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Dysregulation of Serum Gamma Interferon Levels in Vascular Chronic Q Fever Patients Provides Insights into Disease Pathogenesis

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A large community outbreak of Q fever occurred in the Netherlands in the period 2007 to 2010. Some of the infected patients developed chronic Q fever, which typically includes pathogen dissemination to predisposed cardiovascular sites, with potentially fatal consequences. To identify the immune mechanisms responsible for ineffective clearance of *Coxiella burnetii* in patients who developed chronic Q fever, we compared serum concentrations of 47 inflammation-associated markers among patients with acute Q fever, vascular chronic Q fever, and past resolved Q fever. Serum levels of gamma interferon were strongly increased in acute but not in vascular chronic Q fever patients, compared to past resolved Q fever patients. Interleukin-18 levels showed a comparable increase in acute as well as vascular chronic Q fever patients. Additionally, vascular chronic Q fever patients had lower serum levels of gamma interferon-inducible protein 10 (IP-10) and transforming growth factor β (TGF-β) than did acute Q fever patients. Serum responses for these and other markers indicate that type I immune responses to *C. burnetii* are affected in chronic Q fever patients. This may be attributed to an affected immune system in cardiovascular patients, which enables local *C. burnetii* replication at affected cardiovascular sites.

A large community outbreak of Query fever (Q fever), which lasted from 2007 to 2010, took place in the Netherlands (1). In this period, 4,026 cases were reported, of which the majority (2,354) occurred in 2009. Q fever is a zoonotic infection caused by the omnipresent Gram-negative obligate intracellular coccobacillus *Coxiella burnetii*, which infects macrophages and monocytes. After passive entry into the host cell, *C. burnetii* is internalized into phagosomes, which fuse rapidly with lysosomes to form phagolysosomes (2, 3). After exposure, approximately 60% of patients remain asymptomatic. In symptomatic patients with acute Q fever, the most common clinical presentations are isolated fever, pneumonia, and hepatitis. In general, it is a mild disease that resolves spontaneously within 2 weeks. A chronic fatigue syndrome, which may persist for years, has been reported as a clinical complication following acute Q fever. This disease entity should be distinguished from chronic Q fever, which is typically a dissemination of the infection to predisposed sites like defective cardiac valves or aneurysms and vascular grafts, causing potentially fatal endocarditis or vascular infection, respectively (3, 4). The all-cause mortality of chronic Q fever in the Netherlands is 13%, and even 18% in patients with vascular chronic Q fever (5). Because of this high mortality, patients with cardiovascular disease were actively screened for the presence of chronic Q fever during and following the outbreak (6, 7). This screening contributed to the identification of the largest group of chronic Q fever patients diagnosed in a single outbreak.

In chronic Q fever patients, the immune response to *C. burnetii* infection is considered ineffective, possibly due to a defective cell-mediated immune response and absence of formation of granulomas (3, 4, 8, 9). However, the exact mechanism that underlies this defective response has not been elucidated yet. Type I immunity is believed to play a crucial role in the host response against *C. burnetii*. Activation of type I immunity leads to macrophage activation and killing of intracellular bacteria. Gamma interferon (IFN-γ), the key type I cytokine, is produced by T cells and natural killer (NK) cells, which play an important role in host defense against intracellular pathogens (10). In mice, it has been shown that IFN-γ is essential for the early control of *C. burnetii* infection (11). In literature case reports, a sustained clinical response of immunomodulatory therapy with IFN-γ was reported in a child with dual-antibiotic-therapy-resistant chronic multifocal Q fever osteomyelitis (12) as well as a child with multiple-antibiotic-resistant chronic Q fever (13). In addition, it has been shown *in vitro* that *C. burnetii* multiplied in untreated human monocytic cells, while reduced bacterial viability was observed after 24 h of IFN-γ treatment (14). These observations point to an essential role of IFN-γ in immunity against *C. burnetii*. However, whether a defect in type I immunity and IFN-γ production is involved in the development of chronic Q fever is not known.

Only a small proportion of *C. burnetii*-infected patients with cardiovascular risk factors go on to develop chronic Q fever (6, 7).

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Apparently, only a subgroup of these patients is unable to cope with this infection. The present study was designed to identify the immune mechanisms involved in efficient clearance of *C. burnetii* and the nature of the immune defect that hampers clearance in patients that go on to develop chronic Q fever. To this end, we analyzed the immune response of Q fever patients by measuring circulating serum concentrations of a set of cytokines and comparing the responses of patients with acute Q fever, vascular chronic Q fever, and past resolved Q fever.

