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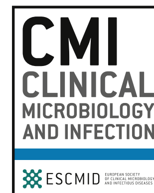

Original article

Involvement of matrix metalloproteinases in chronic Q fever

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Objectives: Chronic Q fever is a persistent infection with the intracellular Gram-negative bacterium *Coxiella burnetii*, which can lead to complications of infected aneurysms. Matrix metalloproteinases (MMPs) cleave extracellular matrix and are involved in infections as well as aneurysms. We aimed to study the role of MMPs in the pathogenesis of chronic Q fever.
Methods: We investigated gene expression of MMPs through microarray analysis and MMP production with ELISA in *C. burnetii*-stimulated peripheral blood mononuclear cells (PBMCs) of patients with chronic Q fever and healthy controls. Twenty single nucleotide polymorphisms (SNPs) of MMP and tissue inhibitor of MMP genes were genotyped in 139 patients with chronic Q fever and 220 controls with similar cardiovascular co-morbidity. Additionally, circulating MMPs levels in patients with chronic Q fever were compared with those in cardiovascular controls with and without a history of past Q fever.
Results: In healthy controls, the MMP pathway involving four genes (*MMP1*, *MMP7*, *MMP10*, *MMP19*) was significantly up-regulated in *C. burnetii*-stimulated but not in *Escherichia coli* lipopolysaccharide -stimulated PBMCs. *Coxiella burnetii* induced MMP-1 and MMP-9 production in PBMCs of healthy individuals (both $p < 0.001$), individuals with past Q fever ($p < 0.05$, $p < 0.01$, respectively) and of patients with chronic Q fever (both $p < 0.001$). SNPs in *MMP7* (rs11568810) ($p < 0.05$) and *MMP9* (rs17576) ($p < 0.05$) were more common in patients with chronic Q fever. Circulating MMP-7 serum levels were higher in patients with chronic Q fever (median 33.5 ng/mL, interquartile range 22.3–45.7 ng/mL) than controls (20.6 ng/mL, 15.9–33.8 ng/mL).
Conclusion: *Coxiella burnetii*-induced MMP production may contribute to the development of chronic Q fever. **A.F.M. Jansen, Clin Microbiol Infect 2017;23:487.e7–487.e13**
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Introduction

Chronic Q fever is a life-threatening condition caused by *Coxiella burnetii*, an obligate intracellular Gram-negative bacterium that infects monocytes and macrophages. Upon exposition, individuals may remain asymptomatic or can develop acute Q fever, often manifested as flu-like illness or an airway infection. In some individuals, a chronic infection develops that may become apparent several years later. In these individuals, *C. burnetii* infects aberrant endovascular foci, such as vascular prostheses and aneurysms. Risk factors for chronic Q fever include valvulopathy, aortic aneurysm and aortic prosthesis [1,2]. To effectively cure these patients, surgical intervention is frequently required in addition to long-term antibiotics. Mortality in both chronic Q fever endocarditis (9.3%) and endovascular infection (18.0%) is high [3]. From 2007 to 2010, a

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large outbreak of Q fever infected many individuals in the Netherlands. Although the epidemic ended in 2010, new chronic Q fever patients are still diagnosed in 2016 [4,5]. How *C. burnetii* persists and eventually causes chronic infection in these patients is still largely unknown.

Matrix metalloproteinases (MMPs) comprise a family of 23 soluble or membrane-bound Ca^{2+}-dependent proteolytic enzymes characterized by a zinc ion at the catalytic site. MMPs were first recognized for their ability to degrade extracellular matrix proteins, but are also involved in cell migration, proliferation and differentiation, angiogenesis, inflammation and cleavage of cytokines [6–8]. They are systemically produced as inactive enzymes (zymogens) that gain activity by various mechanisms and lose activity by tissue inhibitors of MMPs (TIMPs). MMPs have a dual role in the immune response to infection and tissue repair. Migration of immune-competent cells requires MMP-mediated degradation of the extracellular matrix. On the other hand, excess of MMP activity can lead to tissue destruction and hence to the spread of bacteria [9–11] or, as it has been reported for MMP-2 and MMP-9, contributes to the development of vascular aneurysms and aortic dilatation [12–15].

