Coxiella burnetii stimulates production of RANTES and MCP-1 by mononuclear cells: modulation by adhesion to endothelial cells and its implication in Q fever

Soraya Meghari[1], Benoît Desnues[1], Christian Capo[1], Georges E. Grau[1,2], Didier Raoult[1], Jean-Louis Mege[1]

[1] Unité des Rickettsies, CNRS UMR 6020, Université de la Méditerranée, Institut Fédératif de Recherche 48, Faculté de Médecine, 27 bd Jean Moulin, 13385 Marseille Cedex 5, France
[2] *Present address:* Department of Pathology, University of Sydney, Sydney NSW 2006, Australia

Correspondence : Pr J.-L. Mege Jean-Louis
<Mege@medecine.univ-mrs.fr>

ABSTRACT. Q fever is an infectious disease caused by *Coxiella burnetii*, which may become chronic when cytokine network and cell-mediated immune responses are altered. Chemokines, such as Regulated upon Activation, Normal T cell Expressed and Secreted (RANTES, CCL5) and Monocyte Chemoattractant Protein-1 (MCP-1, CCL2), are specialized in the trafficing of peripheral blood mononuclear cells (PBMC), and are associated with T cell polarization that is essential for intracellular survival of *C. burnetii*. The present study investigated whether or not the infection status (no infection and acute or chronic infection with *C. burnetii*) of donors, affected the production of the two chemokines by PBMC with or without stimulation with virulent and avirulent *C. burnetii*. Our findings indicate that *in vitro* exposure to virulent or avirulent *C. burnetii* stimulated the production of RANTES and MCP-1 in PBMC obtained from healthy adults. The co-cultivation of endothelial cells and human PBMC resulted in an increased production of MCP-1 and the up-regulation of RANTES, which were contact-dependent. Unstimulated PBMC from patients with acute or chronic Q fever overproduced MCP-1. Interestingly, the addition of *C. burnetii* resulted in an increased production of RANTES and MCP-1 by PBMC obtained from patients with chronic Q fever, and the co-cultivation of PBMC with endothelial cells amplified increased production of chemokines. Circulating levels of RANTES and MCP-1 were also increased in chronic Q fever. We suggest that the overproduction of RANTES and MCP-1 secondary to the contact of PBMC with endothelium may perpetuate exaggerated inflammatory responses leading to inappropriate PBMC trafficking and to the pathogenesis of Q fever.

Keywords: RANTES, MCP-1, *Coxiella burnetii*, Q fever, leucocytes, endothelium

Q fever is a zoonosis caused by *Coxiella burnetii,* an obligate intracellular bacterium that inhabits monocytes and macrophages [1]. The microorganism is considered as a potential category B biological weapon [2]. The primary infection is symptomatic in a minority of exposed individuals and consists of isolated fever, hepatitis, or pneumonia. This acute form of Q fever is associated with protective immune responses, indicated by the presence of granulomas, and it usually leads to cure [3]. Q fever may become chronic in patients with valvular damage or in immunocompromised patients with lymphomas and in pregnant women [4]. The major clinical form of chronic Q fever is endocarditis. In contrast to the acute Q fever, chronic Q fever is characterized by defective cell-mediated immunity and the lack of granulomas, which are replaced by lymphocyte infiltration and foci of necrosis [5].

Granuloma formation in Q fever is associated with protection as described in other infectious diseases [6]. It is a complex process that requires the activation and recruitment of lymphocytes and monocytes to the site of infection, leading to leucocyte juxtaposition around *C. burnetii*-infected macrophages. Such recruitment needs soluble mediators including chemokines produced by infected cells and/or injured tissues. Chemokines are classified into four subfamilies, CXC, CC, C, and CX_3C, based on the arrangement of positionally conserved cysteine motifs within their structures. Chemokines are involved in constitutive and inducible migration of leucocytes. Each chemokine targets circulating leucocytes: CC chemokines exert their action on multiple leucocyte subtypes, including monocytes, T lymphocytes, dendritic cells, and NK cells, but they are generally inactive on polymorphonuclear cells; C chemokines primarily recruit lymphocytes, and CXC chemokines act on polymorphonuclear cells [7]. Monocyte Chemoattractant Protein-1 (MCP-1, CCL2) and Regulated upon Activation, Normal T cell Expressed and Secreted (RANTES, CCL5) are prototypes of the CC chemokine family. MCP-1 is produced by immune cells, including macrophages, dendritic cells, and non-immune cells such as endothelial cells (EC) and fibro-

doi: 10.1684/ecn.2006.0041

blasts, and its expression is induced by cytokines such as interleukin (IL)-1, Tumor Necrosis Factor (TNF), IL4, and microbial products [8]. MCP-1 interacts with CCR2 that is widely expressed. On the other hand, RANTES is produced by immune cells, including macrophages and T lymphocytes, and non-immune cells such as epithelial cells [9]. RANTES expression is induced by cytokines such as IL1β and TNF. In contrast to MCP-1, RANTES binds different receptors such as CCR1, CCR3, CCR4, and CCR5 [10].