MATERIALS AND METHODS

Serum samples. Included in this retrospective study were sera, obtained from 22 May to 31 May 2009 (weeks 21 and 22), of adult patients (≥18 years) who were diagnosed with PCR-proven seronegative acute Q fever by the Department of Medical Microbiology and Infection Control of the Jeroen Bosch Hospital (JBH; 's-Hertogenbosch, the Netherlands). All sera were referred because of clinical suspicion of acute Q fever. This 10-day period lies in the peak incidence weeks of the 2007-to-2010 acute Q fever outbreak (data from the National Institute for Public Health and the Environment). Serum samples of these patients were screened for IgM phase II antibodies with an enzyme-linked immunosorbent assay (ELISA) (Institut Virion/Serion GmbH, Würzburg, Germany). Following a negative outcome of the IgM phase II ELISA, an in-house PCR for *C. burnetii* DNA had been performed on serum with a positive outcome establishing the diagnosis of acute Q fever (15).

Sera of adult vascular chronic Q fever patients were obtained as part of a call/recall screening program for high-risk patients with an aortic aneurysm or central vascular reconstruction, initiated during and following the outbreak in the areas of the JBH and the neighboring Bernhoven Hospital (locations in Oss and Veghel, the Netherlands) (7). These regions correspond to the center of the epidemic. Screening for IgG phase I and IgG phase II antibodies was performed on serum samples with an immunofluorescence assay (IFA) (Focus Diagnostics, Inc., Cypress, CA, USA). In the case of an IgG phase I titer of ≥1:512, subsequent *C. burnetii* PCR on serum was performed. According to diagnostic criteria established by the Dutch Q Fever Consensus Group, a positive serum PCR in combination with a corresponding serologic profile in a patient with a vascular risk factor is considered diagnostic for proven chronic Q fever (16). Only patients diagnosed with PCR-proven vascular chronic Q fever in a clinically stable situation were analyzed in this study (excluding patients presenting with vascular complications, after recent surgery, or with coinfections).

Sera of adult patients with past resolved Q fever obtained as part of the above-mentioned screening program among patients with an aortic aneurysm or central vascular reconstruction were included in the control group. In these sera, screening for IgG phase I and IgG phase II antibodies revealed low antibody titers indicative of past resolved Q fever infection without indication for chronic Q fever.

After collection, all sera were stored at −20°C.

Ethics statement. In 2009, the Internal Review Board of the JBH approved the anonymous use of discarded blood for validation or research purposes. All patients who donated blood were informed of this possibility and had the right of refusal.

A regional medical ethics committee (Medisch-Ethische Toetsing Patiënten en Proefpersonen [METOPP]) waived the need for informed consent as far as testing of high-risk groups for chronic Q fever was concerned.

Multimarker analysis. From each of the three clinical groups (acute Q fever, vascular chronic Q fever, and past resolved Q fever), 10 serum samples were randomly selected for multianalyte serum analysis. Next, vials containing 125 μl of serum from these samples were blinded and randomized before shipment to the biomarker testing laboratory, Rules-Based Medicine (RBM; Austin, TX, USA). There, a set of 47 inflammation-associated markers were measured by bead-based immunoassay using the Human InflammationMAP v.1.0 marker panel on a Luminex 100 instrument (Luminex, Austin, TX, USA).

For data analysis, eight markers which did not have at least five values in the detectable range in at least one of the experimental groups were discarded from further statistical analysis. Values flagged as below the measurement range were replaced for further calculations by an estimated concentration of 80% of the lowest measurable concentration for that analyte. Further statistical analysis was performed using the statistical software package R (www.r-project.org). Per group, the geometric average concentration per marker was calculated. Differences in marker concentrations between the three groups were determined using a one-way analysis of variance (ANOVA) on log-transformed data. Resulting *P* values were corrected for multiple testing using the false discovery rate (FDR) according to Benjamini and Hochberg (17). An FDR threshold of 10% was used to identify markers with significantly different serum concentrations. For these markers, *post hoc t* tests were performed on the pairwise contrasts among the three experimental groups, again with an FDR threshold of 10% as a significance threshold. Data were visualized as a heat map and by principal component analysis (PCA).

