Coxiella burnetii Survives in Monocytes from Patients with Q Fever Endocarditis: Involvement of Tumor Necrosis Factor

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Endocarditis is the most frequent form of chronic Q fever, an infectious disease caused by *Coxiella burnetii*. As this obligate intracellular bacterium inhabits monocytes and macrophages, we wondered if pathogenesis of Q fever endocarditis is related to defective intracellular killing of *C. burnetii* by monocytes. Monocytes from healthy controls eliminated virulent *C. burnetii* within 3 days. In contrast, monocytes from patients with ongoing Q fever endocarditis were unable to eliminate bacteria even after 6 days. In patients who were cured of endocarditis, the monocyte infection was close to that of control monocytes. This killing deficiency was not the consequence of generalized functional impairment, since patient monocytes eliminated avirulent *C. burnetii* as did control cells. The addition of supernatants of *C. burnetii*-stimulated monocytes from patients with ongoing endocarditis to control monocytes enabled them to support *C. burnetii* survival, suggesting that some soluble factor is responsible for bacterial survival. This factor was related to tumor necrosis factor (TNF): expression of TNF mRNA and TNF release were increased in response to *C. burnetii* in patients with ongoing endocarditis compared to cured patients and healthy controls. In addition, neutralizing anti-TNF antibodies decreased *C. burnetii* internalization, an early step of bacterial killing, in monocytes from patients with ongoing endocarditis but did not affect delayed steps of intracellular killing. We suggest that Q fever-associated activation of monocytes allows the survival of *C. burnetii* by modulating early phases of microbial killing.

Q fever is caused by *Coxiella burnetii*, an obligate intracellular bacterium inhabiting monocytes and macrophages (1). The disease exhibits acute and chronic forms with different courses. Endocarditis is the most frequent clinical expression of chronic Q fever (23). Q fever endocarditis usually occurs in patients with valvular disease and/or alterations in cell-mediated immunity, such as infection by human immunodeficiency virus, lymphoma, chronic renal failure, or pregnancy (21). The medical treatment of endocarditis is long, even when antibiotics are used with chloroquine (24). The evaluation of its efficiency requires prolonged follow-up because of the possibility of late relapses (22).

Macrophages exhibit microbicidal activity which involves binding and phagocytosis of microorganisms and the action of oxidative and nonoxidative compounds within phagocytic vacuoles. Macrophage-mediated killing of microorganisms also requires T-cell-derived cytokines, including gamma interferon (IFN-γ) (25). Hence, patients with IFN-γ receptor deficiency exhibit increased occurrences of mycobacterial infections (2). Conversely, cytokines which down-modulate microbicidal activity of macrophages favor the survival of intracellular microorganisms (3). The survival strategy of *C. burnetii* should interfere with the intrinsic microbicidal activity of macrophages and/or its regulation. Patients with Q fever endocarditis exhibit impaired cell-mediated immunity, including antigen-driven lymphoproliferation (17) and IFN-γ production (14). We recently demonstrated that IFN-γ induces *C. burnetii* killing via apoptosis of infected macrophages (10). The suppression of T-cell responses to *C. burnetii* depends on the release of soluble mediators such as prostaglandins (18) or interleukin-10

(IL-10) (6) by monocytes. Beside their suppressive role, monocytes from patients with Q fever endocarditis overproduce tumor necrosis factor (TNF), a proinflammatory cytokine (5). This may be related to the specific inflammatory syndrome of Q fever endocarditis, consisting of an increase in circulating TNF without variations in cytokine antagonists (7).

This study was undertaken to assess the survival of *C. burnetii* in monocytes from patients with Q fever endocarditis. Control monocytes eliminated *C. burnetii*, whereas those of patients with ongoing Q fever endocarditis did not. This defect was not intrinsic but was related to an increase in TNF production. TNF was involved in this defect mainly by upregulating *C. burnetii* internalization. We suggest that the level of monocyte activation in Q fever determines the survival of *C. burnetii*.

MATERIALS AND METHODS

Patients. Twenty patients, consisting of 13 males and 7 females (mean age, 59 years; range, 39 to 79 years), were included in the study. The diagnosis of endocarditis was based on modified Duke endocarditis service criteria (11). Patients had pathological evidence of endocarditis, a positive echocardiogram, positive blood culture, and high titers of immunoglobulin G (IgG) directed against *C. burnetii*. All of these patients were subjected to valve replacement and medical treatment with doxycycline and chloroquine. They were divided in two groups: one consisting of patients with ongoing endocarditis (*n* = 10) characterized by high titers of specific IgG (mean, 21,000; range, 1,600 to 120,000) and the other made up of patients recently cured of the disease (*n* = 10) and who had low antibody titers (mean, 600; range, 400 to 800). The first group was treated during the course of the study, while treatment of the second group had been stopped at least 3 months before the investigation. Ten healthy subjects, sex and age matched, were included in the study as controls.