RANTES is associated with Th1 responses as suggested by the reduction of type 1 granuloma formation after its neutralization [11]. MCP-1 is preferentially associated with Th2 responses by co-stimulating IL4 production and interfering with IL-12 production and Th1 development [12]. Indeed, MCP-1-deficient mice have diminished Th2 responses [13]. In addition, IFN (interferon)-γ production by T cells in draining lymph nodes, type 1 granuloma formation, and clearance of intracellular pathogens are defective in CCR2-deficient mice [14]. RANTES and MCP-1 are associated with inflammatory disorders and their role is likely in infectious diseases [15]. As both cytokines are essential for the shaping of immune responses, we investigated the production of RANTES and MCP-1 by peripheral blood mononuclear cells (PBMC) in healthy individuals and patients with Q fever. In addition, as mononuclear cells interact with EC during their extravasation from the vascular lumen to peripheral tissues [16], we wondered if the interaction of PBMC with EC affected their ability to produce RANTES and MCP-1. We provide evidence that PBMC-EC contact potentiated chemokine production in controls and increased dramatically this production in patients with chronic Q fever. The overproduction of these chemokines may perpetuate the exaggerated inflammatory response leading to inappropriate PBMC trafficking and, consequently, to the pathogenesis of Q fever.

METHODS

Blood samples

For studying chemokine production by PBMC, blood samples were collected from 10 patients (7 men and 3 women; mean age 60 years; range 37-83 years) with acute Q fever, 8 patients (4 men and 4 women; mean age 50 years; range 25-75 years) with chronic Q fever, and 10 healthy subjects (5 men and 5 women; mean age 42 years; range 23-58 years), after informed consent. To measure circulating chemokines, we also collected blood from 17 patients with acute Q fever (10 men and 7 women; mean age 54 years; range 43-76 years), 33 patients with chronic Q fever (19 men and 14 women; mean age 52 years; range 32-78 years) and 20 healthy controls (10 men and 10 women; 44 years; range 26-59 years). The diagnosis of acute Q fever was based on the detection of phase II *C. burnetii*-specific IgM (mean titer 100; range 50-800) and IgG (mean titer 1200; range 100-3200). The diagnosis of chronic Q fever (*C. burnetii* endocarditis) was based on the modified Duke's University criteria including the presence of phase I *C. burnetii*-specific IgG (mean titer 4000, range 800-12,000) [17]. No co-infection was reported in patients with acute or chronic Q fever.

Preparation of PBMC and other cells

EDTA-anticoagulated blood obtained from the three groups of donors was used and PBMC were separated by Ficoll gradient centrifugation as described previously [18]. Platelets were removed by washing PBMC twice at 260 x *g* for 10 min. The human microvascular EC line (HMEC-1) was kindly provided by Dr. Ades (CDC, Atlanta, GA, USA) and used with his permission. It was cultured as described [19]. EC were seeded on tissue culture flasks coated with 0.2% gelatine in EC growth medium (Cambrex BioSciences, Emerainville, France) supplemented with 10% fetal calf serum (FCS), 1 mM L-glutamine, 1% hydrocortisone, and 1% EC growth factor. After trypsinization, cells were collected and grown to confluence at 37°C in 5% CO_2, and the medium was replaced every other day until the formation of monolayers. L929 cells were subcultured in Eagle MEM containing 25 mM HEPES, 10% FCS, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin (Invitrogen, Eragny, France), after trypsinization once per week.

Preparation of C. burnetii

Virulent *C. burnetii* (phase I organisms) was obtained as previously described [20]. BALB/c mice were injected intraperitoneally with 10^8 *C. burnetii* organisms (Nine Mile strain) contained in 0.5 mL of prewarmed phosphate buffered saline. After 10 days, the mice were killed by cervical dislocation, and their spleens were dilacerated using sterile 70-µm cell strainers in 5 mL of antibiotic-free Eagle MEM supplemented with 4% FCS and 2 mM L-glutamine. Spleen homogenates (1 mL per flask) were added to L929 cell monolayers in antibiotic-free Eagle MEM supplemented with 4% FCS and 2 mM L-glutamine. After 1 week of infection, L929 cells were sonicated and the homogenates were spun down at 300 x *g* for 10 min to discard cell debris, and unpurified phase I bacteria were used for another passage on L929 cells. Avirulent variants of *C. burnetii* (phase II organisms) were cultured in L929 cells by repeated passages [20]. Sonicates of infected cells were spun down at 300 x *g* for 10 min and supernatants containing virulent or avirulent bacteria were centrifuged at 8000 x *g* for 10 min. The bacterial pellet was layered on a 25 to 45% linear Renografin gradient. Next, the gradients were centrifuged and the bacteria were collected, washed, and suspended in HBSS (Invitrogen) before being stored at -80°C. The number of *C. burnetii* organisms in each preparation was determined by Gimenez staining as follows: a predetermined volume of bacterial suspension (5 µL of the serial dilutions) was deposited on diagnostic microscope 6 mm slides (Brand Cel-Line, Menzel, Braunschweig, Germany), dried to perform staining, and the mean number of bacteria was quantified by optical examination of 5 different fields using an eyepiece with a calibrated grid. The bacterial viability was assessed using the LIVE/DEAD *Bac*Light bacterial viability kit (Molecular Probes, Eugene, OR, USA), as previously described [21]. Only preparations containing more than 90% of viable organisms were used.

