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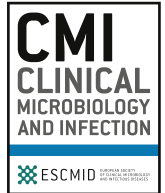

Original article

Intact interferon-γ response against *Coxiella burnetii* by peripheral blood mononuclear cells in chronic Q fever

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ABSTRACT

Objectives: Q fever is caused by *Coxiella burnetii,* an intracellular bacterium that infects phagocytes. The aim of the present study was to investigate whether the *C. burnetii*-induced IFN-γ response is defective in chronic Q fever patients.
Methods: IFN-γ was measured in supernatants of *C. burnetii*-stimulated peripheral blood mononuclear cells (PBMCs) of 17 chronic Q fever patients and 17 healthy individuals. To assess IFN-γ responses, expression profiles of IFN-γ-induced genes in *C. burnetii*-stimulated PBMCs were studied in six patients and four healthy individuals. Neopterin was measured in PBMC supernatants (of eight patients and four healthy individuals) and in sera (of 21 patients and 11 healthy individuals). In a genetic association study, polymorphisms in genes involved in the Th1-cytokine response were analysed in a cohort of 139 chronic Q fever patients and a cohort of 220 control individuals with previous exposition to *C. burnetii*.
Results: IFN-γ production by *C. burnetii*-stimulated PBMCs from chronic Q fever patients was significantly higher than in healthy controls. Many IFN-γ response genes were strongly upregulated in PBMCs of patients. Neopterin levels were significantly higher in PBMC supernatants and sera of patients. The *IL12B* polymorphisms rs3212227 and rs2853694 were associated with chronic Q fever.
Conclusions: IFN-γ production, as well as the response to IFN-γ, is intact in chronic Q fever patients, and even higher than in healthy individuals. Polymorphisms in the IL-12p40 gene are associated with chronic Q fever. Thus, a deficiency in IFN-γ responses does not explain the failure to clear the infection. The genetic data suggest, however, that the IL-12/IFN-γ pathway does play a role. **T. Schoffelen, CMI 2017;23:209.e9–209.e15**
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Introduction

Q fever is a zoonosis caused by the bacterium *Coxiella burnetii*. The infection is transmitted to humans by inhalation of aerosols that contain bacteria from animal manure or birthing fluids [1]. Upon infection, over half of individuals remain asymptomatic, while others develop acute Q fever [1]. Regardless of initial manifestations, a minority of individuals develop chronic infection that may become apparent months or years after initial exposure. Chronic Q fever mainly develops in people with pre-existing valvular disease, aortic aneurysm, or vascular prostheses [2,3], and may present as endocarditis, a mycotic aneurysm, or vascular prosthesis infection. These are life-threatening conditions that require long-term antibiotics and sometimes surgical intervention [3]. During the 2007–2011 Q fever outbreak in the Netherlands, the majority of patients with valvular/vascular risk factors for chronic Q fever cleared the infection [4,5]; however, over 250 of these developed chronic Q fever [6]. It is unknown what explains this difference in susceptibility.

As an obligate intracellular pathogen, *C. burnetii* proliferates in monocytes and macrophages, where it replicates in intracellular

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acidic vacuoles [7]. Containment of intracellular infection by the host's immune system requires a pro-inflammatory response with granuloma formation and killing or control of the bacterium inside activated monocytes/macrophages. The key cytokine in this process is interferon-γ (IFN-γ), a pro-inflammatory cytokine, which activates macrophages, and stimulates immune cells to eliminate or control intracellular pathogens. IFN-γ production is induced by type-1 cytokines secreted by monocytes/macrophages or dendritic cells, most notably interleukin (IL)-12, IL-23, and IL-18.

The important role of IFN-γ in the defence against *C. burnetii* is supported by the high mortality observed in IFN-$\gamma^{-/-}$ mice infected with *C. burnetii* [8]. *In vitro* studies have also shown that IFN-γ induces killing of *C. burnetii* by monocytes, and inhibits growth of *C. burnetii* in mouse fibroblasts [9–11]. It has been reported that chronic Q fever is associated with a defective antigen-driven lymphocyte proliferation to *C. burnetii* antigens, with intact responses to other antigens [12]. A substantial amount of *C. burnetii*-specific IFN-γ is produced by healthy individuals after vaccination with killed *C. burnetii* and after natural infection [13]. Based on these observations, it was assumed that chronic Q fever patients have an inadequate IFN-γ response to *C. burnetii* that leads to persistent infection [8,10,14], but evidence for this hypothesis is lacking. On the contrary, recent studies by us and others have shown high antigen-specific IFN-γ production in chronic Q fever [15,16]. The aim of the present study is to investigate the IFN-γ response to *C. burnetii* in chronic Q fever patients.