Matrix metalloproteinases have been implicated in the pathology of various infections [16–18], including Q fever [10], but their contribution to the pathogenesis of chronic Q fever is largely unknown. To obtain more insight into the role of MMPs in the progression to chronic Q fever, we examined *C. burnetii*-induced transcription of MMP genes, the production of MMPs by peripheral blood cells of healthy individuals, patients with current or past Q fever infection, and patients with chronic Q fever. Additionally, we genotyped polymorphisms in MMP genes and determined serum concentrations of various MMPs in patients with chronic Q fever and controls.

Methods

Subjects

Healthy volunteers ($n = 21$) were hospital personnel or students without a known history of Q fever. Patients with acute Q fever ($n = 8$) were recruited from patients that visited the outpatient clinic of the Radboud University Medical Centre. Individuals with risk factors for chronic Q fever and positive Q fever serology but without evidence of chronic Q fever ($n = 17$) were selected from the cohort that was invited to participate in the Dutch Q fever vaccination campaign [19]. Patients with chronic Q fever ($n = 38$, see Supplementary material, Table S1), diagnosed according to the Dutch consensus guideline [20], were recruited from the participating hospitals. For analysis in serum samples, anonymized aliquots left over after diagnostic procedures were used from seronegative individuals with vascular disease ($n = 10$) and from individuals with positive Q fever serology without progression to chronic Q fever, but with similar cardiovascular co-morbidity ($n = 10$).

For experiments with antibiotics, peripheral blood mononuclear cells (PBMCs) were derived from buffy coats of blood donors (Sanquin, Nijmegen, the Netherlands) with an unknown history of Q fever.

The study was approved by the Medical Ethics Committee Arnhem-Nijmegen (NL35784.091.11). Written informed consent was obtained from all participants, with the exception of anonymized left over specimens from diagnostic procedures, for which written informed consent was waived.

PBMC isolation

The PBMCs from whole blood and buffy coats were isolated by density centrifugation on Ficoll-Paque (Pharmacia Biotech, Piscataway, NJ, USA) [21]. Cells were aspirated and washed twice in sterile PBS and resuspended in RPMI-1640 Dutch modification culture medium (Sigma-Aldrich, St Louis, MO, USA) supplemented with 1% L-glutamine, 1% pyruvate and 1% gentamicin. After isolation, PBMCs were used in gene expression analysis or in PBMC stimulation experiments.

Gene expression analysis

The PBMCs were incubated in a flat-bottom 24-well plate (10^7 cells/mL) in a volume of 1 mL/well in the presence of culture medium (negative control), or stimulated with heat-killed *C. burnetii* Nine Mile RSA 493 phase I (*C. burnetii* NM) 10^7/mL or *Escherichia coli* lipopolysaccharide (LPS) 10 ng/mL. RNA was extracted after 8 h of incubation using the RNeasy MiniKit (Qiagen, Hilden, Germany). Quality and quantity of the samples were assessed with the 2100 Bioanalyzer RNA 6000 Nano LabChip kit (Agilent Technologies, Santa Clara, CA, USA) and Nanodrop, respectively. Whole Genome 4×44K microarrays (Agilent Technologies) were used to analyse PBMC gene expression [22]. R and BIOCONDUCTOR software suites were used to analyse the data. Raw data were preprocessed and a quality check was performed with the *Agi4×44PreProcess* library and quantile normalization was applied. Differential gene expression was performed using *Limma* library [23]. Genes were considered to be differentially expressed when the median absolute fold change (as compared to unstimulated samples) was >2.0 and the false discovery rate was <5%. Selected genes were tested in a functional enrichment analysis with DAVID TOOLS [24], using Gene Ontology and the Kyoto Encyclopaedia of Genes and Genomes pathways. Data were generated in accordance with the Minimum Information About a Microarray Experiment (MIAME) guidelines [25] and were deposited in the National Center for Biotechnology Information's Gene Expression Omnibus, (www.ncbi.nlm.nih.gov/geo/), accessible with number GSE66476.