Co-cultivation of PBMC and EC

EC were seeded in gelatin-coated 96-well culture plates (3 x 10^4 EC/well) and cultured to confluence. To obtain inflammatory EC, they were incubated with 20 ng/mL of human recombinant TNF (R&D Systems, Abingdon, United Kingdom) for 20 h. EC were then co-cultured with PBMC (10^5 PBMC/well) in the presence or absence of virulent or avirulent *C. burnetii* (6 x 10^5 bacteria/well) for different periods. Latex beads (Sigma-Aldrich, Saint Quentin Fallavier, France; 10:1 bead-to-cell ratio) were used as controls.

Assay of chemokines

PBMC supernatants were collected and stored at -80°C before chemokine determination by sandwich enzyme immunoassays. RANTES (detection limit, 5 pg/mL) and MCP-1 (detection limit, 10 pg/mL) assays were provided by Beckman Coulter (Villepinte, France) and BioSource International (Nivelles, Belgium), respectively. The intra- and interspecific coefficients of variation ranged from 5% to 10%.

Quantitative real-time RT-PCR (qRT-PCR) was performed as follows [22]; total cellular RNA was extracted using the Trizol method (Invitrogen), and its integrity and quantity were assessed with the 2100 Bioanalyzer and the RNA 6000 Nano LabChip kit (Agilent Technologies, Palo Alto, CA, USA) after a DNase digestion step. Ten ng of RNA were incubated with reverse transcriptase (Superscript II, Invitrogen) and oligo$(dT)_{12\text{-}18}$ primer. The following nucleotide sequences of primers were used: for RANTES, F5'CTGCTGCTTTGCCTACATTGC3' and R5'GTT CAGGTTCAAGGACTCTCCA-TC3'; for MCP-1, F5'CTTCTGTGCCTGC-TGCTCATAG3' and R5'GAA TCCTGA-ACCCACTTCTGCT3'; for β-actin used as endogenous control, F5'-GTGGGGCGCC-CCAGGCA CCA3' and R5'CTCCTTAATGTCACGCACGATTC3'. Reverse transcriptase was omitted in negative control. qRT-PCR was performed using the LightCycler-FastStart DNA Master SYBR Green System (Roche, Mannheim, Germany). The fold change in the target gene (RANTES or MCP-1) cDNA relative to the β-actin endogenous control was determined as follows: fold change = $2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct$ = (CtTarget - CtActin) of stimulated PBMC - (Ct-Target - CtActin) of unstimulated PBMC. Ct values are defined as the cycle numbers at which the fluorescence signals were detected.

Statistical analysis

Results were expressed as mean ± SD. Results obtained with patients were expressed as median with 25 and 75 percentile distribution, and minimum and maximum values. Quantitative data were compared with the Mann-Whitney *U* test. Differences are considered significant when $p < 0.05$.

RESULTS

C. burnetii stimulated production of RANTES and MCP-1

Unstimulated PBMC from controls transiently released RANTES at 12 h, and this release significantly ($p < 0.05$) increased in response to *C. burnetii (figure 1A)*. MCP-1 was not released by unstimulated PBMC during 48 h of culture but, in the presence of virulent *C. burnetii*, MCP-1 release was demonstrated at 12 h, reached maximum value after 24 h and plateaued thereafter *(figure 1B)*. Avirulent *C. burnetii* also stimulated the release of RANTES (3.6 ± 0.5 ng/mL at 12 h) and MCP-1 (12.2 ± 1.8 ng/mL at 24 h), which was similar to that induced by virulent organisms. In contrast, latex beads had no effect on the release of RANTES (1.6 ± 0.3 ng/mL) or MCP-1 (0.1 ± 0.1 ng/mL). The release of RANTES and MCP-1 by PBMC did not result from exocytosis of preformed chemokines since it was completely inhibited by cycloheximide treatment (data not shown). While *C. burnetii* slightly affected RANTES transcription, as determined by qRT-PCR *(figure 1C)*, it transiently increased the expression of MCP-1 transcripts *(figure 1D)*. Hence, *C. burnetii* stimulated the release of RANTES and MCP-1 independently of bacterial virulence, and markedly affected MCP-1 transcription.

C. burnetii stimulated chemokine production in PBMC interacting with EC

As the interaction of PBMC with EC results in their activation [23], we wondered if such interaction modulated the ability of PBMC from controls to produce RANTES and MCP-1 in response to *C. burnetii*. RANTES and MCP-1 were undetectable in supernatants of human microvascular EC in the presence and absence of *C. burnetii*. In the absence of *C. burnetii*, the co-culture of PBMC with EC increased the release of RANTES: it was detected at 6 h, became maximal at 12 h, decreasing thereafter *(figure 2A)*.