Targeted serum analysis. For both validation of previous results and testing of new hypotheses, serum levels of a smaller number of markers were determined in-house, using sera from 43 acute Q fever patients, 31 vascular chronic Q fever patients, and 10 patients with past resolved Q fever. The vascular chronic Q fever group and the past resolved Q fever groups both included 10 sera also used in the multimarker analysis. Bead-based immunoassays for IFN-γ (Bio-Rad, Veenendaal, the Netherlands), IFN-γ-inducible protein 10 (IP-10) (R&D Systems, Minneapolis, MN, USA), transforming growth factor β1 (TGF-β1) (R&D Systems), and TGF-β2 (R&D Systems) were performed on a Luminex 100 instrument. Serum volumes used were 25 μl for IFN-γ and IP-10 and 20 μl for TGF-β1 and TGF-β2. Interleukin-18 (IL-18) was determined by ELISA (MBL, Woburn, MA, USA) using 20 μl serum. All assays were performed according to the manufacturer's instructions.

Statistical analysis was performed by pairwise comparisons between all groups, using a *t* test on log-transformed values. For this second (targeted) step, a more stringent FDR-corrected *P* value of 5% was used as a significance threshold.

RESULTS

Patients. In the period from 22 May to 31 May 2009, we diagnosed 43 adult patients (24 males [55.8%], mean age of 50 [range, 19 to 82] years) during the early acute stage of acute Q fever as illustrated by the presence of circulating *C. burnetii* DNA (positive PCR) but negative serology (negative IgM phase II ELISA). Follow-up serum samples were received from 30 of these 43 patients. IFA revealed the presence of IgG phase I and/or IgG phase II in all of these follow-up samples, indicating subsequent seroconversion after the initial seronegative phase of the infection.

Screening for chronic Q fever in patients with vascular risk factors identified 31 adult patients (26 males [83.9%], mean age of 71 [range, 53 to 90] years) with PCR-proven vascular chronic Q fever in a clinically stable situation. Vascular chronic Q fever patients were older and significantly more often male than acute Q fever patients.

In addition, this screening program identified 88 patients with vascular risk factors and past resolved Q fever (74 males [84.1%], mean age of 71 [range, 34 to 91] years). Patient characteristics are shown in Table 1.

Circulating *C. burnetii* DNA loads as determined by quantitative PCR (qPCR) during the routine clinical diagnostics were not significantly different between acute and vascular chronic Q fever patients (Table 1).

Immune response. By measuring a large set of cytokines in a

TABLE 1 Patient characteristics in acute and vascular chronic Q fever patients

Characteristic	Value for Q fever group (*n*):			*P* value, acute vs vascular chronic Q fever
	Past resolved (88)	Acute (43)	Vascular chronic (31)	
Gender (% male)	84.1	55.8	83.9	0.013
Age (yr)	71 ± 9	50 ± 16	71 ± 9	<0.001
C. burnetii DNA load (qPCR C_T)[a]	Not determined	35.9 ± 3.9	36.8 ± 4.1	0.36

[a] qPCR threshold cycle (C_T) values are based on the 2-logarithm of the *C. burnetii* DNA quantity, with a lower C_T value indicating a higher load.

small group of patients, we first identified cytokines and other inflammatory markers that were differentially expressed in patients with acute and vascular chronic Q fever. This analysis showed differences in 16 out of 39 analyzed markers (Table 2). Upon comparison to patients with past resolved Q fever, changes in serum concentrations were for most of these markers more pronounced in acute than in vascular chronic Q fever patients. In a PCA visualization, which visualizes samples based on their degree of dissimilarity, the acute Q fever group was clearly distinct from the past resolved Q fever group, whereas the group with vascular chronic Q fever showed a similar overall trend but was not readily distinguishable (Fig. 1).

More detailed data visualization in a heat map indicated that differentially expressed markers could be arranged into groups with distinct expression patterns (Fig. 2). C-reactive protein

TABLE 2 Serum marker analysis results[b]