Materials and methods

Ethics

The ethical committee of Radboud University Medical Center (Nijmegen, the Netherlands) approved the study (NL35784.091.11). Participants provided written informed consent (waiver when deceased ($n=5$), as approved by the ethical committee). Institutional Review boards of participating hospitals approved the inclusion of participants in this study. The study was performed in accordance with the Declaration of Helsinki.

Participants

For peripheral blood mononuclear cells (PBMCs) stimulations, 17 chronic Q fever patients, who visited the internal medicine outpatient clinics of participating hospitals, were included (Table S1). The diagnosis of chronic Q fever was based on the publication of the Dutch chronic Q fever consensus group [17]. Seventeen healthy individuals without known history of Q fever, were included as controls in these experiments.

For genetic analyses, all probable or proven chronic Q fever patients [17], who visited the internal medicine outpatient clinics of participating hospitals, were approached. They were recruited as described before [18], and 139 patients were included. The at risk control group consisted of 220 individuals from the same area with vascular or valvular abnormalities predisposing to chronic Q fever, with serological evidence of exposition to *C. burnetii* (anti-*C. burnetii* phase II IgG antibodies ≥1:32) without clinical symptoms or serological evidence of chronic Q fever. These individuals were recruited as described previously [18].

Bacteria

C. burnetii Nine Mile (NM) phase I (RSA 493) [19] and *C. burnetii* 3262 [20] were cultured at the Central Veterinary Institute (Lelystad, the Netherlands), and the number of *Coxiella* DNA copies determined, as described previously [21]. The *C. burnetii* strains were inactivated by heating 30 minutes at 99°C, and stored at -80°C. Q-vax vaccine (CSL Biotherapies, Victoria, Australia) [22] contains formaldehyde-inactivated *C. burnetii* Henzerling strain phase I in 50 μg/mL, and was used at 100 ng/mL.

PBMCs isolation and stimulation

PBMCs were isolated as previously described [23]. The PBMCs [2.5×10^6/mL] were incubated in RPMI 1640 Dutch modification culture medium (Sigma-Aldrich, St Louis, MO, USA) supplemented with 1% L-glutamine, 1% pyruvate, and 1% gentamicin in a round-bottomed 96-well plate (200 μL/well) at 37°C and 5% CO_2 with heat-killed *C. burnetii* NM (10^7 bacteria/mL and 10^6 bacteria/mL), heat-killed *C. burnetii* 3262 (10^6 bacteria/mL), Q-vax (100 ng/mL) or heat-killed *C. albicans* (ATCC MYA-3573; UC820) (10^5 conidia/mL), or culture medium alone. After 48 hours, supernatants were collected and stored at −20°C.

IFN-γ and neopterin measurements

IFN-γ production was measured in supernatants by enzyme-linked immunosorbent assay (ELISA; Pelikine compact, Sanquin, Amsterdam, the Netherlands). Neopterin was measured in supernatants of eight patients and four healthy individuals and in sera stored at -80°C of 21 chronic Q fever patients and 11 healthy individuals by ELISA (IBL International, Hamburg, Germany).

Gene expression analysis

PBMCs (10^7 cells/mL) of six patients and four healthy individuals were incubated for 8 hours in a flat-bottomed 24-well plate (1 mL/well) at 37°C and 5% CO_2 with heat-killed *C. burnetii* NM (10^7 bacteria/mL), *E. coli* LPS (10 ng/mL) or culture medium alone. RNA was extracted using the RNeasy Mini kit (Qiagen). RNA quality was assessed using the 2100 Bioanalyzer and RNA 6000 Nano LabChip kit (Agilent Technologies), and quantity was assessed using the Nanodrop. Gene expression was analysed using Whole Human Genome 4 × 44K microarrays (Agilent Technologies, Massy, France), representing 45 000 probes and One-color Microarray Based Gene Expression Analysis kit, as previously described [24].

The data were analysed with R and the Bioconductor software suites. Raw data were preprocessed and quality-checked with Agi4x44PreProcess library and normalized through quantile normalization. Differential expression was assessed using Limma library. Genes/probes were considered modulated if any stimulation showed $|FC| > 2$ and corrected $p < 0.01$.

To explore the IFN-γ pathway activation, we performed functional analysis based on Gene Ontology term GO:0034341 ‘response to interferon-gamma’. We explored the activation of this pathway by aggregating standardized (i.e. centre-reduced) expression values within each gene-set of the modulated genes/probes. Minimum Information About a Microarray Experiment (MIAME)-compliant data were submitted to the Gene Expression Omnibus (GEO) (http://www.ncbi.nlm.nih.gov/geo/) with accession number GSE66476.