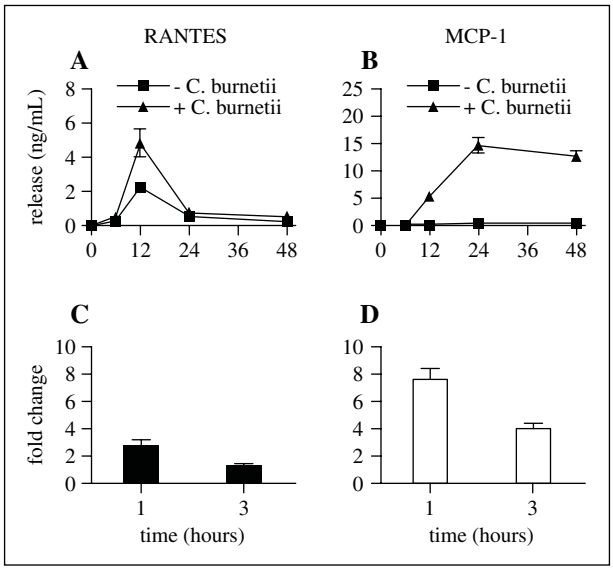

Figure 1

C. burnetii stimulated the production of RANTES and MCP-1.

A, **B**) PBMC from controls were incubated in the presence or absence of *C. burnetii* (bacterium-to-cell ratio of 6:1) for 48 h. Supernatants were collected and assayed for RANTES (**A**) and MCP-1 (**B**) by immunoassays. Results expressed in ng/mL are the means ± SD of 5 experiments.

C, **D**) Transcripts encoding RANTES (**C**) and MCP-1 (**D**) were quantified by qRT-PCR. Results are expressed as the ratio of expression levels in *C. burnetii*-stimulated PBMC *versus* unstimulated PBMC. They are the mean ± SD of 3 experiments.

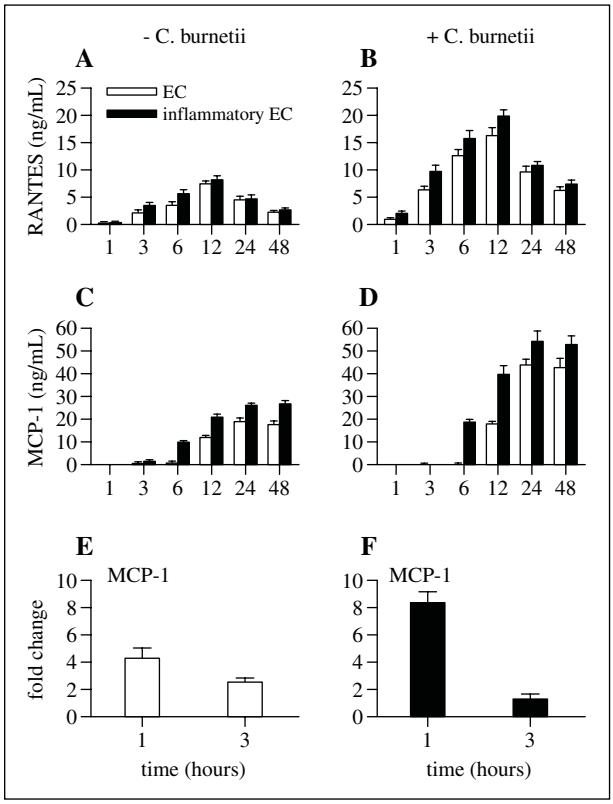

Figure 2
Chemokine production and PBMC-EC interaction.
A-**D**) PBMC from controls were incubated with EC or inflammatory EC in the absence (**A**, **C**) or the presence (**B**, **D**) of *C. burnetii* (bacterium-to-cell ratio of 6:1) for 48 h. Supernatants were collected and assayed for RANTES (**A**, **B**) and MCP-1 (**C**, **D**) by immunoassays. Results expressed in ng/mL are the means ± SD of 5 experiments.
E, **F**) PBMC were unstimulated (**E**) or stimulated with *C. burnetii* (**F**), and transcripts encoding MCP-1 were quantified by qRT-PCR. Results are expressed as the ratio of expression levels in PBMC cultured with EC *versus* PBMC alone. They are the mean ± SD of 3 experiments.

Adding *C. burnetii* to PBMC-EC co-cultures markedly up-regulated the release of RANTES, which was detected 1 h after bacterial stimulation and steadily increased to reach a peak at 12 h *(figure 2B)*. The co-culture of PBMC with EC also resulted in MCP-1 release, even in the absence of *C. burnetii*: the release was detected after 12 h, and remained as a plateau until 48 h *(figure 2*C*)*. *C. burnetii* organisms significantly ($p < 0.05$ at 24 and 48 h) increased the MCP-1 release induced by the interaction of PBMC with EC *(figure 2D)*.

We also wondered if the release of RANTES and MCP-1 by PBMC co-cultured with EC was due to increased gene transcription. The co-culture of PBMC with EC in the presence or absence of *C. burnetii* did not affect the expression of RANTES mRNA when compared to PBMC cultivated alone (data not shown). In the absence of *C. burnetii* stimulation, the expression of MCP-1 mRNA was transiently upregulated in PBMC that interacted with EC, as compared with PBMC alone *(figure 2E)*. In *C. burnetii*-stimulated PBMC that interacted with EC, MCP-1 mRNAs were transiently up-regulated as compared with *C. burnetii*-stimulated PBMC *(figure 2F)*.

