2011.[1] During this period, over 4000 patients with symptomatic acute Q-fever were reported, and it was estimated that over 40,000 individuals experienced a latent infection.[2,3] Although most patients with symptomatic acute Q-fever recover completely with only a serological scar left, infection with *Coxiella burnetii* is notorious for causing long-term sequelae, i.e., chronic Q-fever and Q-fever fatigue syndrome (QFS). Chronic Q-fever, characterized by the persistence of viable *C. burnetii*, may develop in 1–5% of both symptomatic and asymptomatic cases of acute Q-fever. Chronic Q-fever presents mainly as vascular infection,[4] including mycotic aneurysms and infections of vascular prosthesis, and endocarditis.[5] QFS, a debilitating fatigue syndrome following acute Q-fever, may become manifest in approximately 20% of patients.[6–10] Lasting up to 10 years after the acute illness,[11] QFS is considered to be the major cause of the Q-fever-related economical burden following the Dutch outbreak.[12] The pathophysiological mechanisms underlying QFS remain to be elucidated. Interpretations range from compensation-driven and psychogenic perpetuation of the original symptoms,[7] to attribution of the syndrome to cytokine dysregulation due to chronic immune stimulation.[7] The latter might be caused by persisting *C. burnetii*, or by persisting non-infectious *C. burnetii* antigens.[13–18] White blood cells from QFS patients exposed to Q-fever antigens were found to exhibit a marked interleukin-6 (IL-6) production,[13] and the IL-6 production was similar in both chronic Q-fever patients and seropositive controls, which was significantly higher than in seronegative controls.[19] In addition, the group of QFS patients contained significantly more interferon-γ (IFNγ) responders than a group of controls, whilst the proportion of IL-2 responders was lower among QFS patients.[13] IFNγ is a cytokine that plays an important role in the host defence against intracellular bacteria such as *C. burnetii*.[20–23] To date, no diagnostic test is available to diagnose QFS directly and diagnosis partly relies on measurement of *C. burnetii*-specific antibodies, e.g. serology, reflecting humoral immunity. Recently our group developed a *C. burnetii*-specific whole blood IFNγ production assay, which is a promising diagnostic tool for *C. burnetii* infection,[24] with similar performance and practical advantages over serology.[25] In addition, a high IFNγ/IL-2 ratio appeared to be indicative of chronic Q-fever, and may be a useful diagnostic marker for chronic Q-fever and treatment monitoring.[19,26] In addition, as suggested in animal experiments, antigen-specific IFNγ production could also be a useful tool for diagnosis of acute Q-fever.[27]

In the present study, we addressed the question whether there is an aberrant antigen-specific IFNγ production and IFNγ/IL-2 ratio in QFS patients. If so, this might provide additional insight in the potential pathophysiological mechanisms underlying this debilitating long-term complication and might contribute, as immunological markers, to the diagnostic workup of QFS.

Materials and methods

Study population

The study population consisted of QFS patients (n = 20), Q-fever seropositive controls (n = 135), and patients with proven chronic Q-fever (n = 28). All QFS patients were diagnosed with QFS at the Radboud Expertise Centre for Q-fever, Nijmegen, the Netherlands, after a uniform work-up according to the Dutch guideline on QFS.[28] All QFS patients met the following diagnostic criteria: i. fatigue lasted ≥6 months; ii. sudden onset of severe fatigue (defined as a score ≥35 on the subscale fatigue severity of the Checklist Individual Strength (CIS)), or significant increase in fatigue related to a symptomatic acute Q-fever infection; iii. chronic Q-fever and other causes of fatigue, somatic or psychiatric, were excluded; and iv. fatigue resulted in significant functional impairment (defined as a total score ≥450 on the Sickness Impact Profile (SIP)). Blood samples were collected during regular patient care between May 2011 and February 2012. The seropositive controls were anonymously derived from the Dutch Q-fever vaccination campaign, which was organized from January to April 2011[29]; data on their antigen-specific IFNγ production has been published previously.[25] All controls had pre-existing risk factors for development of Q-fever endocarditis or vascular infection, and were Q-fever seropositive ≥1 year after the Q-fever epidemic (IgG phase I or II ≥1:32, but IgG phase I ≤1:512), without clues for persistent Q-fever infection. Chronic Q-fever patients were diagnosed at participating hospitals,[19] and blood samples were collected between December 2010 and March 2012. At the time of sampling, all patients were diagnosed with either Q-fever endocarditis (n = 9) or vascular (prosthesis) infection (n = 18), according to the Dutch guideline on chronic Q-fever[30]; patient characteristics and data on the cytokine production of these patients also have been published before.[19,25]