Genotyping polymorphisms and determining associated IFN-γ response

From outpatient clinic patients, blood was drawn and stored at -80°C. DNA was isolated from these samples using standard methods [25]. Other participants, both patients and control individuals, received a buccal swab kit (Isohelix, Cell Projects Ltd., Harrietsham, UK) to obtain epithelial cells. DNA was isolated using a buccal DNA isolation kit (Isohelix). Single nucleotide

polymorphisms (SNPs) were selected based on known functional effects on gene expression or protein function, published associations with human diseases, and/or haploview data. In total, nine SNPs in *IFNG*, *IFNGR1*, *IL18*, *IL12B*, and *IL12RB1* were genotyped with a Sequenom mass-spectrometry genotyping platform in July 2014. Quality control was performed by duplicating 5% of the samples within and across plates, by incorporating positive and negative control samples and by sequencing samples to verify the various genotypes.

In a subgroup of genotyped control individuals, whole blood was stimulated with *C. burnetii* NM phase I (10^7 bacteria/mL) and *C. burnetii*-induced IFN-γ was measured, as described earlier [16]. Supernatants were stored at −20°C.

Statistical analyses

Median IFN-γ and neopterin concentrations were compared using Mann-Whitney *U* tests, using GraphPad Prism (GraphPad software Inc., version 5). Tests were two-sided and a p-value <0.05 was considered to be statistically significant.

Median standardized expression values of modulated genes present in GO:0034341 were compared between patients and healthy individuals and between stimulations using Wilcoxon matched-pairs signed rank test. A p-value <0.001 (accounting for multiple testing) was considered to be significant.

Hardy-Weinberg equilibrium (HWE) was analysed for all SNPs in the control cohort [26]. Differences in genotype frequencies between patients and controls were analysed by means of a gene dosage model, with Fisher's exact test. Dominant and recessive model analysis was performed by univariate logistic regression, for which ORs and 95% CI are reported. Because the choice of genetic variants was based exclusively on genes with an established role in response to *C. burnetii* recognition, rather than exploratory, no correction for multiple testing was performed. Statistical analyses were carried out with IBM SPSS software (version 20).

Results

Intact C. burnetii—induced IFN-γ production by PBMCs of chronic Q fever patients

The ability of PBMCs of chronic Q fever patients to mount a recall response to *C. burnetii* was investigated by stimulation of PBMCs in the absence of serum for 48 hours with various *C. burnetii*-strains and subsequent measurement of IFN-γ in the supernatants. High IFN-γ production was induced in PBMCs of 17 patients with all *C. burnetii*-strains and this was significantly different from the response of 17 healthy individuals (Fig. 1). Interestingly, inactivated *C. albicans*, known to induce IFN-γ in PBMCs of healthy individuals, led to significant less IFN-γ production in chronic Q fever patients.

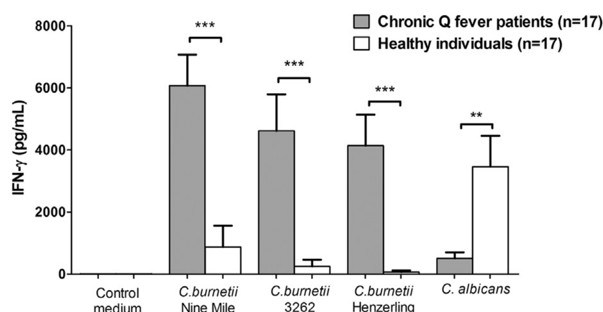

Fig. 1. *C. burnetii*-induced IFN-γ production is increased in chronic Q fever patients compared with healthy individuals. IFN-γ was measured in supernatants of PBMCs stimulated for 48 hours with inactivated *C. burnetii* Nine Mile (10^6 bacteria/mL), *C. burnetii* 3262 (10^6 bacteria/mL), *C. burnetii* Henzerling (100 ng/mL) (all phase I) and *Candida albicans* (10^5 conidia/mL). Values are expressed as mean ± standard error. p-Values are calculated using Mann-Whitney *U* test. **p <0.01, ***p <0.001.

C. burnetii-induced upregulation of IFN-γ response genes in chronic Q fever patients

As chronic Q fever patients produce high amounts of IFN-γ upon *C. burnetii* stimulation, we wondered whether the pathway downstream of IFN-γ would be defective in chronic Q fever patients. In a whole-transcriptome analysis, the transcriptional responses of PBMCs to *C. burnetii* and, for comparison, to *E. coli* LPS were investigated in six patients and four healthy controls.