As with virulent *C. burnetii*, avirulent *C. burnetii* also increased the release of RANTES (17.3 ± 2.1 ng/mL at 12 h) and MCP-1 (39.6 ± 4.1 ng/mL at 24 h) induced by PBMC-EC interaction. Again, latex beads were unable to up-regulate chemokine release when PBMC were co-cultured with EC (for RANTES, 6.7 ± 0.8 ng/mL at 12 h; for MCP-1, 20.5 ± 3.3 ng/mL at 24 h). Using inflammatory EC did not modify RANTES release induced by PBMC stimulated or not by *C. burnetii* (*figure 2A* and *2B*, respectively), but it moderately increased unstimulated and *C. burnetii*-stimulated release of MCP-1 (*figure 2C* and *2D*, respectively). Note that the effect of inflammatory EC was essentially observed during the initial periods of interaction, when the production of chemokines was still low. On the other hand, the physical contact of PBMC with EC is critical for up-regulated release of chemokines, since using porous membranes to separate PBMC from EC led to a chemokine release similar to that observed in the absence of EC *(table 1)*. Next, we wondered whether the up-regulation of RANTES and MCP-1 release was due to PBMC, EC or the PBMC-EC combination. For that purpose, unstimulated or *C. burnetii*-stimulated PBMC and EC were alternatively fixed with 1% formaldehyde, and chemokine production was assessed. The co-incubation of fixed EC with PBMC led to chemokine release; levels of RANTES and MCP-1 were similar to those obtained by PBMC-EC co-culture. Virulent *(table 1)* and avirulent (data not shown) *C. burnetii* increased the release of RANTES and MCP-1 by PBMC incubated with EC or fixed EC in a similar way. In contrast, the co-culture of fixed PBMC with EC completely prevented chemokine release. Hence, chemokine release was due to PBMC, and EC provided a signal amplifying the PBMC response.

Table 1
Chemokine release by PBMC interacting with EC

	- C. burnetii		***+ C. burnetii***	
	RANTES	**MCP-1**	**RANTES**	**MCP-1**
PBMC	2.8 ± 0.7	< 0.005	5.5 ± 0.7	13.7 ± 1.9
PBMC + EC	8.6 ± 0.9	20.2 ± 1.1	19.4 ± 2.5	40.4 ± 3.7
EC-separated PBMC	3.1 ± 0.9	0.2 ± 0.1	5.9 ± 1.0	15.6 ± 2.8
Fixed PBMC	< 0.005	< 0.005	< 0.005	< 0.005
Fixed PBMC + EC	< 0.005	< 0.005	< 0.005	< 0.005
PBMC + fixed EC	7.9 ± 0.8	18.9 ± 0.8	17.8 ± 1.8	37.6 ± 4.2

PBMC from controls were incubated with EC or separated from EC through porous membranes. PBMC and EC were alternatively fixed with 1% formaldehyde, washed and then incubated with EC and PBMC, respectively, in the presence or the absence of *C. burnetii* (bacterium-to-cell ratio of 6:1). Supernatants were collected after 12 and 24 h for RANTES and MCP-1 determination, respectively. Both chemokines were quantified by immunoassays. Results expressed in ng/mL are the mean ± SD of 3 experiments.

Chemokine release in patients with Q fever

We wondered if the release of RANTES and MCP-1 and their modulation by adhesion of PBMC to EC was affected in Q fever. RANTES release by unstimulated PBMC was similar in patients and controls *(figure 3A)* whereas *C. burnetii*-stimulated release was significantly ($p < 0.05$) higher in patients with chronic Q fever than in patients with acute Q fever or controls *(figure 3B)*. In PBMC-EC co-culture experiments, unstimulated *(figure 3C)* and *C. burnetii*-stimulated *(figure 3D)* release of RANTES was significantly higher in PBMC from patients with chronic Q fever than in controls and patients with acute Q fever ($p < 0.05$). The quantities of MCP-1 released by unstimulated PBMC were significantly higher in acute and chronic Q fever ($p < 0.05$) than in controls *(figure 4A)*. In response to *C. burnetii*, they were higher in chronic Q fever than in controls and acute Q fever ($p < 0.05$, *figure 4B*). In PBMC-EC co-culture experiments, MCP-1 release was significantly ($p < 0.05$) increased in patients with chronic Q fever as compared with the two other groups (for unstimulated PBMC, *figure 4C*; for *C. burnetii*-stimulated PBMC, *figure 4D*). These results showed that the *C. burnetii* stimulation of PBMC that had interacted with EC leads to an exaggerated chemokine release in patients with chronic Q fever.

Finally, we determined the circulating levels of RANTES *(figure 5A)* and MCP-1 *(figure 5B)* in Q fever. The circulating levels of RANTES and MCP-1 were similar in healthy controls and patients with acute Q fever. They were significantly ($p < 0.05$) increased in patients with chronic Q fever, suggesting that chemokine production by PBMC and circulating levels of chemokines were related.