PBMC stimulation

For measurement of MMP production by PBMCs, 100 μL containing 5×10^5 PBMCs was incubated with 100 μL of culture medium, or 100 μL containing heat-killed *C. burnetii* NM 2×10^6 bacteria or *E. coli* LPS 2 ng (end concentrations 10^7/mL and 10 ng/mL, respectively). After 24 h, supernatants were stored at −80°C.

To assess the effect of antibiotics on *C. burnetii*-induced MMP production in PBMCs from buffy-coat donors, 100 μL containing 5×10^5 cells was pre-incubated for 1 h at 37°C with culture medium, 5, 10 or 25 mg/L doxycycline (Sigma-Aldrich) or 5 and 10 μg/mL moxifloxacin (VWR, Radnor, PA, USA). In some experiments doxycycline was combined with 1 mg/L hydroxychloroquine (Sigma-Aldrich). Subsequently, *C. burnetii* NM 10^6/mL was added and cells were incubated for 24 h at 37°C and 5% CO_2. Supernatants were stored at −20°C.

In vitro whole blood stimulation

Venous blood was drawn into 5 mL lithium-free heparin tubes (Vacutainer; BD Biosciences, Franklin Lakes, NJ, USA). Blood was diluted 1 : 5 and stimulated according to previously described methods [19] with either culture medium or *C. burnetii* NM (10^7/mL). After a 48 h incubation period, the blood was centrifuged and the supernatants were stored at −80°C.

Measurement of MMPs

MMP-1 and MMP-9 concentrations in supernatants were measured with commercially available ELISA kits (DY901 and

DY911, respectively; R&D Systems, Minneapolis, MN) in accordance with the manufacturers' instructions. The concentration of five MMPs (MMP-1, -2, -7, -9, -10) was determined using a Luminex magnetic bead assay (Merck Millipore, Billerica, MA, USA). Activity of MMP-9 was quantified with a commercially available kit (F9M00, R&D Systems).

Single nucleotide polymorphism analysis

Two cohorts of patients were used for genotype analysis, the first comprised 220 individuals with serologically proven Q fever infection in the past. These individuals had cardiovascular risk factors for chronic Q fever without serological evidence of progression to a chronic infection. The second cohort consisted of 139 patients with chronic Q fever, with either proven or probable disease according to the Dutch consensus guideline [20]. DNA from both patients and controls was isolated from venous blood or epithelial cells from a buccal swab (Isohelix) using standard methods [26]. Single nucleotide polymorphisms (SNPs) were selected based on known functional effects on protein function or gene expression, published associations with human disease and haplotype data (Table 1). The SNPs were genotyped with the Sequenom mass spectrometry genotyping platform as described earlier [27]. Five per cent of the samples was duplicated within and across plates to perform quality control.

Statistical analyses

Statistical analyses were performed using GraphPad Prism v5.03. Differences in response to stimuli between two patient or control groups were analysed with the Mann–Whitney *U* test. Differences between patient or control groups were analysed with the Wilcoxon signed rank test and differences within patient or control groups with the Kruskal–Wallis test, denoted as the H statistic (H). Dunn's multiple comparisons test (Dunn's) was used for post-hoc analysis. The presence of Hardy–Weinberg equilibrium was calculated for all SNPs. A gene dosage model was adopted to determine significance between the two genotype frequencies of both cohorts. With IBM SPSS18 software, a univariate logistic regression was performed using dominant and recessive model analysis, reporting ORs and their 95% CIs. The genetic variants were chosen based on candidate genes that were identified using a gene expression analysis. Therefore, correction for multiple testing was not applied.

Table 1
Genotyped single nucleotide polymorphisms in genes encoding for matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs)

