Monocyte Responses in the Context of Q Fever: From a Static Polarized Model to a Kinetic Model of Activation

Vikram Mehraj, Julien Textoris, Amira Ben Amara, Eric Ghigo, Didier Raoult, Christian Capo, and Jean-Louis Mege

Aix-Marseille University, URMITE, CNRS UMR 7278, IRD 198, INSERM 1095, Marseille, France

Background. Q fever is caused by *Coxiella burnetii*, a bacterium that persists in M2-polarized macrophages. We wondered whether the concept of M1/M2 polarization is applicable to Q fever patients.

Methods. Monocytes from healthy controls were cultured with IFN-γ and IL-4, agonists of M1 and M2 macrophages, respectively, and their gene expression was assessed using whole-genome microarrays. Selected biomarkers were assessed in blood from Q fever patients by real-time reverse transcription polymerase chain reaction (RT-PCR).

Results. Monocytes exhibited early (6-hour) patterns of activation specific to IFN-γ or IL-4 and a late (18-hour) pattern of common activation. Because these responses were not reducible to M1/M2 polarization, we selected biomarkers and tested their relevance in Q fever patients. The early genes *NLRC5*, *RTP4*, and *RHOH*, which were modulated in response to IFN-γ, were up-regulated in patients with acute Q fever, and the expression levels of the late genes *ALOX15*, *CLECSF1*, *CCL13*, and *CCL23* were specifically increased in patients with Q fever endocarditis. The *RHOH* and *ALOX15* genes were associated with the activity of acute Q fever and Q fever endocarditis, respectively.

Conclusions. Our results show that the kinetic model of monocyte activation enables a dynamic approach for the evaluation of Q fever patients.

Keywords. macrophage; microarray; monocyte; Q fever; polarization.

Q fever is caused by *Coxiella burnetii*, an intracellular bacterium known for its myeloid tropism. This disease is characterized by a primary infection that may become chronic in patients with valvulopathy, pregnant women, and immunocompromised patients [1]. Although the primary infection is characterized by a Th1-type protective immune response, the chronic form of the disease is due to impaired cell-mediated immunity [2]. The analysis of *C. burnetii*-macrophage interaction has shown that macrophage polarization is critical for bacterial elimination or persistence [3].

The concept of macrophage polarization is instrumental in analyzing the activation of murine macrophages and, to a lesser degree, human macrophages [4]. M1 macrophages that are stimulated by interferon (IFN)-γ in the presence or absence of lipopolysaccharide (LPS) are considered inflammatory, microbicidal, and tumoricidal. M2 macrophages, which are induced by interleukin (IL)-4/IL-13, IL-10/transforming growth factor (TGF)-β1, immune complexes, apoptotic cells, and corticosteroids, are poorly inflammatory and exhibit immunoregulatory functions [5]. Polarized macrophages are involved in innate immune response to pathogens [6], autoimmune diseases [7], and local immune suppression associated with tumors [8]. We previously showed that *C. burnetii* induces an atypical M2 program in macrophages that combined M2 markers, including TGF-β1, IL-10, CCL18, mannose receptor, and arginase, and some M1 markers, including IL-6 and CCL8 [9]. The uptake of apoptotic lymphocytes by macrophages stimulates an M2 program and the replication of *C. burnetii*. Both events are prevented in the presence of IFN-γ [10].
In vivo, the overexpression of IL-10 in transgenic mice enables bacterial persistence and mimics features of chronic Q fever, including the M2 polarization of macrophages [11]. These reports suggest that the chronic evolution of Q fever is related to the M2 polarization of macrophages, but direct evidence of such a relationship in infected patients is lacking. The concept of the M1/M2 polarization of macrophages seems pertinent only in few clinical situations, and in vivo and in vitro data are often contradictory [12]. In addition, only monocytes, not tissue macrophages, are easily accessible in clinical investigations, and it is not known if the concept of macrophage polarization is applicable to monocytes.

In this study, using a high-throughput microarray approach, we analyzed the transcriptional responses of circulating monocytes to IFN-γ and IL-4, canonical agonists of M1 and M2 macrophages, respectively. When monocytes were stimulated for 6 hours, they exhibited early responses to IFN-γ and IL-4 that were not reducible to M1/M2 polarization. After 18 hours, the monocyte response was no longer polarized, and a late response signature was identified. By selecting early- and late-related genes from each monocyte signature, we showed that specific biomarkers could be used to distinguish acute Q fever and Q fever endocarditis.