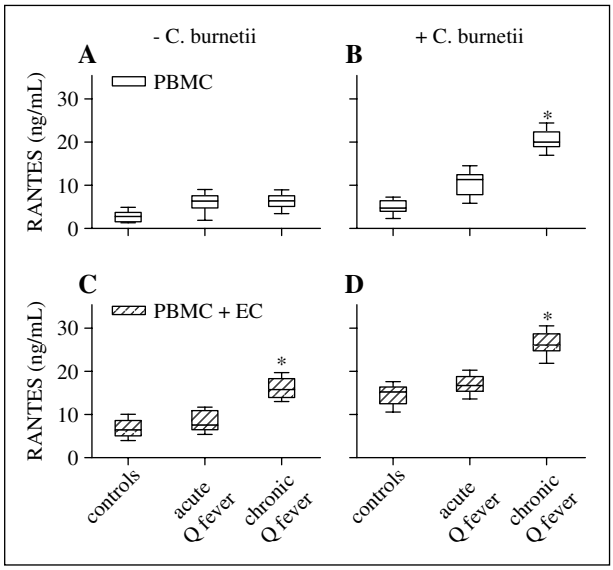

Figure 3
Exaggerated release of RANTES in patients with chronic Q fever. PBMC from controls (n = 10), patients with acute Q fever (n = 10) or with chronic Q fever (n = 8) were cultured without (**A**, **B**) or with (**C**, **D**) EC in the absence (**A**, **C**) or the presence of *C. burnetii* (**B**, **D**). Supernatants were collected after 12 h, and RANTES was quantified by immunoassay. Results in ng/mL are expressed as the median, with 25 and 75 percentile distribution, and minimum and maximum. *$p < 0.05$ represents the comparison between patient groups and the control group.

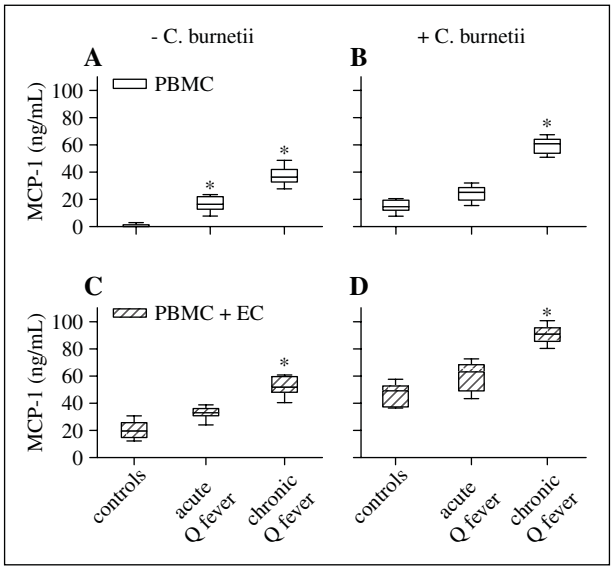

Figure 4
Exaggerated release of MCP-1 in patients with chronic Q fever. PBMC from controls (n = 10), patients with acute Q fever (n = 10) or with chronic Q fever (n = 8) were cultured without (**A**, **B**) or with (**C**, **D**) EC in the absence (**A**, **C**) or the presence of *C. burnetii* (**B**, **D**). Supernatants were collected after 24 h, and MCP-1 was quantified by immunoassay. Results in ng/mL are expressed as the median, with 25 and 75 percentile distribution, and minimum and maximum. * $p < 0.05$ represents the comparison between patient groups and the control group.

DISCUSSION

In this report, we have shown that *C. burnetii* induced sustained MCP-1 release and transiently up-regulated RANTES release by PBMC. Previous data showed that *C. burnetii* elicits the production of inflammatory and immunoregulatory cytokines [24-26]. RANTES was not released from exocytosis granules, in which it has been found in several cell types [27]. RANTES release occurred without changes in transcript levels. This is consistent with the regulatory role of IL-10 that affects RANTES production at the post-transcriptional level [28]. In contrast, MCP-1 release occurred with marked changes in transcript levels, which may be consistent with sustained release of MCP-1. RANTES and MCP-1 release was not related to *C. burnetii* virulence. This suggests that the pathogen-associated molecular patterns shared by virulent and avirulent *C. burnetii* are involved in chemokine release. It is likely that the interaction of *C. burnetii* with PBMC affects the production of RANTES and MCP-1 by a direct effect mediated by pattern recognition receptors such as Toll-like receptor-2 or -4 [29, 30] and/or by an indirect effect mediated by TNF or IL1 [8, 10].

The interaction of PBMC with an EC monolayer modified the unstimulated pattern of RANTES and MCP-1 production, and the ability of PBMC to respond to *C. burnetii*. The adhesion of monocytes to IFN-γ-primed human umbilical vein EC (HUVEC) results in increased release of IL-8 and MCP-1 [23] but, under our conditions, the treatment of HMEC with TNF did not dramatically affect their ability to stimulate chemokine production by PBMC. PBMC-EC contact was sufficient to induce chemokine release, and to prime the response to *C. burnetii*. Cytokine-encoding genes are expressed by monocytes adherent to plastic or tissue matrix [31]. Preliminary results suggested