Marker	Geometric avg/group:			FDR[a]	*Post hoc* test[a]
	Past resolved	Acute	Vascular chronic		
Alpha-2-macroglobulin (mg/ml)	1.17	1.99	1.33	0.158	
Alpha-1-antitrypsin (mg/ml)	2.25	3.72	3.55	0.037 A	BC
Beta-2-microglobulin (μg/ml)	2.03	2.13	2.70	0.337	
Brain-derived neurotrophic factor (ng/ml)	29.3	21.6	21.9	0.133	
Complement C3 (mg/ml)	1.44	2.45	1.67	0.002 A	BD
C-reactive protein (μg/ml)	3.2	157.0	11.4	<0.001 A	BD
Eotaxin-1 (pg/ml)	369	160	334	0.002 A	BD
Factor VII (ng/ml)	661	373	432	0.037 A	B
Fibrinogen (μg/ml)	13.9	25.6	14.5	0.067 A	
Ferritin (ng/ml)	161	638	99	0.001 A	BD
Haptoglobin (mg/ml)	1.87	4.17	2.43	0.163	
Intercellular adhesion molecule 1 (ng/ml)	113	201	199	0.002 A	BC
Gamma interferon (pg/ml)	0.69	8.48	0.73	<0.001 A	BD
Interleukin-1 alpha (pg/ml)	1.27	1.97	1.48	0.163	
Interleukin-1 beta (pg/ml)	1.69	2.16	1.56	0.343	
Interleukin-1 receptor antagonist (pg/ml)	112	403	133	<0.001 A	BD
Interleukin-6 (pg/ml)	4.87	35.26	7.69	0.004 A	BD
Interleukin-8 (pg/ml)	35.2	24.6	94.3	0.129	
Interleukin-10 (pg/ml)	2.92	6.39	3.48	0.159	
Interleukin-12 subunit p70 (pg/ml)	9.9	19.0	10.6	0.082 A	B
Interleukin-15 (ng/ml)	0.37	0.30	0.33	0.723	
Interleukin-17 (pg/ml)	2.42	2.31	1.67	0.413	
Interleukin-18 (pg/ml)	177	291	290	0.082 A	B
Interleukin-23 (ng/ml)	1.84	1.85	1.79	0.989	
Monocyte chemotactic protein 1 (pg/ml)	263	174	221	0.300	
Macrophage inflammatory protein-1 alpha (pg/ml)	56.2	24.9	103.9	0.004 A	BD
Macrophage inflammatory protein-1 beta (pg/ml)	318	489	676	0.103	
Matrix metalloproteinase-2 (ng/ml)	2.01	1.53	1.46	0.163	
Matrix metalloproteinase-3 (ng/ml)	8.16	6.05	8.82	0.447	
Matrix metalloproteinase-9 (ng/ml)	15.6	26.0	23.4	0.098 A	
T-cell-specific protein RANTES (ng/ml)	16.2	11.8	24.8	0.037 A	D
Stem cell factor (pg/ml)	268	199	242	0.244	
Tissue inhibitor of metalloproteinases 1 (ng/ml)	180	210	197	0.273	
Tumor necrosis factor alpha (pg/ml)	2.38	2.84	5.67	0.106	
Tumor necrosis factor receptor 2 (ng/ml)	9.5	13.6	14.1	0.129	
Vascular cell adhesion molecule 1 (ng/ml)	698	847	805	0.413	
Vitamin D-binding protein (μg/ml)	224	239	309	0.343	
Vascular endothelial growth factor (pg/ml)	807	912	957	0.723	
von Willebrand factor (μg/ml)	44.8	52.3	54.9	0.337	

[a] Significance is shown by uppercase letters as follows: A, significant in one-way ANOVA at an FDR of <10%; B, significant for *post hoc* comparison on past resolved versus acute Q fever group (FDR, <10%); C, significant for *post hoc* comparison on past resolved versus vascular chronic Q fever group (FDR, <10%); D, significant for *post hoc* comparison on acute versus vascular chronic Q fever group (FDR, <10%).

[b] A set of 39 serum markers were measured in three clinical groups (each for $n = 10$ samples): past resolved Q fever group, acute Q fever group, and vascular chronic Q fever group.

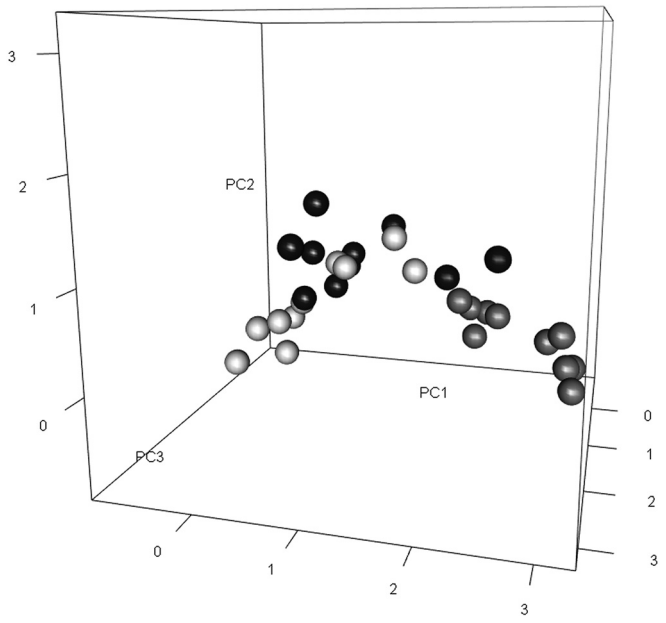

FIG 1 Principal component analysis of markers with different serum levels in patients with acute Q fever, vascular chronic Q fever, and past resolved Q fever. Distances between samples indicate the degree of dissimilarity. Shades of gray indicate patient groups: light gray, past resolved; dark gray, acute; black, vascular chronic Q fever.