METHODS

Patients
This study was approved by the Ethics Committee of the Aix-Marseille University, and written informed consent was obtained from each subject. Q fever diagnosis was based on epidemiological, clinical, and serological criteria [13]. We included 14 patients with acute Q fever with a mean age of 47 years (range, 28–69 years), 13 patients with chronic Q fever and endocarditis with a mean age of 52 years (range, 39–64 years). Six patients with acute Q fever and 9 patients with Q fever endocarditis were investigated twice: at the inclusion, after 5 (3–6) months for acute Q fever and after 16 (8–25) months for Q fever endocarditis. The control group consisted of 10 healthy controls with a mean age of 48 years (range, 32–59 years), 8 patients with classical Whipple’s disease (mean age of 64 years, range: 53–69 years), 7 trauma patients with a mean age of 34 years (range, 24–37 years) and 5 trauma patients with pneumonia (mean age of 36 years, range, 29–43 years) [14]. Samples were handled anonymously without knowledge of disease state until statistical analysis. Peripheral blood was drawn in PAXgene tubes (Qiagen), and RNA was extracted after DNase treatment (see below).

Monocytes and Macrophages
Blood donor buffy coats were provided by the Etablissement Français du Sang (Marseille). Peripheral blood mononuclear cells were subjected to CD14+ magnetic cell sorting (Miltenyi Biotec), which yielded monocytes with a purity higher than 95%, as assessed by flow cytometry. Monocytes (10⁶ cells/assay) were differentiated into macrophages as described elsewhere [9], and after differentiation, more than 95% of cells were macrophages as assessed by CD68 expression and CD14 down-regulation. Monocytes and monocyte-derived macrophages were stimulated with 20 ng/mL recombinant human IFN-γ (PeproTech) or IL-4 (AbCys) for 6 or 18 hours.

Microarrays
RNA was extracted with an RNeasy Mini kit (Qiagen) and analyzed with microarray chips (4×44K Whole Human Genome, Agilent Technologies, Massay, France) as recently described [15]. MIAME-compliant data were submitted to the Gene Expression Omnibus (GEO) of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/geo/) and can be assessed with the GEO series accession number GSE36537.

The data were analyzed with R and the Bioconductor software suites with Significance Analysis of Microarray and a multiclass model integrating the experimental design. Functional enrichment analysis was performed with DAVID as described elsewhere [16]. To assess the M1/M2 polarization status of monocytes, 28 M1 genes and 17 M2 genes (Supplementary Table 1) were manually selected from the literature [4–6] and were found to be modulated in IFN-γ- or IL-4-stimulated macrophages. These genes were plotted above a virtual cell according to their cellular localization. The modulation of gene expression was explored by color coding. The ratio of gene expression in unstimulated cells to gene expression in stimulated cells is coded from red to blue, whereas the ratio of gene expression in IFN-γ-stimulated cells to gene expression in IL-4-stimulated cells is coded from pink to green. Cytoscape [17] and Inkscape were used to design the figures. These 45 genes were also analyzed by quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) to confirm microarray results. The overall correlation coefficient for the 2 techniques was high ($R^2 = 0.64; P = .01$).

qRT-PCR
qRT-PCR experiments were performed with the MMLV RT Kit (Life Technologies) and the 7900HT Fast Real Time PCR System (Applied Biosystems) as recently described [15]. Gene-specific primers were designed with Primer3 [18]. The results were normalized to the result for the housekeeping gene β-actin and are expressed as ΔCt values ($ΔCt = Ct_{Target} - Ct_{Actin}$), where Ct is the number of PCR cycles. Correlation analysis for the microarray and qRT-PCR data was performed with the Spearman correlation coefficient. The significance of the differential expression in patients observed by qRT-PCR was assessed by the Wilcoxon rank sum test with a P-value cutoff of .05.