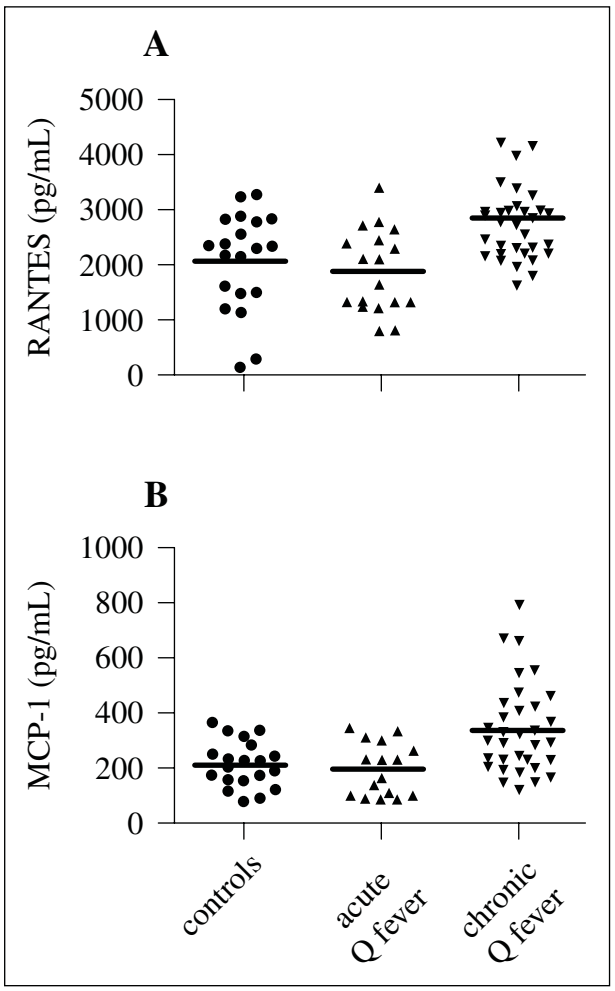

Figure 5
Circulating levels of RANTES and MCP-1 in Q fever.
Plasma from healthy donors, and patients with acute Q fever or chronic Q fever was stored at -80°C before determination of RANTES (**A**) and MCP-1 (**B**) levels by immunoassays. The results are expressed in pg/mL for each individual, and bars represent the median of values.

that the increase in MCP-1 and RANTES release depended on the β2 integrin CD11a/CD18, as shown by experiments with blocking antibodies (data not shown). The production of soluble mediators by EC could not account for the contact-mediated up-regulation of chemokine production. This result disagrees with Lukacs' report in which MCP-1 was released by HUVEC during their interaction with monocytes [23]. Nevertheless, MCP-1- and RANTES-encoding genes were expressed in microvascular EC (data not shown), suggesting that the release process may be dependent on EC type. This provides an additional piece of evidence for physiological differences between macro- and microvascular EC, the latter being more relevant to inflammatory processes [32].

Finally, the production of RANTES and MCP-1 was increased in chronic Q fever and up-regulated after PBMC-EC interaction. It is likely that high amounts of chemokines produced locally interfere with the normal trafficking of leucocytes toward tissue and subsequently with the formation of granulomas. Firstly, MCP-1 is known to be involved in Th2 differentiation [12]. A reorientation of T cell responses toward a Th2 pattern may interfere with protective Th1 immune responses against *C. burnetii*. This is consistent with a recent report describing the role of MCP-1 in susceptibility to tuberculosis [33]. Secondly, it is known that RANTES effects on leucocytes are concentration-dependent. Hence, only high concentrations of RANTES lead to self-aggregation and activate monocytes and T cells [9]. We suggest that high amounts of RANTES and MCP-1 may interfere with directional migration of immune cells and, consequently, with the formation of granulomas. This hypothesis is strengthened by our observation that circulating levels of RANTES and MCP-1 were specifically increased in chronic Q fever.

In this report, we provided evidence that *C. burnetii* stimulates MCP-1 and RANTES release by PBMC, and increases this release when PBMC interact with EC. This mechanism of chemokine release represents an amplification loop that may occur *in vivo*. It is amplified in chronic Q fever in which granuloma formation is defective. We suggest that the overproduction of RANTES and MCP-1 may be deleterious for the adapted traffic of immune cells to the tissues and the formation of granulomas.

Acknowledgements. *This work was supported by a grant from the Programme Hospitalier de Recherche Clinique 2001 «Evaluation des paramètres de microbicidie des macrophages dans la fièvre Q et son suivi». All the authors declare to have no potential conflict of interest.*

REFERENCES

1. Raoult D, Marrie T, Mege JL. Natural history and pathophysiology of Q fever. *Lancet Infect Dis* 2005; 5: 219.
2. Cutler SJ, Paiba GA, Howells J, Morgan KL. Q fever - a forgotten disease? *Lancet Infect Dis* 2002; 2: 717.
3. Maurin M, Raoult D. Q fever. *Clin Microbiol Rev* 1999; 12: 518.
4. Helbig KJ, Heatley SL, Harris RJ, *et al.* Variation in immune response genes and chronic Q fever. Concepts: preliminary test with post-Q fever fatigue. *Genes Immun* 2003; 4: 82.
5. Marrie TJ, Raoult D. Update on Q fever, including Q fever endocarditis. *Curr Clin Top Infect Dis* 2002; 22: 97.
6. Sandor M, Weinstock JV, Wynn TA. Granulomas in schistosome and mycobacterial infections: a model of local immune responses. *Trends Immunol* 2003; 24: 44.
7. Mantovani A. The chemokine system: redundancy for robust outputs. *Immunol Today* 1999; 20: 254.
8. Van Coillie E, Van Damme J, Opdenakker G. The MCP/eotaxin subfamily of CC chemokines. *Cytokine Growth Factor Rev* 1999; 10: 61.
9. Appay V, Rowland-Jones SL. RANTES: a versatile and controversial chemokine. *Trends Immunol* 2001; 22: 83.
10. Ortiz BD, Nelson PJ, Krensky AM. Switching gears during T-cell maturation: RANTES and late transcription. *Immunol Today* 1997; 18: 468.
11. Chensue SW, Warmington KS, Allenspach EJ, Lu B, Gerard C, Kunkel SL, Lukacs NW. Differential expression and cross-regulatory function of RANTES during mycobacterial (type 1) and schistosomal (type 2) antigen-elicited granulomatous inflammation. *J Immunol* 1999; 163: 165.
12. Karpus WJ, Lukacs NW, Kennedy KJ, *et al.* Differential CC chemokine-induced enhancement of T helper cell cytokine production. *J Immunol* 1997; 158: 4129.
13. Gu L, Tseng S, Horner RM, *et al.* Control of TH2 polarization by the chemokine monocyte chemoattractant protein-1. *Nature* 2000; 404: 407.