Monocytes and bacteria. Blood was drawn in EDTA-anticoagulated tubes, and peripheral blood mononuclear cells were separated with Ficoll gradients (Eurobio, Les Ulis, France). Cells were suspended in RPMI 1640 containing 20 mM HEPES (Gibco-BRL, Life Technologies, Cergy-Pontoise, France), 10% fetal calf serum (FCS), 2 mM L-glutamine, 100 U of penicillin per ml, and 100 μg of streptomycin (Gibco-BRL) per ml. Monocytes were purified by incubating 5 × 10^5 peripheral blood mononuclear cells in a glass Labtek chamber/slide (Miles, Naperville, Ill.) for 60 min at 37°C. Nonadherent cells were removed by washing,

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and the remaining cells were designated monocytes because more than 90% of them were CD14$^+$ and had phagocytic characteristics (5).

Virulent *C. burnetii* (Nine Mile strain in phase I; ATTC VR-615) was injected into mice and 10 days later was recovered from spleens and then cultured in mouse L929 fibroblasts maintained in antibiotic-free minimal essential medium (Gibco-BRL) supplemented with 4% FCS and 2 mM L-glutamine for two passages. Avirulent variants were obtained by repeated passages of Nine Mile strain in L929 cells (20). After 1 week, L929 cells were sonicated, and the homogenates were centrifuged at 5,000 × *g* for 10 min. The bacterial pellet was layered on a 25 to 45% linear Renografin gradient and spun down. Purified bacteria were then collected, washed, and suspended in serum-free medium before being stored at −80°C. The concentration of *C. burnetii* was determined by Gimenez staining.

Infection procedure. Monocytes were incubated with *C. burnetii* in phase I (bacterium-to-cell ratio of 200:1) for 24 h in RPMI 1640 containing 10% FCS (10). The cells were washed to remove free bacteria (corresponding to day 0) and cultured for 6 days. As controls, monocytes were incubated with avirulent *C. burnetii* at a bacterium-to-cell ratio of 100:1 for 24 h. As avirulent bacteria were more efficiently internalized by monocytes than virulent organisms (8), we incubated monocytes with a lower number of avirulent *C. burnetii* organisms to obtain similar amounts of infection. In some experiments, a 10-μg/ml concentration of goat antibodies (Ab) neutralizing bioactive TNF or control IgG (R&D Systems, Abingdon, United Kingdom) was added to monocytes before *C. burnetii* infection. Cellular infection was quantified by Gimenez staining. Results were expressed as an infection index, as follows: (number of bacteria per infected cell) × (number of infected cells/100) × 100.

The viability of intracellular bacteria was determined as previously described (10). Briefly, infected monocytes were sonicated, and serial dilutions of homogenates were added to HEL cell monolayers. *C. burnetii* replication was revealed by indirect immunofluorescence with rabbit Ab directed against *C. burnetii*.

Cytokine determination. (i) Immunoassays. Monocytes (2 × 10^5 cells/assay) were incubated with *C. burnetii* (bacterium-to-cell ratio of 200:1) for 24 h. Supernatants were assayed for the presence of TNF and IL-6. The limits of detection of the immunoassay kits (Immunotech, Marseille, France) were 10 and 3 pg per ml, respectively.

(ii) RNA extraction and PCR amplification. Monocytes (5 × 10^5 cells/assay) were incubated with *C. burnetii* (bacterium-to-cell ratio of 200:1) for 3 h. RNA was extracted by using the guanidium-phenol chloroform method (5). cDNA was generated by incubating RNA in a reverse transcriptase mixture. cDNA specimens were amplified in the presence of primers specific for TNF or IL-6. Amplification products were quantified with CytoXpress detection kits (BioSource, Fleurus, Belgium) as previously described (4).

Statistical analysis. Results were calculated as mean ± standard error (SE). The statistical analysis was conducted by analysis of variance. Differences were considered significant when *P* was <0.05.