Serological measurements and detection of *C. burnetii* DNA

IgM and IgG antibodies against *C. burnetii* phase I and phase II antigens were measured by a commercially available immunofluorescence assay (IFA; Focus Diagnostics, Cypress, CA, USA). The PCR assay used to detect DNA of *C. burnetii* in serum was an in-house real time PCR directed against the insertion sequence IS1111a.

In-vitro whole blood stimulation

Whole blood stimulation, followed by measurement of IFNγ and IL-2 production, was done as previously described.[25] In brief, venous blood was drawn into 5 mL endotoxin-free lithium-heparin tubes (Vacutainer, BD Bioscience) and samples were processed within 12 h. Incubation of samples was done as previously described.[25] *C. burnetii* Nine Mile (NM) RSA 493 phase I, heat-inactivated, was used,[25,31] and the mitogen phytohemagglutinin (PHA) (Sigma–Aldrich, St

Louis, MO, USA) as a positive control. As a negative control, incubation with only Roswell Park Memorial Institute medium (RPMI, 1640 Dutch modification, Life Technologies/Invitrogen, Breda, the Netherlands) was performed. After incubation, blood samples were centrifuged at 4656 *g* for 10 min and supernatants were stored at −20 °C until cytokine measurement.

Cytokine measurements

The IFNγ production was measured by enzyme-linked immunosorbent assay (ELISA; Pelikine compact, Sanquin, Amsterdam, the Netherlands), in undiluted whole blood incubated for 24 h either with PHA, or *C. burnetii* NM in all patients, as described.[24,25] IL-2 was measured using a multiplex beads assay (Merck Millipore, Billerica, MA, USA) according to the manufacturer's instructions.

Ethical statement

This study was exempt from ethical approval by the local ethics committee, as there was no additional burden for patients. Samples were obtained during regular patient care after obtaining oral and written informed consent, and, in case of individuals from the Dutch Q-fever vaccination campaign, individuals signed written informed consent to use drawn blood for research purposes.

Statistical analysis

Data were analyzed using Graphpad Prism (Graphpad Software Inc., version 5.03) and SPSS (Version 22.0, SPSS, Inc). The Kruskall—Wallis test was used as non-parametric ANOVA to determine differences between groups. Statistical significance was attained if $p < 0.05$. In case of significance, by post-hoc analysis using Dunn's multiple comparison test was performed to look at pair wise comparisons between the groups, taking into account the number of comparisons made. The correlation between patient characteristics and IFNγ and IL-2 production, and the IFNγ/IL-2 ratio was determined with the non-parametric Spearman's rank correlation coefficient.

Results

Patients and controls

At the time of blood collection, QFS was already diagnosed but treatment had yet to be started (Table 1). The symptom duration of QFS patients, defined as the time of symptom onset until blood sampling, varied between 12 and 51 months (Table 1). All seropositive controls had IgG phase I or phase II titres ≥1:32, but IgG phase I ≤1:512, and none of them showed serological signs of an acute or recent Q-fever infection, reflected by IgM antibodies in absence of IgG

Table 1 Baseline characteristics of 20 patients with Q-fever fatigue syndrome (QFS).