The gene expression profiles overall showed a different activation pattern in unstimulated PBMCs of patients and healthy individuals compared with *E. coli* LPS or *C. burnetii* stimulated PBMCs. PBMCs of patients stimulated with *C. burnetii* showed a very distinct activation pattern (Fig. 2(a)). In the heat-map, the patients' PBMCs stimulated with *C. burnetii* clustered together (Fig. 2(b)). We focused our analysis on IFN-γ response genes. First, we specifically looked at genes that are described to be activated by IFN-γ in PBMCs and monocytes [27] (Table 1). We found that these were mostly upregulated in *C. burnetii*-stimulated PBMCs of chronic Q fever patients, in contrast to PBMCs of healthy individuals, while *E. coli* LPS-stimulated PBMCs did not show a different expression profile. Second, we assessed the transcriptional modulation of the IFN-γ response pathway (GO term GO:0034341 'response to interferon-gamma'). One-hundred and fifty-one genes of GO:0034341 were represented by probes in the microarray (Table S2). Of these, 54 probes in 42 genes were modulated. The median standardized expressions of the 54 probes, representing the relative activation of the IFN-γ response pathway, are shown in Fig. 3. The highest activation of the IFN-γ response was found in *C. burnetii*-stimulated PBMCs of patients. Moreover, *C. burnetii* stimulation showed a significantly higher activation in patients' PBMCs compared with PBMCs of healthy individuals (p <0.001), whereas there was no significant difference in activation on *E. coli* LPS stimulation between patients and healthy individuals (p >0.001).

Neopterin is increased in chronic Q fever

Neopterin is a highly stable molecule and its biosynthesis is seen as a marker of activation of the cellular immune system, in particular by IFN-γ. In the course of a cellular immune reaction, neopterin can be measured in serum. It is produced *in vitro* by macrophages after stimulation with IFN-γ. We found significantly higher concentrations of neopterin in serum of 21 chronic Q fever patients than in 11 healthy individuals (Fig. 4(a)). In addition, in the supernatant of *C. burnetii* NM-stimulated PBMCs of chronic Q fever patients, neopterin levels were significantly higher than those of healthy individuals (Fig. 4(b)), while in supernatants of *C. albicans*-stimulated PBMCs, neopterin concentrations were similar.

Polymorphisms in IL12B are associated with chronic Q fever and are associated with decreased IFN-γ production

We investigated whether subtle, common genetic variations in the type-1 cytokine pathway, that affect IFN-γ production or response, are associated with the risk for development of chronic Q fever. We performed a genetic association study using a cohort of chronic Q fever patients and appropriate control individuals as described previously [18]. In total, 139 (92 proven and 47 probable) chronic Q fever patients and 220 control individuals without