RESULTS

Killing of *C. burnetii* by monocytes is defective in Q fever endocarditis. Monocytes were incubated with *C. burnetii* in phase I for 24 h (day 0), and the infection of monocytes was assessed at days 3 and 6 postinfection (p.i.). An incubation time of 24 h was required to obtain the infection of 75% of monocytes with one to two bacteria per cell. While this initial incubation allows the measurement of the early phase of killing, the infection after 3 and 6 days is an assessment of the delayed phase of intracellular killing. At day 0, the bacterial phagocytosis in monocytes from patients with ongoing endocarditis (active patients) was different from that in monocytes from cured patients or controls (Fig. 1A). The initial infection index was significantly (*P* < 0.005) higher in monocytes from active patients (250 ± 35) than in control monocytes (115 ± 12) or in monocytes from cured patients (136 ± 15). After 3 days, the number of bacteria was decreased by 75% in control monocytes, and only 15% of bacteria present at day 0 were found after 6 days (Fig. 1B). The decrease in the bacterial count resulted from an alteration of *C. burnetii* viability, as assessed by culturing homogenates from infected monocytes on HEL cells (105 ± 35 vacuoles per shell vial at day 0 and 20 ± 9 vacuoles per shell vial at day 6 for control monocytes). In contrast, the number of intact bacteria remained constant during the first 3 days p.i. and decreased slightly thereafter (15% inhibition) in monocytes from active patients. Bacterial viability was not altered after 3 days of infection (123 ± 38 vacuoles per shell vial), but it was slightly decreased at 6 days p.i. (95 ± 32 vacuoles per shell vial). In monocytes from cured
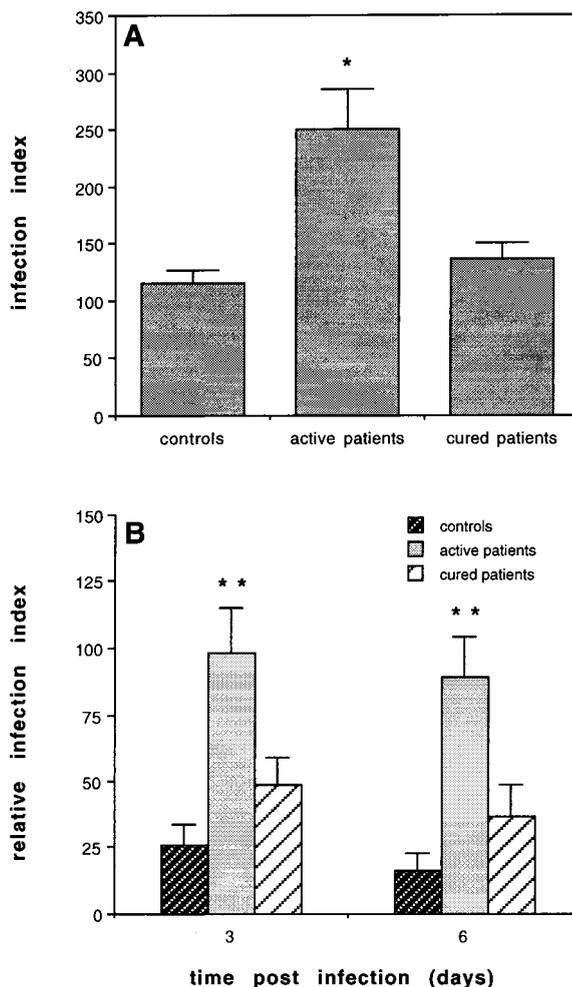

FIG. 1. *C. burnetii* survival in monocytes from patients with Q fever endocarditis. Monocytes from controls (*n* = 10), patients with ongoing Q fever endocarditis (active patients) (*n* = 10), or cured patients (*n* = 10) were incubated with *C. burnetii* at a bacterium-to-cell ratio of 200:1. Monocyte infection was determined at day 0 (A) and at 3 and 6 days p.i. (B) by Gimenez staining. In panel A, results (means ± SEs) are expressed as an infection index; in panel B, they are expressed as a relative infection index compared to values at day 0. *, *P* < 0.005; **, *P* < 0.01 (for the comparison of active patient values with control values).

patients, the number of bacteria declined by 55% after 3 days and slowly decreased thereafter (70% inhibition after 6 days). At each time, the infection index was significantly lower (*P* < 0.01) in monocytes from cured patients than in monocytes from active patients. We then wondered if impaired killing of *C. burnetii* by patient monocytes was specific. Avirulent variants of *C. burnetii* were incubated with monocytes according to the procedure described for virulent bacteria. At day 0, the phagocytosis indices in control and patient cells were similar (132 ± 18 and 156 ± 24, respectively). The number of avirulent bacteria was decreased by 70% after 3 days and by 80% after 6 days in monocytes from active patients, cured patients, and healthy controls (Table 1). Our results indicate that ongoing Q fever endocarditis is associated with specific defective killing of virulent *C. burnetii*.