Gene	SNP ID	Gene region	Nucleotide change[a]	Amino acid change
MMP1	rs144393	440 bp upstream	T>C	NA
MMP1	rs7125062	Intron 4	T>C	NA
MMP2	rs1053605	Exon 5	C>T	Thr174Thr
MMP2	rs243865	1 kb upstream	C>T	NA
MMP3	rs679620	Exon 9	G>A	Lys45Glu
MMP3	rs522616	600 bp upstream	T>C	NA
MMP7	rs11568818	180 bp upstream	A>G	NA
MMP8	rs3765620	Exon 10	A>G	Thr32ILe
MMP8	rs1940475	Exon 9	T>C	Lys64Glu
MMP9	rs17576	Exon 6	A>G	Gln279Arg
MMP10	rs486055	Exon 9	C>T	Arg53Lys
MMP12	rs12808148	300 bp downstream	T>C	NA
MMP12	rs2276109	30 bp upstream	A>G	NA
MMP13	rs2252070	80 bp upstream	A>G	NA
MMP13	rs671188	3 kb upstream	T>C	NA
TIMP1	rs2070584	330 bp downstream	T>G	NA
TIMP1	rs4898	Exon 5	T>C	Phe124Phe
TIMP2	rs7212662	Intron 2	T>G	NA
TIMP2	rs2277698	Exon 3	G>A	Ser101Ser
TIMP3	rs738992	Intron 1	T>C	NA

Abbreviations: SNP, single nucleotide polymorphism; ID, identification number; NA, not applicable.
[a] The first nucleotide is the most common nucleotide.

Results

Transcriptome analysis shows C. burnetii-induced up-regulation of MMP genes

In a whole transcriptome microarray analysis, transcriptional responses of PBMCs to heat-killed *C. burnetii* and *E. coli* LPS were determined in healthy controls ($n = 4$) and in patients with chronic Q fever ($n = 6$). In PBMCs of healthy volunteers, we identified a cluster of 32 genes (45 probes) that were significantly up-regulated after *C. burnetii* stimulation, but not after *E. coli* LPS stimulation (see Supplementary material, Table S2). In this set of genes there was a significant enrichment of collagen catabolic processes (false discovery rate < 0.01) with four genes involved (*MMP1*, *MMP7*, *MMP10* and *MMP19*). In PBMCs of patients with chronic Q fever, these genes were also significantly up-regulated after stimulation with *C. burnetii*, although *MMP7* and *MMP10* were also significantly up-regulated in response to *E. coli* LPS. Three additional MMPs genes encoding MMP-8, MMP-9 and MMP-14 were also up-regulated at least twofold in stimulated PBMCs from healthy controls and patients with chronic Q fever (both by *C. burnetii* and *E. coli* LPS) (see Supplementary material, Table S3).

Coxiella burnetii stimulation of whole blood and PBMCs results in production of MMP-1 and MMP-9 in healthy controls and patients with chronic Q fever

To determine which MMPs are produced by PBMCs stimulated with *C. burnetii*, we assessed by Luminex assay the release in the supernatant of MMP-1, MMP-2, MMP-7, MMP-9 and MMP-10 using cells from eight healthy controls, eight past Q fever individuals with vascular co-morbidity, and eight patients with chronic Q fever (see Supplementary material, Fig. S1). Only MMP-1 and MMP-9 production was significantly increased. Based on these results, we further focused on the production of MMP-1 and MMP-9 measured by ELISA in both supernatant of PBMCs and whole blood cultures. The response to *C. burnetii* was clearly increased in healthy controls (MMP-1: $H = 19.45$, $p < 0.001$, Dunn's $p < 0.001$, MMP-9: $H = 20.00$, $p < 0.001$, Dunn's $p < 0.001$) and patients with chronic Q fever (MMP-1: $H = 14.07$: $p < 0.001$, Dunn's: $p < 0.001$, MMP-9: $H = 15.69$ $p < 0.001$, Dunn's $p < 0.001$) and more pronounced compared with the LPS response (MMP-1 and MMP-9: healthy controls and patients with chronic Q fever Dunn's: $p > 0.05$) (Fig. 1). The *C. burnetii*-induced MMP-1 and MMP-9 production did not differ between PBMCs from patients with chronic Q fever and healthy controls or past Q fever-infected individuals. (Wilcoxon signed rank test, $p > 0.05$).

Similar to what was found in PBMCs, *C. burnetii*-stimulated whole blood cultures of healthy controls ($n = 12$), patients with acute Q fever ($n = 8$), past Q fever-infected individuals ($n = 8$) and patients with chronic Q fever ($n = 10$) showed an increase in MMP-1 and MMP-9 compared with unstimulated cultures ($H = 79.27$, $p < 0.001$, Dunn's: healthy controls $p > 0.05$, acute Q fever $p < 0.01$, past Q fever $p < 0.01$, chronic Q fever $p < 0.05$ and MMP-9: $H = 43.22$, $p < 0.001$, Dunn's healthy controls $p < 0.01$, acute Q fever patients $p > 0.05$, past Q fever patients $p < 0.01$, chronic Q fever patients $p < 0.01$) (data not shown).