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RESULTS

Kinetics of Transcriptional Responses of Monocytes to IFN-γ and IL-4

The transcriptional responses of monocytes and macrophages to IFN-γ or IL-4 were investigated using whole-genome microarrays. The overall transcriptional response showed a completely different activation pattern in monocytes and macrophages. Principal component analysis confirmed that the cell type (monocytes or macrophages), time (6 or 18 hours), and type of stimulation (IFN-γ or IL-4) influenced transcriptional modulation (Figure 1A and 1B). Based on a multiclass model that integrates the cell type (macrophages or monocytes), length of stimulation (6 or 18 hours) and type of stimulation (IFN-γ or IL-4), 15 619 probes were modulated with an absolute FC of at least 2.0 and a false discovery rate (FDR) < 1% (assessed with significance analysis of microarray). When filtering the dataset for an absolute FC ≥ 4.0 and a FDR < 1% 4807 modulated probes (3406 genes) were identified. These 4807 modulated probes are represented as a heatmap (Figure 1C). Modulated genes were then filtered to retain those genes specifically modulated in monocytes (Figure 1D). We identified 587 probes whose targets were modulated in IFN-γ- or IL-4-stimulated monocytes compared with unstimulated monocytes. The early genes (6-hour stimulation) that were specifically up-regulated in response to IFN-γ or IL-4 included the targets of 59 probes (46 annotated genes) and the targets of 61 probes (39 annotated genes), respectively. In contrast to the early responses of monocytes to IFN-γ and IL-4, the patterns of the late response (18-hour stimulation) of monocytes to IFN-γ and IL-4 were indistinguishable and were characterized by a specific cluster of up-regulated genes that corresponded to 74 probes and 57 annotated genes (Figure 1D). Taken together, these results revealed a biphasic response of monocytes to IFN-γ and IL-4.

Polarization of Early and Late Monocyte Responses

We wondered whether the polarization observed in macrophages is applicable to the early and late responses of monocytes. We studied the expression in monocytes of 28 and 17 genes considered to be markers of M1 and M2 polarization in...

Figure 1. Principal component analysis and heatmap representation of modulated probes. Monocytes and macrophages were stimulated with IFN-γ or IL-4 (20 ng/mL), and microarray analysis was performed after RNA extraction. A and B, The impacts of the cell type and the length of stimulation (A), and those of cell stimulation by IFN-γ or IL-4 (B) were analyzed by principal component analysis. The distance along each axis represents the amount of variance in gene expression explained by the corresponding factor. C, The 4807 probes modulated in monocytes and macrophages stimulated for different periods by IFN-γ or IL-4 (|FC|>4 and FDR < 1%) are represented as a heatmap with probes in rows and samples in columns. Dendrograms show the results of the hierarchical clustering of the samples (top) and genes (left) (average linkage based on the Pearson correlation distance). D, Only genes modulated in stimulated monocytes compared with unstimulated monocytes were retained. Up-regulated genes specific to early stimulation with IFN-γ or IL-4 and those genes specifically up-regulated after 18 hours were selected. Specific signatures are identified as black lines on the right hand side. Gene expression levels are color-coded from blue to red. Abbreviations: FDR, false discovery rate; IFN, interferon; IL, interleukin.
macrophages [4–6]. In the early response of monocytes to IFN-γ, 16 M1 genes were up-regulated, but only one M2 gene was up-regulated (Figure 2A). In the early response of monocytes to IL-4, 10 M2 genes, but only 3 M1 genes, were up-regulated (Figure 2C). Hence, the early responses of monocytes to IFN-γ and IL-4 showed a clear dichotomy in the expression of M1 and M2 genes (Figure 2E). We then investigated whether the specific signatures of monocytes induced by IFN-γ and IL-4 included M1/M2 genes. In the early signature induced by IFN-γ (which included 46 genes), only 11 genes were M1 genes. Similarly, the early signature induced by IL-4, composed of 39 genes, included only 2 M2 genes, demonstrating that the early responses of monocytes are not reducible to M1/M2 polarization.

In the late response of monocytes to IFN-γ, most M1 genes that were up-regulated in the early response to IFN-γ returned to their basal levels or were down-regulated. Only one M1 gene remained up-regulated. In addition, 8 M2 genes were up-regulated in the late response to IFN-γ (compare Figure 2A and 2B). The late response of monocytes to IL-4 was characterized by the up-regulation of one M1 gene, the down-regulation of 14 M1 genes, and the up-regulation of 10 M2 genes (compare Figure 2C and 2D). The modulation of M1 and M2 genes was remarkably similar in the late responses of monocytes to IFN-γ and IL-4 (Figure 2F), demonstrating that the late responses of monocytes were not polarized as the responses of macrophages.