(CRP) and IL-6 serum levels were very strongly increased in the acute Q fever group compared to a smaller increase in the vascular chronic Q fever group. Four markers (IL-18, matrix metalloproteinase-9 [MMP9], alpha-1-antitrypsin [AAT], and intercellular adhesion molecule 1 [ICAM1]) had increased expression at comparable levels in both acute and vascular chronic Q fever patients. Several markers showed an increase in the acute Q fever group but no substantial change in the vascular chronic Q fever group. These markers included IL-1 receptor antagonist (IL-1RA), complement C3, IL-12 subunit p70 (IL-12p70), ferritin, IFN-γ, and fibrinogen. The difference was most pronounced for IFN-γ. Two markers, macrophage inflammatory protein-1 alpha (MIP1α) and Regulated on Activation, Normal T cell Expressed and Secreted (RANTES), had lower serum levels in acute Q fever patients but increased levels in the vascular chronic Q fever group. Finally, serum levels of factor VII and eotaxin-1 were decreased in both acute and vascular chronic Q fever groups compared to patients with past resolved Q fever (Table 2; Fig. 2).

Because *C. burnetii* is an intracellular pathogen and its clearance is probably induced via the Th1 route, we further explored our finding of low IFN-γ levels in the vascular chronic Q fever group in larger groups of patient sera. In addition, several other cytokines involved in IFN-γ signaling were measured in these larger patient groups. These included IL-18, a cytokine known to induce the production of IFN-γ by NK cells and T cells; IP-10, a chemoattractant for activated T cells expressed in many Th1-type inflammatory diseases (18) which is also regulated by IL-18; and TGF-β1, because it is known to reduce IFN-γ production (19).

These analyses again showed significantly higher levels of IFN-γ in the acute Q fever group (12.30 pg/ml) than in the vascular chronic Q fever (2.72 pg/ml) or the past resolved Q fever (1.75 pg/ml) group, both differences being significant at an FDR-corrected *P* value of <0.001. Differences between vascular chronic Q fever and past resolved Q fever patients were not significant (FDR-corrected *P* value of 0.32) (Table 3).

In the analysis of the larger groups, IL-18 again showed significantly increased levels in acute and vascular chronic Q fever patients, compared to past resolved Q fever patients. Levels in acute and vascular chronic Q fever patients were 593 and 761 pg/ml, respectively, which were not significantly different (FDR = 0.062).

Serum levels of IP-10 were higher in patients with acute Q fever (161 pg/ml) than in vascular chronic Q fever patients (93 pg/ml) and past resolved Q fever patients (43 pg/ml) (both significant at an FDR of <0.01). The difference between the vascular chronic Q fever and past resolved Q fever groups was also significant (FDR = 0.023).

TGF-β1 levels were decreased in vascular chronic Q fever patients (11.9 ng/ml) compared to acute Q fever patients (141.4 ng/ml) or past resolved Q fever patients (112.5 ng/ml). For this marker, the difference between the vascular chronic Q fever group and both other groups was significant at an FDR of <5% (Table 3). Also, TGF-β2 levels were decreased in vascular chronic Q fever patients (209 pg/ml) compared to acute Q fever patients (769 pg/ml) or past resolved Q fever patients (577 pg/ml), with the levels in the acute Q fever group significantly different from both other groups (Table 3).

DISCUSSION

In this study, we set out to identify the immune mechanisms involved in clearance of *C. burnetii*, and the nature of the immune defect that hampers such clearance in patients who go on to develop chronic Q fever, within the context of this study more specifically vascular chronic Q fever. A particular strength of our study is that we determined serum levels of over 40 different cytokines and other inflammatory markers, allowing us to relate the various responses to each other. To our knowledge, this study is the first one describing such a comprehensive serum profiling in Q fever patients.