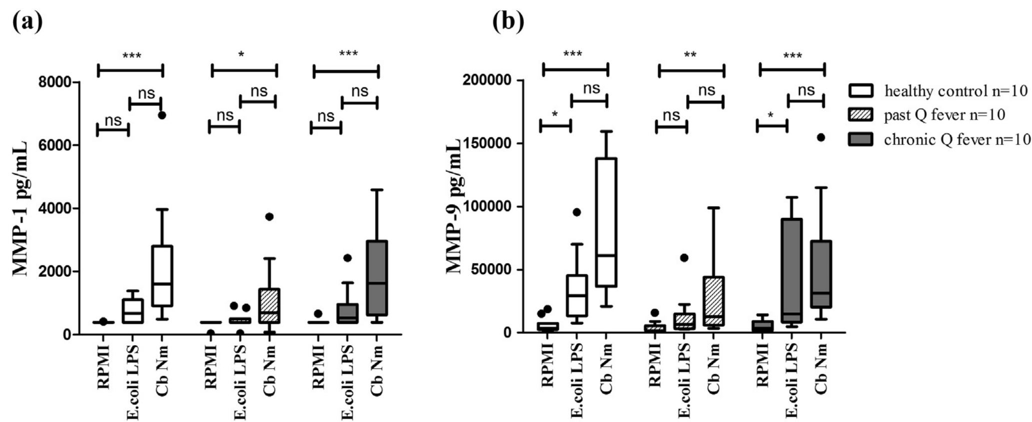

Fig. 1. Matrix metalloproteinase 1 (MMP-1) and MMP-9 production in peripheral blood mononuclear cells (PBMCs). MMP-1 and MMP-9 production after *Escherichia coli* lipopolysaccharide (LPS) and *Coxiella burnetii* stimulation in PBMCs. Healthy controls ($n = 10$), individuals with past Q fever ($n = 10$), patients with chronic Q fever ($n = 10$). (a) MMP-1 concentration in PBMCs incubated with either culture medium, *E. coli* LPS 10 ng/mL or *C. burnetii* Nine Mile 10^7/mL. (b) MMP-9 concentration in PBMCs stimulated with either culture medium, *E. coli* LPS or *C. burnetii*. Cb Nm 1×10^7, C. burnetii Nine Mile concentration 1×10^7/mL. Medians with 1.5 interquartile range are shown. Analysis was performed using the Wilcoxon signed rank test for difference in stimuli between groups, the Kruskal–Wallis test with Dunn's Multiple Comparison test was used to determine differences within groups. *p < 0.05, **p < 0.01, *** p < 0.001, ns, not significant.

SNPs in MMP genes are more common in patients with chronic Q fever

We assessed whether SNPs in MMP genes were more common in patients with chronic Q fever than in controls, indicating a risk for the development of chronic Q fever. For the genetic association analysis, two cohorts were used. The first cohort comprised 139 proven or probable patients with chronic Q fever. They were compared to a cohort of 220 controls with a positive serology for *C. burnetii* without progression to chronic Q fever, but with similar cardiovascular co-morbidity. Polymorphisms were successfully genotyped in genes encoding for the soluble MMPs, MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-12, MMP-13 and TIMP-1, TIMP-2, TIMP-3. For each polymorphism >93% of participants were genotyped. All SNPs were in Hardy–Weinberg equilibrium in the control group, except for *TIMP2* (rs2277698), which was therefore excluded. Genotyping revealed an association between chronic Q fever and *MMP7* (rs11568818, A>G) and *MMP9* (rs17576, A>G). In *MMP7*, the investigated SNP (rs11568818), is located in the promoter region and the G allele was more prevalent in patients with chronic Q fever (p < 0.05, OR 1.63, 95% CI 1.01–2.66). In *MMP9* rs17576 the G allele was more prevalent in patients with chronic Q fever compared with the controls (p < 0.05, OR 1.67, 95% CI 1.08–2.58). The other genotyped *MMP* and *TIMP* polymorphisms were not differentially distributed.