As a consequence, we hypothesized that the M1/M2 transcriptional program of macrophages may be insufficient to account for the clinical manifestation of Q fever. To test this hypothesis, we selected 6 M1-related genes (IL15, CXCL9, IL2RA, IDO1 [NDO], TNF, TNFSF10 [TRAIL]) and 6 M2-related genes (CHN2, CTSC, CD209 [DC-SIGN], FN1, HRH1, SLCA47), and we assessed their expression levels in Q fever patients. The expression levels of M1 and M2 genes (with the exception of CD209) were similar in healthy controls and patients with acute Q fever. In patients with Q fever endocarditis, the expression levels of 4 M1 genes were up-regulated, and the expression level of one M2 gene was down-regulated relative to the expression levels in healthy controls (Supplementary Figure 1). These results show that the expression of the M1/M2 genes does not characterize the evolution of Q fever.

**Functional Classification of Early and Late Monocyte Responses**

Because the majority of the genes up-regulated in the early and late signatures of monocytes were not M1/M2 genes, we wondered if these genes belonged to distinct functional groups. Among the early genes specifically up-regulated in response to IFN-γ, we identified 6 different clusters including “apoptosis,” “complement,” “defense response,” “IFN-related genes,” “Jak/Stat pathway,” and “lipid metabolism.” Six other functional clusters were identified among the early genes that were specifically up-regulated in response to IL-4. They included “cell adhesion,” “C-type lectin,” “cytoskeleton,” “endocytosis and vesicular transport,” “response to wounding,” and “RNA binding.” The late response of monocytes was organized in 5 different clusters including “Ig domain,” “immune response,” “Golgi apparatus,” “signal transduction,” and “Wnt pathway” (Table 1). These results demonstrated that the early and the late responses of monocytes to IFN-γ and IL-4 were characterized by specific functional signatures that may be useful to define new biomarkers of Q fever.

**Specific Biomarkers of Q Fever**

Among the early annotated genes, we selected 4 genes representative of the early IFN-γ response (NLRC5, RTP4, C1S, GCH1) and 4 genes representative of the early IL-4 response (ITGB3, TREMI, NEDD4L, RHOH), which are not described as M1 or M2 markers. The expression levels of these genes were compared between healthy controls and Q fever patients using qRT-PCR. In acute Q fever, 2 early genes associated with the IFN-γ response, NLRC5 and RTP4, were significantly up-regulated. This up-regulation was specific of acute Q fever. Indeed, the expression of NLRC5 and RTP4 genes was not modulated in patients with Q fever endocarditis relative to healthy controls (Figure 3). In patients with Whipple’s disease, an infectious disease due to an intracellular bacterium with macrophage tropism, NLRC5, RTP4, and RHOH genes were not modulated (Supplementary Figure 2). In trauma patients with or without pneumonia, who exhibited an intense inflammatory response, NLRC5 and RTP4 genes were down-regulated, suggesting that the modulation of early genes in acute Q fever was not a consequence of inflammatory response. Interestingly, the ITGB3 and TREMI genes, which were associated with the early response to IL-4, were significantly down-regulated in patients with acute Q fever compared with controls. Only one early gene associated with the IL-4 response, RHOH, was significantly up-regulated in patients with acute Q fever (Figure 3B). Therefore, the up-regulation of NLRC5, RTP4, and RHOH genes may be considered biomarkers of acute Q fever.