Gender & age (yr)	Symptom duration[a] (months)	CIS fatigue	SIP total score	PCR serum	IFA IgM phase I	IFA IgM phase II	IFA IgG phase I	IFA IgG phase II	ELISA	CFA
W, 45	32	54	587	Negative	Negative	Negative	1:64	1:128	Negative	Negative
M, 55	35	51	1726	Negative	1:32	1:256	1:512	1:512	Positive	40
M, 57	18	49	1037	Negative	Negative	Negative	1:128	1:128	Negative	Negative
M, 64	37	47	2376	Negative	Negative	1:128	1:128	1:512	Dubious	40
M, 58	35	56	1583	Negative	1:64	Negative	1:32	1:128	Negative	Negative
W, 58	36	56	1205	Negative	Negative	1:128	Negative	1:32	Positive	Negative
M, 44	49	55	888	Negative	1:256	1:256	1:128	1:1024	Positive	80
M, 49	20	55	1374	Negative	1:16	1:32	Negative	1:16	Negative	Negative
M, 57	24	49	1792	Negative	Negative	Negative	1:128	1:128	Negative	Negative
M, 47	12	41	641	Negative	Negative	Negative	1:32	1:32	Negative	Negative
W, 48	16	41	1115	Negative	1:128	1:512	1:256	1:512	Positive	40
M, 46	17	50	546	Negative	Negative	Negative	1:256	1:512	Negative	10
M, 56	30	54	1408	Negative	1:64	1:128	1:512	1:512	Positive	40
M, 42	27	56	578	Negative	1:128	1:32	1:128	1:256	Negative	Negative
M, 59	28	45	1801	Negative	Negative	1:32	1:512	1:512	Dubious	40
M, 38	30	56	634	Negative	1:16	Negative	1:512	1:1024	Negative	80
W, 49	21	45	953	Negative	1:32	1:64	1:64	1:256	Dubious	20
W, 51	29	44	527	Negative	Negative	Negative	1:128	1:256	Dubious	20
M, 57	51	46	1389	Negative	1:16	Negative	1:128	1:256	Negative	80
W, 23	23	56	1194	Negative	1:16	Negative	Negative	1:16	Positive	Negative

Abbreviations: *QFS* = Q-fever fatigue syndrome; *CIS* = Checklist Individual Strength, subscale fatigue; *SIP* = Sickness Impact Profile; *PCR* = Polymerase chain reaction, in-house real time PCR directed against the insertion sequence IS1111a; *IFA* = Immunofluorescence assay (Focus Diagnostics, California, U.S.A), detecting IgM and IgG antibodies against phase I- and phase II-antigens; *ELISA* = Enzyme-linked immunosorbent assay (Panbio®, Australia, *Coxiella burnetii* (Q Fever) IgM ELISA, a screenings test directed against IgM phase II; *CFA* = Complement fixation assay (CFA) (Virion-Serion, Würzburg, Germany) directed against *C. burnetii* phase II antigens; *M* = Man; *W* = Woman.

[a] Symptom duration: time onset of symptoms until blood sampling.

antibodies. The mean age of QFS patients was 50.2 yrs (SD 9.3), which was significantly younger ($p < 0.001$) than 60.8 years (SD 15.1) and 66.2 years (SD 11.8) for the seropositive controls and chronic Q-fever group, respectively. There was no correlation between age and IFNγ production (Spearman's rank correlation coefficient −0.71, $p = 0.341$), between age and IL-2 production (Spearman's rank correlation coefficient −0.002, $p = 0.978$), and between age and IFNγ/IL-2 ratio (Spearman's rank correlation coefficient 0.060 ($p = 0.466$)). All groups had a predominant male distribution, with 70% being male in the QFS group, 78% in the seropositive control group, and 79% in the chronic Q-fever group (*not significant*).

IFNγ and IL-2 production and IFNγ/IL-2 ratio

Aspecific PHA-induced IFNγ production was similar in QFS patients, seropositive controls, and chronic Q-fever patients (Table 2, Fig. 1A). Specific stimulation with *C. burnetii* NM for 24 h in QFS patients showed a median IFNγ production of 319.5 pg/ml, which was significantly higher ($p < 0.01$) than in seropositive controls (median 120 pg/ml), but not significantly different from chronic Q-fever patients (median 2846 pg/ml) ($p = 0.110$) (Fig. 1B). No significant difference was observed in IL-2 production between QFS patients (median 104.5 pg/ml), seropositive controls (median 81 pg/ml), and chronic Q-fever patients (median 82.5 pg/ml) (Fig. 1C). The IFNγ/IL-2 ratio was calculated for each individual. The IFNγ/IL-2 ratio in QFS patients was not significantly different from seropositive controls, but significantly lower than the ratio found in chronic Q-fever patients ($p < 0.01$) (Fig. 1D).

