Overexpression of the Per2 Gene in Male Patients with Acute Q Fever

Vikram Mehraj,1 Julien Textoris,1,2 Christian Capo,1 Didier Raoult,1 Marc Leone,1,2 and Jean-Louis Mège1

1Unité de Recherche sur les Maladies Infectieuses Tropicales et Emergentes, Aix-Marseille Université, Marseille, France, and 2Service d’Anesthésie et de Réanimation, Hôpital Nord, Assistance Publique–Hôpitaux de Marseille, France

The prevalence of Q fever is higher in men than in women. Because the expression of circadian clock genes differs in male and female mice infected with Coxiella burnetii, we hypothesized that circadian genes are differently modulated in men and women with Q fever. The expression of the Per2 gene was significantly increased in males with acute Q fever compared with healthy volunteers. No significant difference was observed in females. We showed for the first time that gender altered the expression of a circadian gene, Per2, in an infectious disease.

The clinical expression of Q fever, a worldwide zoonosis due to Coxiella burnetii, is affected by various host factors, including gender and age [1]. The male-to-female ratio of patients admitted to the hospital is 2.45 in adults [1]. In parallel, the rate of Q fever-related complications is higher in males than in females [1]. Men represent 75% of the patients diagnosed with C. burnetii endocarditis, the major manifestation of chronic Q fever [1].

In mice, it has been shown that sex hormones play a role in the pathophysiology of C. burnetii infection. The bacterial load is increased in ovariectomized mice, reaching levels found in males, and estradiol administration restores the bacterial load to levels found in intact females [2]. We have also shown that major components of the circadian rhythm pathway are affected by C. burnetii infection in females. The transcriptional expression of Clock and Arntl genes is down-regulated, whereas that of Per2 gene is upregulated in females but not in males [3]. It is likely that the circadian rhythm and the immune response are related [4]. Rodent models show that the circadian oscillation of clock genes affects interferon-γ production through Per2 regulation or the activity of natural killer cells [5, 6].

To our knowledge, little is known regarding the relation between clock gene expression and human infectious diseases. This relation has never been investigated in Q fever patients. Here, we hypothesized that the expression of the Clock, Arntl, and Per2 genes may be affected by C. burnetii infection in patients with acute and chronic Q fever. We showed that the expression of the Per2 gene was specifically increased in men with acute Q fever. This result may indicate for the first time that a gene involved in the circadian clock is differentially modulated in men and women infected with a bacterial pathogen such as C. burnetii.

METHODS

The study was conducted with the approval of the Ethics Committee of the Aix-Marseille University, Marseille, France. Informed written consent was obtained from each participant. Blood samples from patients undergoing infectious disease consultations and healthy volunteers were collected in PAXgene Blood RNA tubes (Qiagen, Courtaboeuf, France). The samples were collected from 9:00 am to 11:00 am. The diagnosis of Q fever was performed as reported previously [7]. Q fever patients were split into “Acute” and “Endocarditis” according to their underlying disease [8]. The gene expression in blood samples was determined with real-time quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) as recently described [9]. In brief, total RNA was extracted after DNase digestion, according to the manufacturer’s recommendations (Invitrogen, Life Technologies, Saint-Aubin, France). Real-time PCR with cDNA templates was performed using Light Cycler-FastStart DNA MasterPLUS SYBR Green I (Roche Diagnostics, Basel, Switzerland). The primers were designed with the free Web software Primer3 (http://frodo.wi.mit.edu/). The primers consisted of the forward sequences 5’-CGGAGTTAGAGATGG-TGGAAGA-3’, 5’-TACTGAGGAAAAGGGAGGAGAGG-3’ and 5’-CCCTCTACCTGCTCAAA-GAAAA-3’ and the reverse sequences 5’-GGGACTGGAAA-ATGCTGAGTT-3’, 5’-AAGGATA-ACAGCAAACGACAGGGG-3’ and 5’-GCCCTCTGCTCT-ACAAAAACAA-3’ for the Per2, Clock, and Arntl genes, respectively. Quantitative RT-PCR experiments were performed using the gene encoding β-actin as the
reference housekeeping gene. For each patient, the cycle threshold (CT) values of the genes of interest were normalized with the patient’s CT values of β-actin to calculate the ΔCT. The results are expressed as the median and were compared using the nonparametric Mann–Whitney U test. A P value less than .05 was considered significant.