The defect in *C. burnetii* killing is reproduced by supernatants from patient monocytes. We wondered if defective bacterial killing of monocytes was intrinsic or might be reproduced by soluble mediators. For that purpose, monocytes from healthy controls were treated with supernatants obtained by

TABLE 1. Avirulent *C. burnetii* killing in Q fever endocarditis[a]

Group	Relative infection index (%)[b] at day:	
	3	6
Controls	29 ± 11	12 ± 7
Active patients	37 ± 10	21 ± 8
Cured patients	28 ± 8	13 ± 7

[a] Monocytes from controls and patients were incubated with avirulent *C. burnetii* at a bacterium-to-cell ratio of 100:1 for 24 h at 37°C. Monocyte infection was determined by Gimenez staining at 3 and 6 days p.i.

[b] Compared to day 0. Results are means ± SEs for five individuals per group.

culturing monocytes from controls, active patients, or cured patients with *C. burnetii* for 24 h at 37°C. *C. burnetii* was then added to treated cells at a bacterium-to-cell ratio of 200:1, and the infection was assessed for 6 days as described above. The uptake of *C. burnetii* was significantly ($P < 0.02$) higher in cells treated with monocyte supernatants from active patients than in cells treated with control supernatants (Fig. 2A). While the bacterial number steadily decreased in monocytes treated with supernatants from controls, it remained constant after 3 and 6 days in cells treated with supernatants from active patients (Fig. 2B). When monocytes were pretreated with supernatants from cured patients, the phagocytosis of *C. burnetii* (Fig. 2A) and the bacterial number at 3 and 6 day p.i. (Fig. 2B) were similar to those observed in cells treated with supernatants from control monocytes. Hence, monocyte supernatants from patients with ongoing endocarditis were able to induce defective intracellular killing in monocytes.

C. burnetii stimulates cytokine overproduction in patient monocytes. We previously found that patients with Q fever endocarditis exhibit an increase in spontaneous TNF production by monocytes (5). We investigated *C. burnetii*-stimulated production of TNF in monocytes from controls and patients. The expression of cytokine transcripts was studied by using a quantitative method (Table 2). In the absence of *C. burnetii*, the amounts of TNF mRNA were significantly higher in active patients than in controls ($P < 0.05$). After 3 h of stimulation with *C. burnetii* at a bacterium-to-cell ratio of 200:1, TNF transcripts dramatically increased, but their levels were significantly higher in active patients than in controls ($P < 0.01$). In cured patients, the amounts of TNF transcripts, spontaneously produced or induced by *C. burnetii*, were near the control values. Cytokine secretion was assessed in supernatants from monocytes stimulated with *C. burnetii* for 24 h (Table 2). Monocytes from active patients spontaneously released more TNF than those from controls ($P < 0.01$). When monocytes were stimulated with *C. burnetii*, the levels of secreted TNF were higher in active patients than in controls ($P < 0.04$). The TNF release by monocytes stimulated or not stimulated by *C. burnetii* returned to normal values in patients who had recovered from the disease. The increase in *C. burnetii*-stimulated TNF production was specific, since the amounts of IL-6 transcripts were similar in unstimulated monocytes from active patients (142 ± 31 copies per ng of RNA) and controls (175 ± 27 copies per ng of RNA). Active patients and controls expressed IL-6 transcripts in response to *C. burnetii* in a similar way (437 ± 25 and 518 ± 50 copies per ng of RNA, respectively). No difference was observed between spontaneous and *C. burnetii*-induced releases of IL-6 in active patients and controls (142 ± 31 versus 175 ± 27 pg/ml and 437 ± 25 versus 518 ± 50 pg/ml, respectively). Taken together, our data indicate that defective elimination of *C. burnetii* in patients with active endocarditis is associated with a specific increase in TNF production.