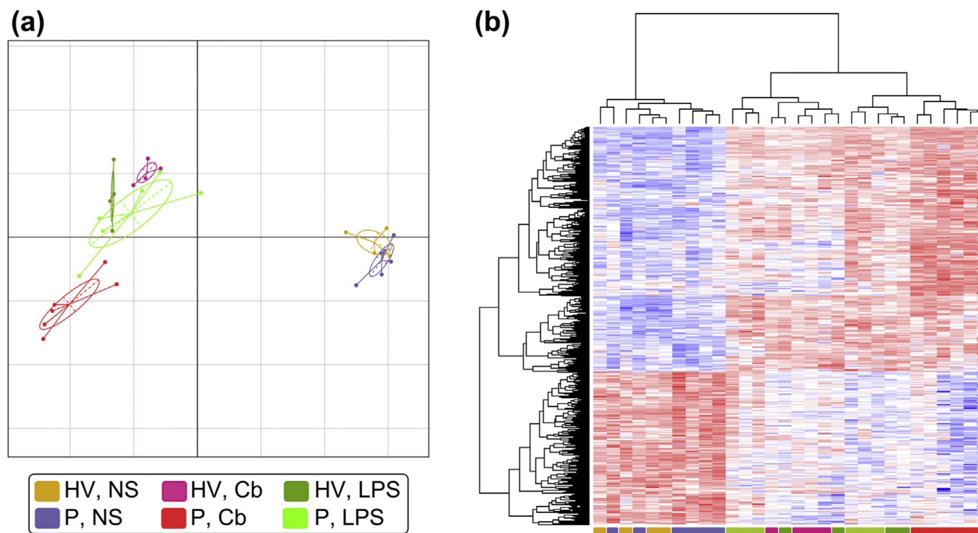

Fig. 2. Distinct transcriptional profile of *C. burnetii*-stimulated PBMCs of chronic Q fever patients. PBMCs of six chronic Q fever patients (P) and four healthy volunteers (HV) were either not stimulated (NS), or stimulated with heat-inactivated *C. burnetii* Nine Mile (10^7 bacteria/mL) (Cb), or stimulated with *E. coli* LPS (10 ng/mL) (LPS) for 8 hours. RNA was extracted and a microarray was performed. (a) Graphical representation of the samples based on the correspondence analysis of the modulated probes. The samples are coloured according to the group (patients versus healthy volunteers) and stimulus (not stimulated, *C. burnetii* or LPS). (b) Heatmap representation of the modulated probes in stimulated PBMCs compared with unstimulated PBMCs. The probes are shown in the rows and the samples in the columns. The expression levels are colour-coded from blue to red. Below the columns, samples are colour-coded as in A.

Table 1
Modulation of interferon-γ related genes in PBMCs of chronic Q fever patients and healthy individuals stimulated by *C. burnetii* or *E. coli* LPS

Symbol HGNC	Probe ID	Stimulation with *C. burnetii*		Stimulation with *E. coli* LPS	
		Chronic Q fever patients	Healthy individuals	Chronic Q fever patients	Healthy individuals
BCL6	A_23_P57856	2.16	1.67	2.32	2.36
CD69	A_23_P87879	2.83	1.18	1.35	1.41
CISH	A_24_P97465	3.19	1.02	1.08	1.23
CXCL9	A_23_P18452	62.5	0.45	1.24	4.53
CXCL10	A_24_P303091	16.5	0.20	0.64	1.67
CXCL11	A_23_P125278	15.5	0.10	0.70	1.45
GBP1	A_23_P62890	11.2	0.62	2.05	4.16
GBP2	A_23_P85693	2.75	0.88	1.31	1.97
HCAR3	A_23_P64721	5.35	2.42	2.59	2.47
ICAM1	A_23_P153320	6.66	3.09	3.87	3.65
IRF1	A_23_P41765	3.80	1.17	1.28	2.26
IRF8	A_23_P332190	3.16	0.72	1.09	1.36
RHOH	A_23_P58132	2.39	0.84	1.23	1.11
RPS9	A_32_P223456	1.14	1.14	1.04	0.92
SERPING1	A_23_P139123	4.23	0.20	0.66	1.09
SOCS1	A_23_P420196	7.33	2.78	4.04	3.94
STAT1	A_24_P274270	2.00	0.54	1.29	2.21
UBE2L3	A_32_P100428	1.39	1.10	1.14	1.31
UBE3A	A_24_P207150	1.10	1.06	0.89	1.06
VAMP5	A_23_P39840	2.43	0.68	0.89	1.21

PBMCs of six chronic Q fever patients and four healthy individuals were stimulated with heat-inactivated *C. burnetii* Nine Mile (10^7 bacteria/mL) or *E. coli* LPS (10 ng/mL) for 8 hours. The median ratios of gene expression in stimulated PBMCs to unstimulated PBMCs are shown. Listed genes are induced by interferon-γ in PBMCs or monocytes, as described by Waddell *et al.* [27]. HGNC, HUGO Gene Nomenclature Committee; ID, identification number.

chronic Q fever but with serological evidence of *C. burnetii* exposure and a risk factor for chronic Q fever were included. Genotyping of patients and controls was successful for all polymorphisms (Table S3) in genes encoding IFN-γ (*IFNG*), IFN-γ receptor chain 1 (*IFNGR1*), IL-18 (*IL18*), IL-12p40 (*IL12B*), and the IL-12 receptor β1 chain (*IL12RB1*). For each polymorphism, >92% of the participants were genotyped. All SNPs were in Hardy-Weinberg equilibrium in the control group.

In the gene dosage analysis, genotyping revealed an association between chronic Q fever and both *IL12B* polymorphisms: *IL12B* rs2853694 (p 0.006) and *IL12B* rs3212227 (p 0.004). No associations were observed between other polymorphisms and presence of chronic Q fever (Table S4). Subsequently, *IL12B* rs2853694 was found to be significantly differently distributed in a recessive model analysis, leading to increased risk of chronic Q fever (p 0.001; OR 2.18, 95% CI 1.35–3.53). *IL12B* rs3212227 distribution was significantly different in a dominant model analysis, with protective effect of the C allele (p 0.004; OR 0.50, 95% CI 0.31–0.80) (Table S4). These two polymorphisms are not strongly linked (r^2=0.248).