A similar procedure was used to define biomarkers from the late signature of monocytes using eight genes belonging to different clusters. In acute Q fever patients, 5 genes (ALOX15, CLEC4F, CCL23, BACE1, RAMP1) were not differentially regulated relative to the expression levels in healthy controls (Figure 4), demonstrating that late biomarkers were unable to distinguish acute Q fever. By contrast, the ALOX15, CLEC4F, CCL13, CCL23, and RAMP1 genes were significantly up-regulated in patients with Q fever endocarditis relative to healthy controls. Interestingly, the expression levels of ALOX15, CLEC4F, CCL13, and CCL23 were significantly higher in patients with Q fever endocarditis than in patients with acute Q fever (Figure 4), suggesting that these genes may serve as specific biomarkers of Q fever endocarditis. We also found that 2 late genes, GALNTL18 and WNT5B, which were not modulated in
Figure 2. Gene modulation of M1/M2 markers in stimulated monocytes. Monocytes were stimulated with IFN-γ or IL-4 for 6 hours. Genes involved in macrophage polarization (n = 45) were manually selected from the literature and plotted against a virtual cell according to their GO cellular component annotation. A–D, The ratios of gene expression in monocytes stimulated with IFN-γ (A and B) or IL-4 (C and D) for 6 (A and C) and 18 (B and D) hours to the gene expression in unstimulated monocytes are color-coded from blue (FC = −5) to red (FC = +5) at each node. E and F, M1/M2 markers were plotted against a virtual cell, and the ratios of the expression of these markers in monocytes stimulated with IFN-γ or IL-4 are color-coded from green (FC IFN-γ/IL-4 = −5) to pink (FC IFN-γ/IL-4 = +5). Genes in white were up-regulated similarly by IFN-γ and IL-4. Abbreviations: IFN, interferon; IL, interleukin.
Q fever endocarditis patients, were down-regulated in acute Q fever patients, providing indirect evidence that late genes may be biomarkers of Q fever endocarditis. Again, late genes were not modulated in patients with Whipple’s disease. In trauma patients with or without pneumonia, these genes were down-modulated compared with healthy controls (Supplementary Figure 2). These results demonstrate that ALOX15, CCL13, and CCL23 genes were specifically up-regulated in Q fever endocarditis.

Finally, we assessed the modulation of putative biomarkers during the follow-up of patients with Q fever. In patients with
acute Q fever, the expression of NLRC5 and RTP4 genes remained up-regulated 5 months after the initial inclusion whereas the expression of RHOH gene was similar to controls after 5 months (Figure 5A). The modulation of CCL23, CLEC4F, ALOX15, and CCL13 genes was assessed in patients with Q fever endocarditis 16 months after the inclusion. The expression of CCL13 and CCL23 genes remained unchanged. By contrast, the expression of ALOX15 and CLEC4F genes decreased with time, but only the expression of ALOX15 gene returned to control levels (Figure 5B). Taken together, our results support the hypothesis that the kinetics model of monocyte activation is useful to assess the clinical manifestation of Q fever.

DISCUSSION

The M2 polarization of macrophages has been considered critical for the persistence of C. burnetii [6, 9, 11]. Nevertheless, this concept needs to be confirmed in circulating monocytes. Different constraints impair the evaluation of patients with Q fever based on the concept of macrophage polarization. The isolation of macrophages is adapted for pathophysiological studies but is not convenient for routine use in clinical practice. As a consequence, the use of whole blood may be a practical alternative but requires the validation of monocyte biomarkers. In this study, we investigated the responses of monocytes to IFN-γ and IL-4, and we used these data to guide our analysis of whole blood from patients with Q fever.

The responses of monocytes to IFN-γ and IL-4 consisted of early and late phases of activation, a pattern that did not fit with the M1/M2 polarization model at the transcriptional level. The hallmarks of M1/M2 polarization were found in the early phases of monocyte activation, but the second phase of monocyte activation was common to the different modes of stimulation. Our results were consistent with the modulation of early and late genes in macrophages stimulated with LPS [19] and the transient polarization observed in murine monocytes stimulated with Listeria monocytogenes [20].

In the early phase of the monocyte response to IFN-γ, we identified one set of up-regulated genes that included M1 genes, interferon-response genes, and genes related to inflammatory signaling, complement, and apoptosis. A second set of genes that were specifically up-regulated in IL-4-stimulated monocytes included M2-related genes, and a series of genes partitioned into different clusters associated with lectins, cell adhesion, endocytosis, and the cytoskeleton. By contrast, the late response of monocytes was characterized by the up-regulation of numerous genes independent of the agonist, IFN-γ or IL-4. This late response was also distinct from the LPS tolerance that appears late in the inflammatory response and is associated with an M2 program [21]. The late response of monocytes may be considered a termination program with only some features
of the M2 program. Our results were consistent with data obtained in a murine model of resolving and nonresolving peritonitis. In this model, peritoneal macrophages from mice with sustained inflammation may be referred to as M1 macrophages, and macrophages from mice with resolving inflammation produce high levels of immunoregulatory cytokines but also present hallmarks of M1 macrophages [22]. A recent transcriptional analysis confirmed that the peritoneal macrophages collected from mice with resolving inflammation exhibit a unique phenotype that is inconsistent with the conventional M1/M2 macrophage classification [23]. These results suggest a high level of plasticity in macrophages. The existence of a unique termination program in monocytes stimulated with IFN-γ and IL-4 may represent a parsimonious way to control the monocyte activation induced by a great variety of cytokines and infectious agents.