TNF is involved in defective killing of *C. burnetii* by patient monocytes. The role of TNF in impaired killing of *C. burnetii* was assessed by using neutralizing anti-TNF Ab, which completely inhibited *C. burnetii*-stimulated TNF secretion at 10 μg/ml (data not shown). First, monocyte supernatants from active patients were pretreated with anti-TNF Ab (or goat IgG) and then added to control monocytes before *C. burnetii* infection. The increase in bacterial phagocytosis and bacterial number at 3 and 6 days p.i. was prevented (data not shown). Second, anti-TNF Ab or control IgG was added to patient monocytes before the infection with *C. burnetii*. In active patients, 10 μg of anti-TNF Ab per ml decreased the uptake of *C. burnetii* by 36% ± 5% (Fig. 3A). The blocking Ab did not affect the bacterial number at 3 and 6 days p.i. in active patients or in cured patients (Fig. 3B). These results indicate that TNF in involved in defective killing of *C. burnetii* during Q fever endocarditis by acting mainly on bacterial uptake.
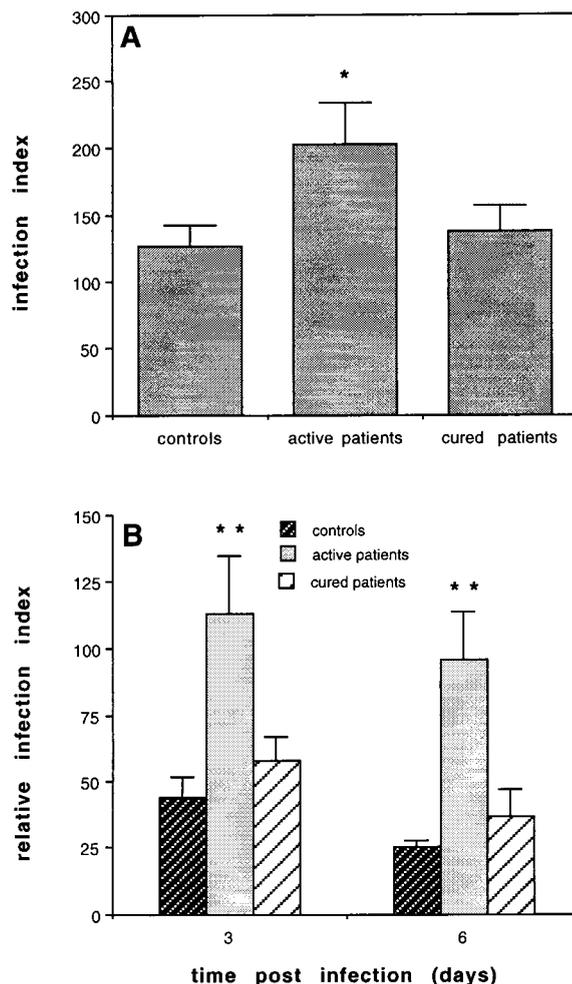

FIG. 2. Effect of monocyte supernatants on *C. burnetii* survival. Control monocytes were pretreated with supernatants of monocytes from healthy individuals ($n = 3$), active patients ($n = 3$) or cured patients ($n = 3$) which had been stimulated by *C. burnetii* (bacterium-to-cell ratio of 200:1). The cells were then infected with virulent bacteria as described for Fig. 1. Monocyte infection was determined at day 0 (A) and at 3 and 6 days p.i. (B) by Gimenez staining. In panel A, results (means ± SEs) are expressed as an infection index; in panel B, they are expressed as a relative infection index compared to values at day 0. *, $P < 0.02$; **, $P < 0.01$ (for the comparison of active patient values with control values).

TABLE 2. TNF production in Q fever endocarditis[a]

Group	No. of TNF copies/ng of RNA[b]		TNF secretion (pg/ml)[b]	
	Without C. burnetii	With C. burnetii	Without C. burnetii	With C. burnetii
Controls	7.4 ± 0.8	146 ± 25	185 ± 16	1,999 ± 430
Active patients	13.6 ± 1.4*	440 ± 95***	396 ± 59***	4,826 ± 897**
Cured patients	11.2 ± 3.2	229 ± 79	195 ± 49	2,202 ± 506

[a] Monocytes were stimulated or not stimulated with *C. burnetii* at a bacterium-to-cell ratio of 200:1 for 3 h. RNA was extracted and incubated with a reverse transcriptase mixture. After incubation with specific primers, the cDNAs were amplified and the expression of TNF transcripts was quantitated. Supernatants of monocyte cultures were collected after 24 h and assayed for the presence of TNF by immunoassay.

[b] Results are expressed as means ± SEs. *, $P < 0.05$; **, $P < 0.04$; ***, $P < 0.01$ (for the comparison of active patient values with control values). Each group contained 10 individuals.