Next we investigated the functional consequences of these two genetic variations by assessing the *C. burnetii* induced IFN-γ production in whole blood samples from control individuals stratified for *IL12B* genotypes either in a recessive model (rs2853694; low-risk AA/AC versus high-risk CC) or in a dominant model

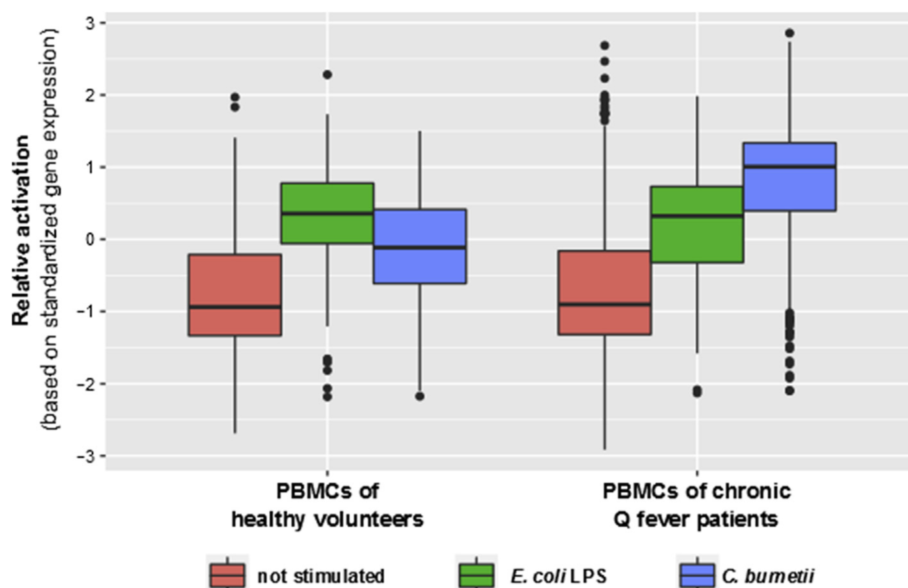

Fig. 3. Chronic Q fever patients show increased relative activation of the 'response to interferon-gamma' pathway (Gene Ontology GO-term GO:0034341). PBMCs of four healthy volunteers and six chronic Q fever patients were either not stimulated, stimulated with *E. coli* LPS (10 ng/mL), or stimulated with heat-inactivated *C. burnetii* Nine Mile (10^7 bacteria/mL) for 8 hours. RNA was extracted and a microarray was performed. The relative activation of the 'response to interferon-gamma' pathway was based on median standardized gene expression of the 54 probes in the microarray that corresponded to 42 genes mapped in GO:0034341 *and* were significantly modulated in stimulated PBMCs ($|FC|>2$ and $p<0.01$) (see Table S1 for a list of these 54 probes). The horizontal lines present the median, the boxes present the interquartile range (IQR), and the whiskers present 1.5*IQR. Outliers (outside the whiskers) are plotted as dots. *C. burnetii*-stimulated PBMCs of patients showed significantly higher relative activation than those of healthy individuals (Wilcoxon test; $p<0.001$), whereas there was no significant difference in relative activation on *E. coli* LPS stimulation between patients' and healthy individuals ($p>0.001$).

(rs3212227; high-risk AA versus low-risk AC/CC) (Fig. S1). The mean *C. burnetii*-stimulated IFN-γ production in individuals with rs2853694 CC genotype (243 pg/mL) and AA/AC genotype (563 pg/mL) was not significantly different (*p* 0.13). Individuals with rs3212227 AC/CC low-risk genotypes and CC genotypes did not show statistically different mean IFN-γ production either (573 pg/mL versus 428 pg/mL, *p* 0.40).

Discussion

We investigated the *C. burnetii*-induced IFN-γ production and response in chronic Q fever patients. We show that PBMCs of chronic Q fever patients are not only capable of high IFN-γ production in response to *C. burnetii in vitro*, but also display upregulation of IFN-γ response genes and of the highly IFN-γ-dependent neopterin. In addition, we found in a large group of chronic Q fever patients ($n=139$) and control individuals that *IL12B* polymorphisms are associated with progression to chronic Q fever.