Correlations between patient characteristics and cytokine measurements

Correlations between the most important characteristics of QFS patients (Table 1) and the measured cytokine productions were assessed (Table 3). The duration of symptoms did not significantly correlate with IFNγ production, but did so with IL-2 production ($p = 0.032$); it negatively correlated with the IFNγ/IL-2 ratio ($p = 0.025$). No correlation was found between the level of fatigue and IFNγ or IL-2 production, as well as the IFNγ/IL-2 ratio. A positive correlation was found between the level of perceived disabilities, reflected by the SIP total score, and IL-2 production ($p = 0.047$), but no correlation was found with either IFNγ production or the IFNγ/IL-2 ratio. Finally, no correlation was found between the IgG phase I titres and either IFNγ or IL-2 production, or the IFNγ/IL-2 ratio.

Discussion

In this study we assessed the antigen-specific IFNγ production and IFNγ/IL-2 ratio in *C. burnetii*-stimulated whole blood of QFS patients. We found that the IFNγ production of QFS and chronic Q-fever patients was not significantly

Table 2 IFNγ and IL-2 production in 20 patients with Q-fever fatigue syndrome (QFS).

Patients	IFNγ production (pg/ml)				IL-2 production (pg/ml)	Ratio IFNγ/IL-2
Gender & age	RPMI	PHA [10 μg/ml]	*C. burnetii* NM [10^7/ml]	*C. burnetii* NM [10^7/ml] - RPMI	*C. burnetii* NM [10^7/ml]	*C. burnetii* NM [10^7/ml]
W, 45	8	551	231	223	103	2.2
M, 55	22	477	356	334	107	3.1
M, 57	8	933	5347	5339	170	31.4
M, 64	10	935	5142	5132	820	6.3
M, 58	17	80	234	217	299	0.7
W, 58	29	5600	915	886	141	6.3
M, 44	8	5000	389	381	287	1.3
M, 49	8	236	266	258	59	4.4
M, 57	8	148	192	184	96	1.9
M, 47	8	125	500	492	16	30.8
W, 48	8	248	270	262	114	2.3
M, 46	12	953	4545	4533	78	58.1
M, 56	21	538	1754	1733	47	36.9
M, 42	39	311	2683	2644	106	24.9
M, 59	8	83	135	127	39	3.3
M, 38	8	146	643	635	362	1.8
W, 49	20	23	25	5	22	0.2
W, 51	19	2320	146	127	245	0.5
M, 57	20	81	325	305	16	19.1
W, 23	23	956	102	79	100	0.8

Net IFNγ production is shown after 24 h incubation of whole blood with PHA or *C. burnetii* NM. Furthermore, net IL-2 production is shown after 24 h incubation of whole blood with *C. burnetii* NM.

Abbreviations: *IFNγ* = Interferon-gamma; *IL* = Interleukin; *QFS* = Q-fever fatigue syndrome; *PHA* = Phytohemagglutinin; *C. burnetii* = *Coxiella burnetii*; *NM* = Nine Mile strain; *RPMI* = Roswell Park Memorial Institute medium, (1640 Dutch modification, Life Technologies/Invitrogen, Breda, the Netherlands); *M* = Man; *W* = Woman.