We determined the consequences of the rs17576 SNP with respect to the production of MMP-9 in *C. burnetii*-stimulated PBMCs from patients with chronic Q fever ($n = 11$) and found that patients carrying the risk allele G ($n = 9$) did not differ in MMP-9 production from patients with the wild-type AA ($n = 2$, p > 0.05). *MMP9* rs17576 leads to an amino acid change in the substrate binding region of MMP-9 [28,29], therefore we checked whether the activity of MMP-9 was affected by the SNP and studied the *C. burnetii*-induced MMP-9 activity in ten healthy individuals, with known genotypes for *MMP9* rs17576 (five wild-type individuals, four with the risk genotype AG and one with genotype GG). Following *C. burnetii* stimulation, MMP-9 activity could be detected (compared with unstimulated PBMCs, p < 0.001) but activity did not differ between the wild-type (median 12.5 ng/mL; interquartile range (IQR) 8.7–17.10 ng/mL) and genotypes with the risk allele (median 16.2 ng/mL, IQR 11.1–18.9 ng/mL) (p > 0.05).

MMP plasma and serum concentrations in patients and healthy individuals

We performed pilot experiments to determine which MMPs can be measured in serum and plasma from patients with chronic Q fever, identified during or after the last Dutch outbreak. Serum samples were available in this cohort, but plasma samples had not been collected on a large scale. Only the MMPs that showed no large variation between serum and plasma were eligible for further assessment in the left-over serum samples of patients [30,31]. The latter was the case for MMP-2, MMP-7 and MMP-10 (see Supplementary material, Fig. S2). MMP-1 and MMP-9 showed differences between serum and plasma from different collection tubes and were therefore considered not suitable for comparisons.

Circulating concentrations of MMP-2, MMP-7 and MMP-10 were assessed in serum of controls with vascular diseases without a history of Q fever, controls with vascular disease and a past Q fever infection and patients with chronic Q fever (Fig. 2). MMP-2 concentrations were increased in individuals with past Q fever infection compared with healthy controls (p 0.03) and patients with chronic Q fever (p 0.05). Compared with healthy controls, serum levels of MMP-7 were higher in patients with chronic Q fever (median 20.6 ng/mL, IQR 15.9–33.8 ng/mL; median 33.5 ng/mL, IQR 22.3–45.7 ng/mL, respectively, p 0.03, Mann–Whitney *U*-test). MMP-10 serum concentrations were not different between the groups.

Doxycycline but not other antibiotics, inhibit MMP-1 production

All patients with chronic Q fever enrolled in the study were treated with doxycycline or a combination of doxycycline and hydroxychloroquine during blood sampling. Tetracyclines, including doxycycline, are known to inhibit MMP transcription and activity [32]. To assess the effect of doxycycline on *C. burnetii*-induced MMP production, PBMCs from blood donors were stimulated with *C. burnetii* in the presence of doxycycline. During chronic Q fever treatment, serum concentrations of doxycycline are 5–10 mg/L, therefore these concentrations as well as 25 mg/L were used in the experiments. After 24 h, cells treated with 25 mg/L of doxycycline produced less MMP-1 (Mann–Whitney *U* test, p < 0.05), whereas the MMP-9 concentration was only slightly decreased ($n = 9$; p < 0.05, see Fig. 3). Hydroxychloroquine