Previously, it was assumed that chronic Q fever patients have an inadequate T-cell derived IFN-γ production in response to *C. burnetii* infection. However, the current study could not confirm this assumption. In contrast, our findings suggest that *C. burnetii*-induced IFN-γ production in chronic Q fever is increased. In a previous study, Koster *et al.* [12] found that lymphocytes of chronic Q fever patients fail to proliferate *in vitro* in response to *C. burnetii* antigens. In one of these patients, this was demonstrated 5 years after the endocarditis was treated with antibiotics and cardiac valve replacement. This unresponsiveness was antigen-specific, as lymphocyte proliferation in response to *Candida* antigens was preserved [12]. The method of studying *C. burnetii*-specific adaptive immune responses used by Koster *et al.*, that is lymphocyte proliferation, is different from our recent studies in which we showed increased IFN-γ production by *ex vivo C. burnetii*-stimulated whole blood of chronic Q fever patients [28]. In our present study, we confirmed these findings in PBMCs cultured for 48 hours in the presence of three different *C. burnetii* strains. The absence of autologous serum in these cultures shows that the IFN-γ production observed is not dependent on anti-*C. burnetii* antibodies.

Surprisingly, we found decreased IFN-γ response to *Candida albicans* in chronic Q fever patients compared with healthy individuals. This indicates that PBMCs of patients with an active chronic Q fever infection are strongly responsive to *C. burnetii* in a specific fashion, at the expense of responsiveness to other stimuli. More research is necessary to validate this surprising finding and to unravel the mechanism behind it.

Transcriptome analysis revealed that many IFN-γ responsive genes are upregulated in *C. burnetii* stimulated PBMCs of chronic Q fever patients. In addition, IFN-γ-dependent transcription factors were specifically upregulated in *C. burnetii* stimulated PBMCs of patients, and not in healthy individuals (data not shown). This shows that the IFN-γ signalling pathway is intact in chronic Q fever.

The high neopterin levels *in vivo*—the measurements in serum—and *in vitro*—in *C. burnetii*-stimulated PBMCs—indicate that macrophages of chronic Q fever patients are activated by IFN-γ. This confirms that the IFN-γ signalling pathway is intact. In a previous study, mean neopterin levels in plasma of 13 acute and 23 chronic Q fever patients were also found to be increased (5.4 and 5.1 ng/mL, respectively) compared with 17 healthy individuals (2.1 ng/mL), although these differences were not significant [29].

Interestingly, our findings that the IFN-γ pathway in response to *C. burnetii* is intact in chronic Q fever patients can be added to the observation that anti-*C. burnetii* antibody titres are high in chronic Q fever patients [30]. Taken together, we conclude that we have not found evidence for an impaired adaptive immune response in chronic Q fever. As these patients are not able to kill the pathogen, apparently the antibody response and the strong IFN-γ response do not lead to adequate bactericidal effects. Why these hosts fail to eliminate the bacteria is still enigmatic.

IL-23 and IL-12 are critical in the innate immune response to pathogens to induce production of IFN-γ. An essential component of both IL-23 and IL-12 is IL-12p40. In the present study, we found that the presence of SNPs in the promoter (rs2853694) and at the