DISCUSSION

In this study, we found that monocytes from patients with Q fever endocarditis were unable to eliminate *C. burnetii*, in contrast to control monocytes. This finding is complementary with early observations of immune dysfunction in Q fever, in which monocytes from patients with endocarditis are involved in the suppression of T-cell-mediated responses (18). This defect was a consequence of the activity of Q fever, since it was corrected in patients who had recovered from infection. It did not result from a generalized deficiency of bacterial killing, since patient monocytes eliminated avirulent *C. burnetii* and *Legionella pneumophila* (data not shown). This finding is reminiscent of data obtained for leprosy, in which monocytes of lepromatous leprosy patients support the growth of *Mycobacterium leprae* but restrict the multiplication of other bacteria susceptible to intracellular killing (15). The monocyte defect in *C. burnetii* killing affected early and delayed steps of microbicidal activity. *C. burnetii* uptake was significantly higher in monocytes from active patients than in monocytes from controls and cured patients. This finding may be related to the finding by Chaturvedi and Newman that a low growth of *Mycobacterium avium* or *Histoplasma capsulatum* is associated with a low phagocytosis by macrophages in human immunodeficiency virus-infected patients (9). However, the relationship between initial and delayed steps of bacterial killing is complex. Alveolar macrophages, which ingest more mycobacteria than monocytes, support a less efficient replication (13). The H37Ra and H37Rv strains of *Mycobacterium tuberculosis* are similar in their capacities to enter human macrophages, but they exhibit distinct survival patterns (27, 28). Our results show that the delayed step of microbicidal activity against *C. burnetii* was altered in monocytes from patients with Q fever endocarditis. This step of bacterial killing involves oxidative and nonoxidative mechanisms. The generation of reactive oxygen intermediates is apparently not required for *C. burnetii* killing. Indeed, monocyte production of reactive oxygen intermediates was similar in patients and healthy controls (data not shown). In addition, we showed that monocytes from patients with chronic granulomatous disease, which are known to be unable to produce reactive oxygen intermediates, eliminated *C. burnetii* as did control cells (10). It is likely that nonoxidative mechanisms such as intracellular traffic and vacuole environment (12) are modified in monocytes during Q fever endocarditis. The efficiency of chloroquine, which is known to alkalinize phagosomes, in Q fever treatment (24) supports this hypothesis.

The defect in *C. burnetii* killing by patient monocytes might be related to our experimental procedure, which was based on

the culture of monocytes in the absence of lymphocytes. The coculture of monocytes with autologous lymphocytes did not restore *C. burnetii* killing of patient monocytes (data not shown). The defect in *C. burnetii* killing was not intrinsic, since it was reproduced by monocyte supernatants from patients with ongoing Q fever endocarditis. Thus, soluble mediators may be responsible for the lack of *C. burnetii* killing by monocytes. We provided evidence that TNF is largely involved in this defect. First, monocytes from patients with ongoing endocarditis, which were not competent to eliminate *C. burnetii*, overexpressed TNF transcripts and secreted high levels of TNF in response to *C. burnetii*. The TNF production by monocytes from cured patients, which efficiently killed bacteria, was similar to that by control monocytes. These results may be related to the increase in spontaneous production of TNF by monocytes from patients with ongoing endocarditis (5). Second, the
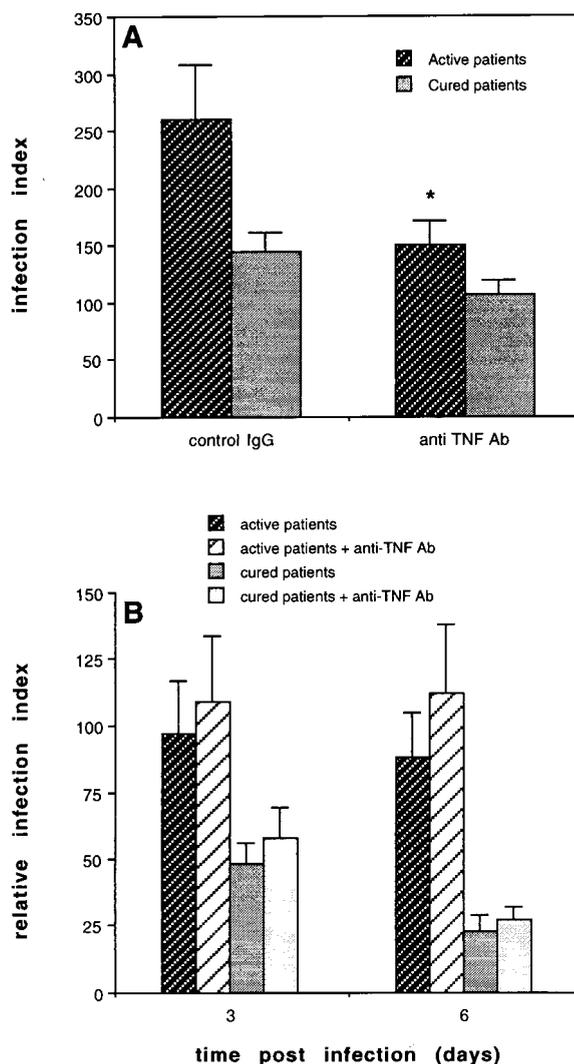