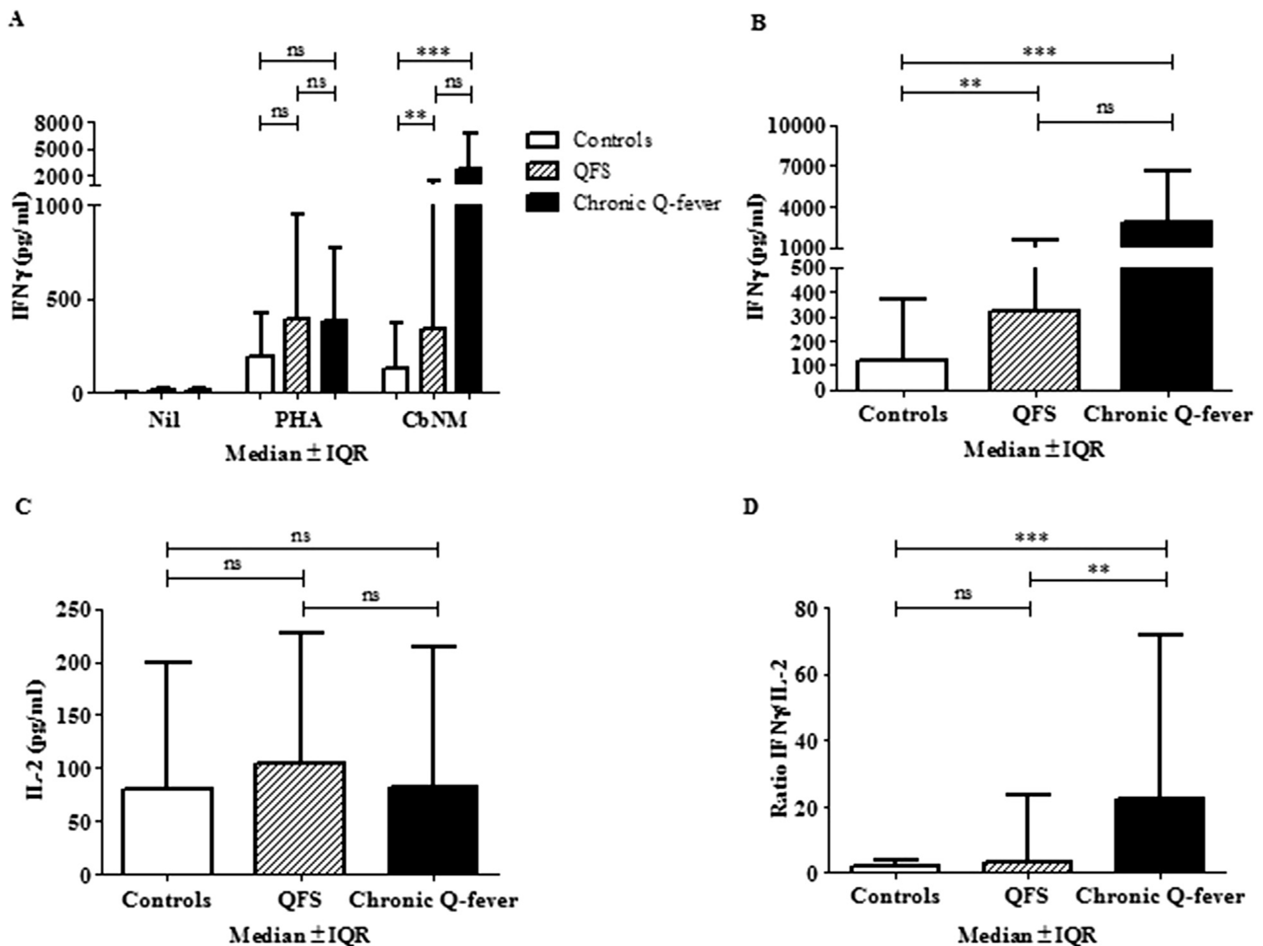

Figure 1 IFNγ and IL-2 production in Q-fever fatigue syndrome (QFS) patients, chronic Q-fever patients and Q-fever seropositive controls. (A) Comparable aspecific PHA-induced IFNγ production between QFS patients, seropositive controls and chronic Q-fever patients after 24 h incubation of whole blood. There is no significant difference in specific *Cb*NM-induced IFNγ production between QFS and chronic Q-fever patients. (B) *Cb*NM-induced IFNγ production (stimulated minus unstimulated) after 24 h incubation of whole blood, showing a significant difference in IFNγ production between seropositive controls and QFS and chronic Q-fever patients, with an increasing trend of IFNγ production towards chronic Q-fever patients. (C) *Cb*NM-induced IL-2 production (stimulated) between seropositive controls, QFS patients and chronic Q-fever patients after 24 h incubation of whole blood. (D) IFNγ/IL-2 ratio, showing a significant difference between chronic Q-fever patients and both seropositive controls and QFS patients. A trend towards a higher IFNγ/IL-2 ratio is observed towards chronic Q-fever patients. Median ± IQR are shown. The Kruskall–Wallis test was used, and, in case of significance, post-hoc analysis using the Dunn's multiple comparison test was performed to look at pair wise comparisons between the groups, taking into account the number of comparisons made. Abbreviations: *IFNγ* = Interferon-gamma; *IL* = Interleukin; *QFS* = Q-fever fatigue syndrome; *PHA* = Phytohemagglutinin; *CbNM* = *Coxiella burnetii* Nine Mile; *ns* = not significant; *IQR* = Interquartile range; *controls* = seropositive controls. **p-Value <0.01. ***p-Value <0.001.