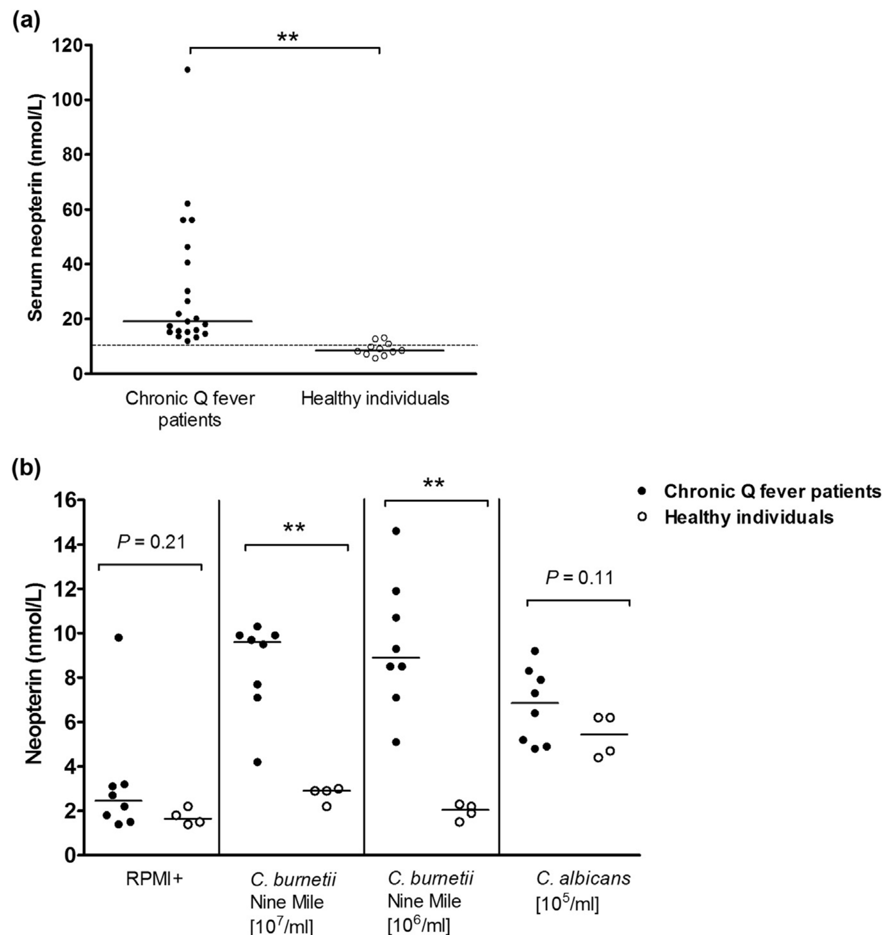

Fig. 4. *C. burnetii*-induced neopterin is significantly increased in chronic Q fever patients. (a) Neopterin was measured in serum of chronic Q fever patients (n=21) and healthy individuals (n=11). (b) Neopterin was measured in supernatant of PBMCs of patients (n=8) and of healthy individuals (n=4) after stimulation for 48 hours with inactivated *C. burnetii* Nine Mile (NM) phase I (10^7 bacteria/mL), *C. burnetii* NM phase I (10^6 bacteria/mL), and *C. albicans* (10^5 bacteria/mL). Medians of healthy individuals and patients are compared by Mann-Whitney *U* test. ** p <0.01.

3′UTR (rs3212227) of the IL-12p40 gene, *IL12B*, was associated with the development of chronic Q fever. For both SNPs, association with leprosy and tuberculosis—both caused by *Mycobacteria* which are also intracellular pathogens—has been described [31–34]. For the management of *C. burnetii* infection in patients at risk, these polymorphisms may be incorporated in risk stratification for development of chronic Q fever.

It might well be that the difference in IFN-γ response to *C. burnetii* in chronic Q fever patients is related to genetic polymorphisms in *IL12B*. However, we found that healthy individuals with the risk genotype of the polymorphisms did not significantly differ in *in vitro* IFN-γ production on *C. burnetii* stimulation. Hence we were unable to prove that these polymorphisms have an impact on the susceptibility to *C. burnetii* through production of IFN-γ. These polymorphisms may nevertheless have an effect *in vivo* on production of IFN-γ or other cytokines and on proliferation and differentiation of various T cell subsets. The high IFN-γ production on *C. burnetii* stimulation in chronic Q fever patients is measured during an active chronic infection and most likely results from an ongoing stimulation of adaptive immune responses. In this light it would be interesting to compare initial IFN-γ responses between acute Q fever patients who eventually develop a chronic infection and those who do not. Honstettre *et al.* described a trend of reduced IL-12p40 release by PBMCs during acute Q fever in patients with valvulopathy, of whom 50% subsequently developed chronic Q fever [35].

There are some considerations that should be taken into account when interpreting our results. First of all, we studied the immune response of circulation blood cells to *C. burnetii*. Although difficult to perform, it may be more relevant to study the local immune response in *C. burnetii*-infected vascular walls or valvular tissue, where these are mostly low-grade infections in which systemic immune activation is apparently not effective in clearing the local infection. The local immunological processes are likely to be crucial for survival of *C. burnetii* at predilection sites such as defective cardiac valves and aneurysmatic vascular wall. Immunohistochemical studies of *C. burnetii*-infected cardiac valves showed small, focal collections of infected mononuclear phagocytes [36]. Further identification of these cells and their immunological environment could help us to understand the local persistence of *C. burnetii*.

Second, the model that we used to study the IFN-γ response to *C. burnetii*, that is *in vitro* stimulation of PBMCs with heat-inactivated bacteria, might not reflect the processes *in vivo*. In particular, relatively high doses of *C. burnetii* were used to stimulate the PBMCs in culture, compared with the low antigen load in serum during Q fever infection.

In conclusion, the present study shows that IFN-γ production and response to IFN-γ are intact in chronic Q fever patients and do not explain the failure to clear the infection. Our genetic analyses, showing that *IL12B* polymorphisms are associated with chronic Q fever, also points to the IFN-γ pathway, although the functional consequences of these polymorphisms in Q fever remain unclear.

Transparency declaration

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cmi.2016.11.008.

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