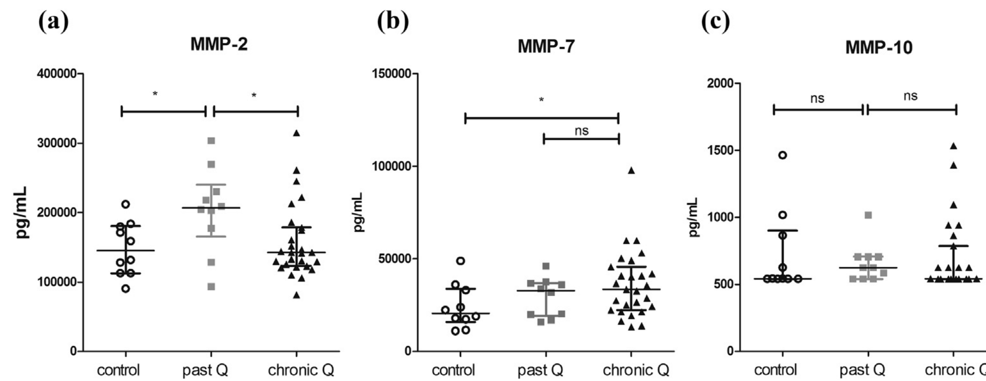

Fig. 2. Serum concentrations of matrix metalloproteinase 2 (MMP-2), MMP-7 and MMP-10 in control individuals with similar vascular disease ($n = 10$), past Q fever infected individuals with vascular disease ($n = 10$) and patients with chronic Q fever ($n = 27$) serum obtained within 1.5 years after diagnosis. Medians with interquartile range are indicated. $^{*}p < 0.05$; ns, $p > 0.05$.

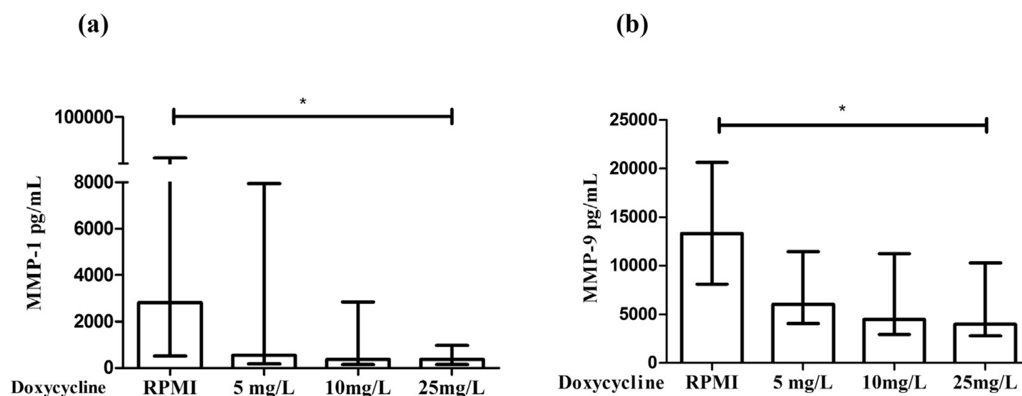

Fig. 3. Matrix metalloproteinase 1 (MMP-1 and MMP-9) production in peripheral blood mononuclear cells (PBMCs) pre-incubated with doxycycline. (a) MMP-1 and (b) MMP-9 concentrations in PBMCs ($n = 9$) of healthy blood donors, pre-incubated with doxycycline and stimulated with *Coxiella burnetii* NM in 10^7/mL. Medians with interquartile range are provided. Asterisks indicate significant p-values ($p < 0.05$) compared with culture medium (RPMI). $^{*}p < 0.05$.

treatment of PBMCs did not result in a reduction of MMP ($p > 0.05$), neither did it have an additive effect on MMP production under doxycycline treatment ($p > 0.05$). Due to side effects or low response to doxycycline, some patients switched to other drug regimens such as moxifloxacin, a fluoroquinolone. Moxifloxacin did not change the *C. burnetii*-stimulated MMP-1 or MMP-9 production (data not shown).

Discussion

In the current study, we investigated through several immunological and genetic approaches the role of MMPs in the pathophysiology of chronic Q fever. We show that the expression of certain MMP genes (*MMP1*, *MMP7*, *MMP8*, *MMP9*, *MMP10*, *MMP14* and *MMP19*) is up-regulated upon stimulation with heat-killed *C. burnetii*. This leads in turn to the release of at least MMP-1 and MMP-9. For gene expression as well as protein production, the *C. burnetii*-induced MMP response is more pronounced than the LPS response. MMP transcripts are up-regulated in both healthy controls and patients with chronic Q fever and do not differ between them. In accordance with these results we did not detect a difference between patients and controls in MMP protein production. In addition, we demonstrate that MMP-7 serum concentrations are higher in patients with chronic Q fever than controls with similar cardiovascular co-morbidity. Although MMPs have been studied in other infectious diseases, only one paper addressed the involvement of MMPs in Q fever and reported elevated MMP-2 and MMP-9 in sera of patients with acute Q fever [10].