A considerable number of markers showed different levels in acute Q fever patients compared to vascular chronic Q fever patients. Of these markers, CRP and IL-6 were strongly increased in the acute Q fever group and to a lesser extent in the vascular chronic Q fever group compared to the past resolved Q fever group. Recent findings obtained in acute Q fever patients from the same outbreak also showed correlated responses between these two acute-phase response markers (20). We also show here that a set of CC-type chemokines (MIP1α, RANTES, and eotaxin-1) shows downregulation in acute Q fever patients and a heterogeneous response in the group of vascular chronic Q fever patients. These chemokines share their capacity to attract eosinophils, and their common downregulation in the acute Q fever group points toward a skewing of the systemic chemokine profile away from a Th2 toward a Th1 response, which is in agreement with the type I response found in acute Q fever patients. Most other inflammatory markers had increased levels in the acute and vascular chronic Q fever groups, with levels in the vascular chronic patients either similar to the acute Q fever group or intermediate between the acute and past resolved Q fever groups. These, together, indicate a weaker overall inflammatory response in vascular chronic Q fever patients.

The main finding, however, of this study was that patients with vascular chronic Q fever display reduced IFN-γ produc-

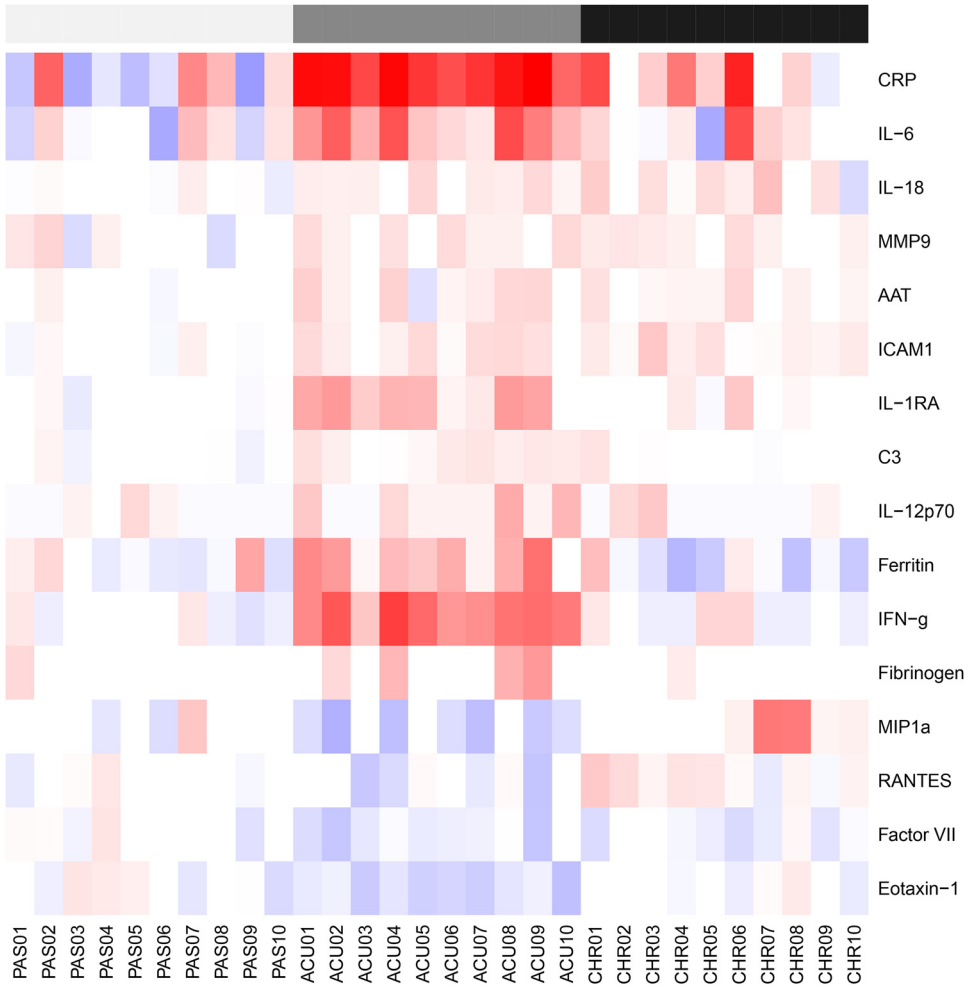

FIG 2 Heat map of serum markers with significantly different (FDR, 10%) serum concentrations in patients with acute Q fever (ACU01 to -10), vascular chronic Q fever (CHR01 to -10), and past resolved Q fever (PAS01 to -10). Serum levels are given in comparison to the average of the past resolved Q fever patient group. The color scale runs from red (increased levels) via white (unchanged) to blue (decreased levels). Colors in the top bar indicate patient groups and correspond to those in Fig. 1. AAT, alpha-1-antitrypsin; C3, complement C3; CRP, C-reactive protein; ICAM1, intercellular adhesion molecule 1; IFN-g, gamma interferon; IL-1RA, interleukin-1 receptor antagonist; IL-6, interleukin-6; IL-12p70, interleukin-12 subunit p70; IL-18, interleukin-18; MIP1a, macrophage inflammatory protein-1 alpha; MMP9, matrix metalloproteinase-9; RANTES, Regulated on Activation, Normal T cell Expressed and Secreted.

tion. This could indicate a potential cause of the inadequate immune response postulated in patients with chronic Q fever (3, 4). As is shown in mice, IFN-γ seems to play a key role in the early control of Q fever. In mice, low levels of IFN-γ resulted in aberrant macrophage activation and the lack of killing of *C. burnetii* (11).

TABLE 3 Targeted serum analysis[a]

Marker	Geometric avg/group (*n*): Past resolved (10)	Acute (43)	Vascular chronic (31)	FDR for comparison: Past resolved with acute	Past resolved with vascular chronic	Acute with vascular chronic
IFN-γ (pg/ml)	1.75	12.30	2.72	<0.001	0.320	<0.001