FIG. 3. Effect of anti-TNF Ab on *C. burnetii* killing. Monocytes from active ($n = 5$) and cured ($n = 5$) patients were incubated with *C. burnetii* in the presence of anti-TNF neutralizing Ab or control IgG (10 μg/ml) at day 0 (A), and monocyte infection was monitored for 3 and 6 days (B) at 37°C. Monocyte infection was determined as described for Fig. 1. In panel A, results (means ± SEs) are expressed as an infection index; in panel B, they are expressed as a relative infection index compared to values at day 0. *, $P < 0.02$ (for the comparison of values in the presence of anti-TNF Ab with values in the presence of control IgG).

treatment of monocyte supernatants with anti-TNF Ab abrogated their ability to promote *C. burnetii* survival in monocytes. The effect of TNF on *C. burnetii* survival is distinct from the usual role of TNF in containment of intracellular microorganisms (16). For instance, strains of *M. tuberculosis* recently characterized as having increased virulence elicited higher TNF levels than classical mycobacterial strains, but all of the strains grew in human monocytes at similar rates (19). However, IFN-γ, which stimulates TNF expression in macrophages, causes increased growth of *M. tuberculosis* in human cells (26). The multiplication of virulent strains of *M. tuberculosis* in monocytes has been related to the production of TNF (27). We suggest that the ability of *C. burnetii* to stimulate TNF production may serve primarily to promote pathogenesis rather than protection. On the other hand, TNF most probably affects the early phase of the *C. burnetii* killing process. Indeed, blocking secreted TNF with anti-TNF Ab decreased bacterial uptake by monocytes of patients with ongoing endocarditis, but it did not enable monocytes to kill *C. burnetii*. Thus, it is likely that other factors related to monocyte activation interfere with delayed phases of *C. burnetii* killing. IFN-γ alone is not a good candidate, since its addition to patient monocytes infected with *C. burnetii* did not correct the deficiency in bacterial killing (data not shown).

We found that ongoing Q fever endocarditis is characterized by a deficiency of intracellular killing of *C. burnetii* by monocytes. This defect is largely corrected in cured patients. It is not intrinsic but involves secreted TNF. The secreted TNF primarily affects *C. burnetii* uptake but not delayed steps of intracellular killing. This finding assigns a role in pathogenesis of Q fever endocarditis to TNF and monocyte activation.