We show that subtle variations in *MMP7* and *MMP9* are more common in patients with chronic Q fever than in the control group with past Q fever and similar cardiovascular co-morbidity. *MMP7* and *MMP9* polymorphisms have been associated with susceptibility to various malignancies [33–35] and cardiovascular diseases [36]. In infectious diseases, these associations have been less extensively investigated and the genotyped SNPs were not yet found to be associated with susceptibility to infection. In *Helicobacter pylori* infection, an SNP in the promoter region of *MMP7* (rs11568818) leads to increased risk of precancerous lesions [37]. Genetic variations in *MMP9* have a protective role in malarial disease [38] and carrying SNP rs17576 reduces the risk of disease-specific sequelae after ocular *Chlamydia trachomatis* infection [39]. Polymorphisms in these MMP genes are associated with an increased risk of development of chronic Q fever. The genetic variant in *MMP9* (rs17576) is noteworthy, because it results in a change from a positively charged amino acid to an uncharged amino acid, located in the domain required for substrate binding [28,29]. In a small group of ten healthy volunteers with known genotypes for this MMP-9 SNP, however, we did not find a significant difference in activity.

The involvement of MMPs in chronic Q fever is not surprising as both are associated with aneurysms and infective endocarditis. Chronic Q fever develops in patients with cardiovascular risk factors, which include aneurysms and valvulopathy [1,2]. *MMP9*

expression is up-regulated in the tissue of abdominal aortic aneurysms [40,41] and is associated with the initiation and expansion of aortic aneurysms [41,42]. Circulating MMP-9 concentrations have been found to be higher in individuals with abdominal aortic aneurysms [14]. The pathology of infective endocarditis is also thought to involve MMPs [43] and circulating MMP-9 concentrations in infective endocarditis are associated with the risk of embolic events [44]. Additionally, doxycycline has been suggested to lower MMP-9 gene expression and protein production in aortic aneurysm tissue in humans [45].

We demonstrate that doxycycline, belonging to the tetracyclines, can inhibit *C. burnetii*-induced MMP-1 and MMP-9 production in PBMCs. Maitra *et al.* showed that chemically modified tetracyclines are able to inhibit MMP production independently from their antimicrobial effect [46]. Doxycycline acts by down-regulating gene transcription and its effects on activity are due to the chelation of zinc and calcium ions that are necessary for enzymatic activity [32]. We showed only a partial inhibition of MMP-9 production by doxycycline. The inhibitory effect on MMP activity may, however, have an additive effect to the decreased production. The inhibitory effect on MMP production and activity may therefore prevent or halt tissue destruction of the vascular wall in patients with chronic Q fever.

The strength of the current study is that for the genetic risk evaluation we had access to the largest cohort ever of patients with chronic Q fever and a control group of individuals with past Q fever infection and cardiovascular co-morbidity. Drawbacks of the study are that we did not have insight into the serological Q fever status of all healthy controls and we had only access to sera and not to appropriately anti-coagulated plasma for the measurement of circulating MMP concentrations. Preanalytical conditions such as blood specimen collection are critical for reliable determination of MMPs. Dependent on the type, anticoagulants may elicit further release of certain MMPs by blood cells [30,47,48]. Therefore, only MMP-2, MMP-7 and MMP-10 could be measured and the protein concentrations of MMP-1 and MMP-9, possibly more relevant for the disease, could not be determined. The other MMPs that were identified in the gene expression analysis (*MMP8*, *MMP14*, *MMP19)* were not analysed due to the absence of commercially available assays. Lastly, the use of doxycycline by all patients might also have reduced the production and serum concentration of MMPs.

In conclusion, *C. burnetii* is able to induce MMP gene expression in and protein production by peripheral blood immune cells. Sera of patients with chronic Q fever contain higher concentrations of MMP-7 compared with healthy controls and genetic variants in MMPs (*MMP7* and *MMP9*) are associated with chronic Q fever. Taken together, our findings suggest that *C. burnetii*-induced production of MMPs may play a role in chronic Q fever.

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.2017.01.022.

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