IL-18 (pg/ml)	325	593	761	0.005	<0.001	0.062
IP-10 (pg/ml)	43.0	161.3	93.3	<0.001	0.023	0.004
TGF-β1 (ng/ml)	112.5	141.4	11.9	0.055	0.029	<0.001
TGF-β2 (pg/ml)	577	769	209	<0.001	0.050	<0.001

[a] Serum markers were measured in three clinical groups: past resolved Q fever group, acute Q fever group, and vascular chronic Q fever group.

One of the cytokines consistently induced in both acute and vascular chronic Q fever is IL-18. This cytokine, formerly known as IFN-γ-inducing factor, is mainly produced by cells of the macrophage lineage with a wide range of biologic activities that include the induction of IFN-γ production in T cells and NK cells (21, 22). Increased circulating IL-18 levels have been reported in viral infections, in which context recently markedly elevated levels during acute Epstein-Barr virus (EBV) infections and EBV-associated diseases were shown (23). Phylogenetically, the nearest relatives to *C. burnetii* are the *Legionellaceae*. These microorganisms share similar intracellular lifestyles and utilize common genes to infect alveolar macrophages (24). It has been shown previously that IL-18 plays a key role in modulating induction of IFN-γ in the murine lung in response to *Legionella pneumophila* infection (25). The role of IL-18 in immunity against *C. burnetii* has, however, not been investigated.

In our study, IL-18 serum levels were increased at comparable

levels in both acute and vascular chronic Q fever patients compared to past resolved Q fever patients, a finding which was validated in the second larger serum analysis. Thus, it appears that although one of the cytokines involved in activation of IFN-γ production was present, this did not result in IFN-γ production in vascular chronic Q fever patients, whereas acute patients readily produced IFN-γ.

In addition to IL-18, also IL-12p70, which is known to induce the production of IFN-γ by NK cells and T cells (26), and IL-23, another cytokine involved in the Th1 immune response by activating inflammatory cells that is required for induction of chronic inflammation and granuloma formation (27), were measured in the initial biomarker panel. The latter did not show any signs of differential expression among the three patient groups. (FDR-corrected *P* value, 0.98). IL-12p70 showed an increased level in the acute Q fever group compared to the other two groups. However, it should be noted that this difference was only borderline significant (FDR = 8%) and that this cytokine was not consistently present within the detectable range (8 times in acute Q fever patients, 4 times in vascular chronic Q fever patients, and 4 times in past resolved Q fever patients), so some caution needs to be taken in interpreting these findings.

Taken together, IL-18, IL-12p70, and IL-23 did not show clearly significant differences in serum levels between acute and vascular chronic Q fever patients, and therefore, these cytokines cannot be held responsible for the observed difference in IFN-γ levels in patients with acute and vascular chronic Q fever.