We showed that the model of M1/M2 polarization was not applicable to monocyte responses in the context of Q fever. We selected M1-related and M2-related genes and tested these genes in whole blood. The expression levels of these genes were similar in patients with acute Q fever and healthy controls. Only a minority of these genes were up-regulated in patients with Q fever endocarditis. Such findings demonstrate that Q fever patients exhibited an activation program that cannot be classified as an M1 or M2 pattern, in contrast to the result of in vitro and in vivo studies using macrophages [9, 10, 24]. The

Figure 5. Expression of biomarkers according the evolution of Q fever. A, The expression of potential biomarkers of acute Q fever was analyzed in blood from healthy donors (1), patients with acute Q fever tested at the inclusion (2) and 5 months after inclusion (3). B, The expression of potential biomarkers of Q fever endocarditis was analyzed in blood from healthy donors (1), patients with Q fever endocarditis tested at the inclusion (2) and 16 months after inclusion (3). Individual results of qRT-PCR data are shown. Horizontal bars represent the median of each group, and significant differences between groups are shown. Abbreviation: qRT-PCR, quantitative real-time reverse transcription polymerase chain reaction. *P-value < .05; **P-value < .01.
differences in the transcriptional programs of monocytes and macrophages may explain why attempts to use markers defined as M1/M2 for macrophages are unsuccessful in clinical studies [25, 26]. Only rare reports suggest that monocytes are polarized in infectious diseases. In addition, even though monocytes from patients with Whipple’s disease overexpress CD163, other markers of M2 cells are lacking [27]. In patients with active tuberculosis [28] or in children vaccinated with BCG [29], a transient M1 profile is identified, whereas in vitro studies with macrophages suggest M2 polarization [12]. By contrast, monocytes stimulated with Orientia tsutsugamushi and patients with scrub typhus both exhibit an M1-type program [30].

Interestingly, the identification of early and late signatures of in vitro-stimulated monocytes allowed the identification of Q fever biomarkers. Some early genes specifically associated with acute Q fever, such as NLRC5 and RTP4, were up-regulated by IFN-γ, suggesting that acute Q fever is controlled at least in part by an IFN-γ-mediated immune response. That these genes were not modulated in patients with Q fever endocarditis confirmed the defective Th1-response reported for Q fever endocarditis [31]. We also found that genes associated with IL-4 response, such as ITGB3 and TREMI, were down-regulated in patients with acute Q fever, reinforcing our hypothesis that acute Q fever is associated with an efficient immune response. Moreover, we found that some late genes, including ALOX15, CLEC4F, CCL13, and CCL23, were specifically associated with Q fever endocarditis. Note that a few late genes were also related to the M2 program, a result that might lead to incorrect conclusions about monocyte activation in the context of Q fever endocarditis. The modulation of early genes in acute Q fever and late genes in Q fever endocarditis seemed to be specific of Q fever because these genes were not modulated in patients with another infectious disease, the Whipple’s disease, and patients with acute pathologies such as trauma or pneumonía. The follow-up of patients with Q fever revealed that the expression of RHOH and ALOX15 genes was normalized after several months of disease’s evolution, suggesting that these genes may be considered as biomarkers of the activity of acute Q fever and Q fever endocarditis, respectively.

In conclusion, we demonstrated that the concept of macrophage polarization is not useful to describe monocyte activation and is irrelevant to explore patients at the bedside. The kinetic approach of monocyte activation we developed allowed the identification of new biomarkers of acute Q fever and Q fever endocarditis, respectively. Additionally, 2 biomarkers were associated with the activity of the disease.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of

Notes

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References