RESULTS

Our cohort consisted of 14 men (mean age of 51 ± 4 years), including 9 patients with acute Q fever and 5 with Q fever–related endocarditis; 6 women (mean age of 35 ± 3 years), including 5 patients with acute Q fever and 1 with Q fever–related endocarditis; and 12 healthy volunteers, including 6 men (mean age of 41 ± 4 years) and 6 women (mean age of 39 ± 5 years). With respect to gender, age did not differ significantly between the patients and the healthy volunteers. In contrast, among the patients, the men were older than the women (51 ± 4 vs 35 ± 3 years; P = .02). No difference was noted between men and women in the healthy volunteers.

In men, the expression of the Per2 gene was significantly higher in the patients with acute Q fever than in the healthy volunteers (FC = 3.1; P = .01). No significant difference of expression was observed between Q fever–related endocarditis and healthy controls (P = .18) (Figure 1A). In women, the Per2 expression ratio between acute Q fever and healthy controls did not reach a significant level (FC = 1.8; P = .18) (Figure 1B). In contrast to the expression of the Per2 gene, the expression of the Clock and Arntl genes was not affected in men or women with Q fever compared with healthy volunteers (Figure 1C–F).

DISCUSSION

To our knowledge, we showed significant changes in circadian clock–related gene expression in a human infectious disease for the first time. Indeed, the expression of the Per2 gene was increased in men but not in women with acute Q fever. This result confirmed that C. burnetii infection partly affects the circadian clock, as demonstrated in mice [3]. This effect was independent of the time of blood collection because blood was sampled within a 2-hour period.

The human infection shows striking differences compared with the murine model of C. burnetii infection. First, the expression of the Per2 gene is upregulated in female mice [3], but it is obvious that the circadian clock is inverted in rodents and humans [10]. Second, the expression of the Arntl and Clock genes is modulated in female mice infected with C. burnetii. However, this finding was not confirmed in the present study. These differences may be induced by specificities at the tissue level. Indeed, the expression of the Per2, Arntl, and Clock genes was studied in the liver of mice, whereas blood samples were collected in humans. Note that significant differences have been reported in the expression of the Clock gene between peripheral tissues and blood [11].

In humans, the exposure to C. burnetii is similar for both genders, although the prevalence of Q fever is higher in men than in women [1]. This explains the small number of women included in our study. Murine models have shown that the bacterial load is higher in the spleen of males than in females [2]. In Drosophila, phagocytosis is circadian-regulated by the protein Timeless. This protein appears to modulate an upstream event in phagocytosis [12]. Because the survival of C. burnetii in macrophages is mediated by a subversion of receptor-mediated phagocytosis [13], this result suggests that the impaired bacterial clearance in males may be related to these circadian clock alterations [2, 3]. Future studies are required to test this hypothesis.
Interestingly, the expression of the Per2 gene was increased in men with acute Q fever but not in those with C. burnetii endocarditis, suggesting that the expression of the Per2 gene is related to the clinical status of the patient. It has been demonstrated that the immune response of patients with acute Q fever is largely different from that of patients with C. burnetii endocarditis [8].

Among the factors affecting the chronic evolution of Q fever, aging may be essential. Indeed, in mice, aging is associated with a reduced amplitude of circadian clock–related gene expression [14]. Age affects both the expression of circadian clock–related genes and the response of mice to C. burnetii infection [15]. In our cohort, the female patients were younger than the male patients. Future studies are required to identify the correlation between age and gender in the expression of the Per2 gene.

In conclusion, we reported for the first time that the expression of the Per2 gene was increased in men with acute Q fever but not in women. This gender dimorphism may be related to the higher incidence and severity of Q fever in men than in women. This finding provides new perspectives to investigate the pathophysiology of Q fever.

Notes

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