Furthermore, we have found significantly lower levels of IP-10 in patients with vascular chronic Q fever than in patients with acute Q fever. It is known that inflammation is associated with secretion of IP-10 from leukocytes like neutrophils, eosinophils, and monocytes as well as epithelial, endothelial, and stromal cells and keratinocytes in response to IFN-γ (28). For instance, it has been shown that impaired IP-10 production led to susceptibility to *L. pneumophila* infection (29). Apparently, induction of IFN-γ and IP-10 is reduced in vascular chronic Q fever, even though the signal from the IFN-γ- and IP-10-inducing cytokine IL-18 is present, indicating a dysregulation of the type I cytokine response. A hypothesis that would fit with these observations is that IL-18 responsiveness is reduced in (vascular) chronic Q fever, at least as far as the response to *C. burnetii* is involved.

One of the cytokines that is implicated in modulating IL-18 responsiveness is TGF-β, which is a pleiotropic cytokine produced by T cells and macrophages (30). Under inflammatory conditions, in the presence of other cytokines such as IL-6 and IL-4, TGF-β can promote further inflammation and augment autoimmune conditions (31). In mice, TGF-β interferes with IL-18-induced IFN-γ production by reducing the number of IL-18 receptors at the cell surface and, thereby, preventing induction of T-bet expression (32). In addition, TGF-β reduces IFN-γ production in response to the engagement of the Fc receptor CD16 on NK cells (19). However, by enhancing survival of memory $CD8^+$ T cells, once an antigen is detected, it increases the production of IL-17 and IFN-γ (33). Furthermore, it has been shown that TGF-β inhibits the killing of the intracellular microorganisms *Trypanosoma cruzi*, *Mycobacterium avium*, and *Mycobacterium tuberculosis*. In supernatants of unstimulated peripheral blood mononuclear cells from patients with Q fever endocarditis, TGF-β levels were significantly increased compared with control supernatants (34).

We showed that the levels of TGF-β in patients with vascular chronic Q fever were significantly lower than those in patients with acute Q fever. Our results seem to be contrary to what could be expected based on previous research. However, our findings are shown to be highly significant (FDR-corrected *P* value, $<10^{-5}$). A possible explanation may be that *in vivo* TGF-β is acting locally, which might explain differences between literature findings based on cytokine production by specific types of immune cells under controlled conditions as opposed to systemic levels of serum cytokines measured in our study.

A difference between local and systemic response in chronic Q fever can most likely be attributed to perturbed responses by *C. burnetii*-infected macrophages. Such a defect in local macrophage cellular immunity points toward a change in macrophage M1/M2 polarization. A relation between macrophage polarization and disease course has, for example, been observed in leprosy (35) as well as Whipple's disease (36), both caused by intracellular pathogens.

Indeed, literature data indicate that *C. burnetii* activates an atypical M2 program in macrophages, which may account for the capacity of *C. burnetii* to survive in macrophages (37). *C. burnetii*-stimulated macrophages produced high levels of TGF-β1 (37), and we hypothesize that this may account for the increase in serum TGF-β found in acute Q fever patients. The lack of systemic increase in serum TGF-β in vascular chronic Q fever patients might be explained by such responses being confined to sites associated with chronic Q fever like aneurysms, or cardiac valves in the case of Q fever endocarditis. Interestingly, it has been reported that patients with cardiac valve lesions have elevated levels of apoptotic leukocytes in the circulation and that apoptotic cell binding induces a nonprotective M2 program in *C. burnetii*-stimulated monocytes and macrophages (38). Patients with cardiovascular disease may, therefore, have a skewed immune system as a consequence of their existing illness, which creates a suitable environment for *C. burnetii* replication and the pathogenesis of chronic Q fever (38). This provides an explanation for the incidence of chronic Q fever among cardiovascular patients. Interestingly, *in vitro* data show that IFN-γ redirects monocytes and macrophages toward an M1 program and leads to reduced counts of *C. burnetii* (38). Moreover, two isolated case studies have been reported in which IFN-γ was used to treat forms of chronic Q fever (12, 13). This suggests that research into IFN-γ therapy in chronic Q fever patients might be valuable.

The multimarker serum screening approach used in this study allowed us to identify several markers in type I immunity that were affected in vascular chronic Q fever. Despite the significant differences between specific cytokines in acute and vascular chronic Q fever, a limitation of our study is the lack of consecutive determination of these cytokines in subsequent sera. These results could have affected the precise understanding of the immune response in acute and vascular chronic Q fever.

In conclusion, we found significantly lower serum levels of IFN-γ in patients suffering from vascular chronic Q fever, despite the presence of similar IL-18 levels and significantly reduced levels of TGF-β, in comparison to acute Q fever patients. This cytokine profile indicates a dysregulation of the systemic and probably also local type I response to *C. burnetii*, which could explain the inadequate immune response in these patients.

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