different, but for both significantly increased compared to seropositive controls. In addition, the IFNγ/IL-2 ratio in QFS patients was similar to that in seropositive controls, but lower than in chronic Q-fever patients. Of note, no differences in IL-2 production between the three groups were found. These results suggest that *C. burnetii*-induced IFNγ production and IFNγ/IL-2 ratio may discriminate seropositive controls from QFS and chronic Q-fever patients.

At present, the measurement of the specific humoral immune response, i.e. serology, has a central position in the diagnosis of Q-fever, but it is increasingly accepted that cell-mediate immune responses are also relevant to describe the anti-*C. burnetii* host response. However, the precise relationship between T-cell function and protective immunity remains unknown. Memory T lymphocytes can be broadly divided in central memory T-cells, which lack immediate effector function and mainly secrete IL-2, and effector memory T-cells, displaying immediate effector function, e.g. IFNγ and IL-2 secretion.[32] IFNγ plays a pivotal role in protective immunity against many intracellular bacteria, but is also a marker of infection, immunity, and the extent of immune-mediated pathology.[20]

It has been proposed that full activation of the macrophage by IFNγ is required to eliminate *C. burnetii*, and that the phase 1 antigen can promote downregulation of IFNγ by lymphocytes, perhaps by modulating IL-2 production.[33] This

Table 3 Correlations between patient characteristics and IFNγ and IL-2 production in Q-fever fatigue syndrome (QFS) patients.

Patient characteristics	Duration of symptoms (months)[a]		CIS fatigue score		SIP score		IFA IgG phase 1 titres	
Cytokine production	Correlation (ρ)[b]	p-Value	Correlation (ρ)[b]	p-Value	Correlation (ρ)[b]	p-Value	Correlation (ρ)[b]	p-Value
IFNγ (pg/ml)	−0.235	0.320	−0.077	0.748	0.028	0.907	0.600	0.242
IL-2 (pg/ml)	0.480	0.032[c]	−0.106	0.657	0.449	0.047[c]	−0.086	0.919
IFNγ/IL-2 ratio	−0.498	0.025[c]	0.112	0.637	−0.147	0.535	0.314	0.564

Abbreviations: *IFNγ* = Interferon-gamma; *IL* = Interleukin; *QFS* = Q-fever fatigue syndrome; *CIS* = Checklist Individual Strength, subscale fatigue; *SIP* = Sickness Impact Profile; *IFA* = Immunofluorescence assay (Focus Diagnostics, California, U.S.A), detecting IgM and IgG antibodies against phase I- and phase II-antigens.

[a] Symptom duration: time onset of symptoms until blood sampling.

[b] Calculated using Spearman's rank correlation coefficient (ρ).

[c] Significant correlation of $p \leq 0.05$.