14. Kurihara T, Warr G, Loy J, *et al.* Defects in macrophage recruitment and host defense in mice lacking the CCR2 chemokine receptor. *J Exp Med* 1997; 186: 1757.

15. Rot A, von Andrian UH. Chemokines in innate and adaptive host defense: basic chemokinese grammar for immune cells. *Annu Rev Immunol* 2004; 22: 891.

16. Imhof BA, Aurrand-Lions M. Adhesion mechanisms regulating the migration of monocytes. *Nat Rev Immunol* 2004; 4: 432.

17. Fournier PE, Casalta JP, Habib G, *et al.* Modification of the diagnostic criteria proposed by the Duke Endocarditis Service to permit improved diagnosis of Q fever endocarditis. *Am J Med* 1996; 100: 629.

18. Capo C, Zugun F, Stein A, *et al.* Upregulation of tumor necrosis factor α and interleukin-1β in Q fever endocarditis. *Infect Immun* 1996; 64: 1638.

19. Ades EW, Candal FJ, Swerlick RA, *et al.* HMEC-1: establishment of an immortalized human microvascular endothelial cell line. *J Invest Dermatol* 1992; 99: 683.

20. Capo C, Moynault A, Collette Y, *et al. Coxiella burnetii* avoids macrophage phagocytosis by interfering with spatial distribution of complement receptor 3. *J Immunol* 2003; 170: 4217.

21. Ghigo E, Capo C, Tung CH, Raoult D, *et al. Coxiella burnetii* survival in THP-1 monocytes involves the impairment of phagosome maturation: IFN-γ mediates its restoration and bacterial killing. *J Immunol* 2002; 169: 4488.

22. Desnues B, Raoult D, Mege JL. IL-16 is critical for *Tropheryma whipplei* replication in Whipple disease. *J Immunol* 2005; 175: 4575.

23. Lukacs NW, Strieter RM, Elner V, *et al.* Production of chemokines, interleukin-8 and monocyte chemoattractant protein-1, during monocyte:endothelial cell interactions. *Blood* 1995; 86: 2767.

24. Dellacasagrande J, Ghigo E, Hammami SM, *et al.* αvβ3 integrin and bacterial lipopolysaccharide are involved in *Coxiella burnetii*-stimulated production of tumor necrosis factor by human monocytes. *Infect Immun* 2000; 68: 5673.

25. Ghigo E, Capo C, Raoult D, *et al.* Interleukin-10 stimulates *Coxiella burnetii* replication in human monocytes through tumor necrosis factor down-modulation: role in microbicidal defect of Q fever. *Infect Immun* 2001; 69: 2345.

26. Shannon JG, Howe D, Heinzen RA. Virulent *Coxiella burnetii* does not activate human dendritic cells: role of lipopolysaccharide as a shielding molecule. *Proc Natl Acad Sci USA* 2005; 102: 8722.

27. Tang YQ, Yeaman MR, Selsted ME. Antimicrobial peptides from human platelets. *Infect Immun* 2002; 70: 6524.

28. Wright KM, Friedland JS. Complex patterns of regulation of chemokine secretion by Th2-cytokines, dexamethasone, and PGE2 in tuberculous osteomyelitis. *J Clin Immunol* 2003; 23: 184.

29. Honstettre A, Ghigo E, Moynault A, *et al.* Lipopolysaccharide from *Coxiella burnetii* is involved in bacterial phagocytosis, filamentous actin reorganization, and inflammatory responses through Toll-like receptor 4. *J Immunol* 2004; 172: 3695.

30. Zamboni DS, Campos MA, Torrecilhas AC, *et al.* Stimulation of Toll-like receptor 2 by *Coxiella burnetii* is required for macrophage production of pro-inflammatoty cytokines and resistance to infection. *J Biol Chem* 2004; 279: 54405.

31. Haskill S, Johnson C, Eierman D, *et al.* Adherence induces selective mRNA expression of monocyte mediators and proto-oncogenes. *J Immunol* 1988; 140: 1690.

32. Grau GE, Lou J. TNF in vascular pathology: the importance of platelet-endothelium interactions. *Res Immunol* 1993; 144: 355.

33. Flores-Villanueva PO, Ruiz-Morales JA, Song CH, *et al.* A functional promoter polymorphism in monocyte chemoattractant protein-1 is associated with increased susceptibility to pulmonary tuberculosis. *J Exp Med* 2005; 202: 1649.