is however difficult to reconcile with the finding that chronic Q-fever patients exhibit a very high specific IFNγ production. It has been postulated that distinct IFNγ/IL-2 functional profiles correlate with different models of infection.[20] This concept is supported by previous findings, showing a high IL-2 production in seropositive controls, assumed to have cleared the infection successfully, and high IFNγ and low IL-2 production in chronic Q-fever patients.[19] Interestingly, our study revealed that QFS patients had a markedly higher *C. burnetii*-specific IFNγ production than seropositive controls. In addition, the IFNγ production in QFS patients and chronic Q-fever patients did not significantly differ, although there was a trend that QFS patients had lower IFNγ production than chronic Q-fever patients, and it can be expected that with larger numbers of patients these differences would become significant. In that case, it is tempting to hypothesize that QFS represents an altered cell-mediated immunity in the spectrum of Q-fever related syndromes, i.e. an inactive state without viable *C. burnetii* in contrast to chronic Q-fever. The combined use of IFNγ production and IL-2 production allows a better distinction between QFS patients, seropositive controls, and chronic Q-fever patients.[19] Also, a positive correlation between IL-2 production and both symptom duration and level of perceived disabilities was found, suggesting that QFS patients slowly attain an inactive state of infection, with a subsequent negative correlation between symptom duration and IFNγ/IL-2 ratio. Similarly, resolution of fatigue in the acute sickness response appeared to be associated with improvement of cell-mediated immunity.[34] The IFNγ/IL-2 ratio was proposed as an additional diagnostic marker for chronic Q-fever,[19] and our results indicate that the IFNγ/IL-2 ratio also discriminates between QFS and chronic Q-fever patients, but not between QFS patients and seropositive controls. Our data are supported by another study in the literature, showing IFNγ upregulation and IL-2 downregulation in QFS patients compared to control groups.[13] All these results point to an altered cell-mediated immune response in those who do not recover completely, implicating that both antigen-specific IFNγ production and IFNγ/IL-2 ratio might be used as immunological marker in the diagnostic workup of QFS. Although the results are strikingly similar, both our study and that of Penttila et al.[13] deal with low numbers of patients. Thus further confirmation is needed.

Other limitations of our study are that the cytokine studies in the seropositive controls and chronic Q-fever patients were performed earlier and derived from published studies of our group.[19,25] Ideally, these studies should have been done completely in parallel to avoid laboratory artefacts. However, the determination of IFNγ production is a standard procedure and therefore inter- and intra-individual variation is limited. In addition, the best control group for comparison with QFS patients would be patients with a previous Q-fever infection with asymptomatic recovery, i.e., without QFS or other co-morbidity. In contrast, the seropositive controls were anonymously derived from a vaccination campaign; these subjects had an indication for vaccination but were not vaccinated because of positive Q-fever serology. We cannot exclude that some of these patients suffered from fatigue. Finally, IL-6 production was not measured though it has been found that the IL-6 production was accentuated in QFS patients, with a significant correlation with total symptom scores,[13] and also higher in chronic Q-fever patients and seropositive controls compared to seronegative controls.[19]

Thus, it is too early to advice the usage of the immunological assays described here in a routine clinical setting. To overcome the mentioned limitations, and to investigate whether the IFNγ production assay or IFNγ/IL-2 ratio, and other cytokines such as IL-6, would be useful in clinical practice for diagnosing QFS, i.e. regardless of the time-point of sampling, a case–control study with comparison of QFS patients, CFS patients, seropositive controls without co-morbidity, and healthy controls will be performed in the near future.

Conclusion

In conclusion, the IFNγ production in QFS patients is significantly higher than in seropositive controls, and the IFNγ/IL-2 ratio is significantly lower than in chronic Q-fever patients.

Further investigation in larger cohorts of QFS patients is warranted, as these results point to an altered cell-mediated immunity in QFS, and hence opens up avenues for better understanding the pathogenesis of this enigmatic complication of Q-fever and of other fatigue syndromes.

Acknowledgment

The authors wish to thank all participating hospitals in facilitating collection of blood samples of chronic Q-fever patients.

List of abbreviations

C. burnetii *Coxiella burnetii*
CFS Chronic fatigue syndrome
CIS Checklist Individual Strength
ELISA Enzyme-linked immunosorbent assay
IFA Immunofluorescence assay
IFNγ Interferon-gamma
IL Interleukin
NM Nine Mile
PHA Phytohemagglutinin
Radboudumc Radboud university medical center
RPMI Roswell Park Memorial Institute medium
SIP Sickness Impact Profile
QFS Q-fever fatigue syndrome

Authors' contribution

SK, CB, TS, and MvD planned and designed the study, and have been involved in the analysis and interpretation of data. SK and RR drafted the manuscript. RR was also involved in the analysis and interpretation of data. SK, TS and CB collected samples of patients at the outpatient clinic. TS performed the experiments, and was involved in data collection, as well as in drafting and critical revision of the manuscript. JvdM, MN, CB, and MvD participated in interpretation of results. Furthermore, they provided critical revisions to the first drafts of the manuscript. All authors read and approved the final manuscript.

Potential conflicts of interest

Conflicts of interest: none. The authors declare that the final manuscript has not been submitted or accepted for publication elsewhere.

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