REFERENCES

1. **Baca, O. G., and D. Paretsky.** 1983. Q fever and *Coxiella burnetii*: a model for host-parasite interaction. Microbiol. Rev. **47:**127–149.
2. **Bach, E. A., M. Aguet, and R. D. Schreiber.** 1997. The IFNγ receptor: a paradigm for cytokine receptor signaling. Annu. Rev. Immunol. **15:**563–591.
3. **Bläuer, F., P. Groscurth, M. Schneemann, G. Schoedon, and A. Schaffner.** 1995. Modulation of the antilisterial activity of human blood-derived macrophages by activating and deactivating cytokines. J. Interferon Cyt. Res. **15:**105–114.
4. **Brunet, P., C. Capo, J. Dellacasagrande, X. Thirion, J. L. Mege, and Y. Berland.** 1998. IL-10 synthesis and secretion by peripheral blood mononuclear cells in haemodialysis patients. Nephrol. Dial. Transplant. **13:**1745–1751.
5. **Capo, C., F. Zugun, A. Stein, G. Tardei, H. Lepidi, D. Raoult, and J. L. Mege.** 1996. Upregulation of tumor necrosis factor alpha and interleukin-1β in Q fever endocarditis. Infect. Immun. **64:**1638–1642.
6. **Capo, C., Y. Zaffran, F. Zugun, P. Houpikian, D. Raoult, and J. L. Mege.** 1996. Production of interleukin-10 and transforming growth factor β by peripheral blood mononuclear cells in Q fever endocarditis. Infect. Immun. **64:**4143–4147.
7. **Capo, C., N. Amirayan, E. Ghigo, D. Raoult, and J. L. Mege.** 1999. Circulating cytokine balance and activation markers of leukocytes in Q fever. Clin. Exp. Immunol. **115:**120–123.
8. **Capo, C., F. P. Lindberg, S. Meconi, Y. Zaffran, G. Tardei, E. J. Brown, D. Raoult, and J. L. Mege.** Subversion of monocyte functions by *Coxiella burnetii*: impairment of the cross-talk between αvβ3 integrin and CR3. J. Immunol., in press.
9. **Chaturvedi, S., and S. L. Newman.** 1997. Modulation of the effector function of human macrophages for *Histoplasma capsulatum* by HIV-1. J. Clin. Invest. **6:**1465–1474.
10. **Dellacasagrande, J., C. Capo, D. Raoult, and J. L. Mege.** 1999. IFN-γ-mediated control of *Coxiella burnetii* survival in monocytes. The role of cell apoptosis and TNF. J. Immunol. **162:**2259–2265.
11. **Fournier, P. E., J. P. Casalta, G. Habib, T. Messana, and D. Raoult.** 1996. Modification of the diagnostic criteria proposed by the Duke endocarditis service to permit improved diagnosis of Q fever endocarditis. Am. J. Med. **100:**629–633.
12. **Heinzen, R. A., M. A. Scidmore, D. D. Rockey, and T. Hackstadt.** 1996. Differential interaction with endocytic and exocytic pathways distinguish parasitophorous vacuoles of *Coxiella burnetii* and *Chlamydia trachomatis*. Infect. Immun. **64:**796–809.
13. **Hirsch, C. S., J. J. Ellner, D. G. Russel, and E. A. Rich.** 1992. Complement receptor-mediated uptake and tumor necrosis factor-α-mediated growth inhibition of *Mycobacterium tuberculosis* by human alveolar macrophages. J. Immunol. **152:**743–753.
14. **Izzo, A. A., and B. P. Marmion.** 1993. Variation in interferon-gamma responses to *Coxiella burnetii* antigens with lymphocytes from vaccinated and naturally infected subjects. Clin. Exp. Immunol. **94:**507–915.
15. **Kaplan, G., and G. E. Hancock.** 1989. Macrophages in leprosy, p. 459–468. *In* M. Zembala and G. L. Asherson (ed.), Human monocytes. Academic Press, London, United Kingdom.
16. **Kaufmann, S. H. E.** 1993. Immunity to intracellular bacteria. Annu. Rev. Immunol. **11:**129–163.
17. **Koster, F. T., J. C. Williams, and J. S. Goodwin.** 1985. Cellular immunity in Q fever: specific lymphocyte unresponsiveness in Q fever endocarditis. J. Infect. Dis. **152:**1283–1289.
18. **Koster, F. T., J. C. Williams, and J. S. Goodwin.** 1985. Cellular immunity in Q fever: modulation of responsiveness by a suppressor T cell-monocyte circuit. J. Immunol. **135:**1067–1072.
19. **Manca, C., L. Tsenova, C. E. Barry, A. Bergtold, S. Freeman, P. A. J. Haslett, J. M. Musser, V. H. Freedman, and G. Kaplan.** 1999. *Mycobacterium tuberculosis* CDC 1551 induces a more vigorous response in vivo and in vitro, but is not more virulent than other clinical isolates. J. Immunol. **162:**6740–6746.
20. **Meconi, S., V. Jacomo, P. Boquet, D. Raoult, J. L. Mege, and C. Capo.** 1998. *Coxiella burnetii* reorganization of the actin cytoskeleton in human monocytes. Infect. Immun. **66:**5527–5533.
21. **Raoult, D.** 1990. Host factors in the severity of Q fever. Ann. N.Y. Acad. Sci. **590:**33–38.
22. **Raoult, D.** 1993. Treatment of Q fever. Antimicrob. Agents Chemother. **37:**1733–1736.
23. **Raoult, D., and T. J. Marrie.** 1995. Q fever. Clin. Infect. Dis. **20:**489–496.
24. **Raoult, D., P. Houpikian, H. Tissot Dupont, J. M. Riss, J. Arditi-Djiane, and P. Brouqui.** 1999. Treatment of Q fever endocarditis: comparison of two regimens containing doxycycline and ofloxacin or hydroxychloroquine. Arch. Int. Med. **159:**167–173.
25. **Rook, G. A. W.** 1989. Intracellular killing of microorganisms, p. 291–302. *In* M. Zembala and G. L. Asherson (ed.), Human monocytes. Academic Press, London, United Kingdom.
26. **Rook, G. A. W., and B. R. Bloom.** 1994. Mechanisms of pathogenesis in tuberculosis, p. 485–501. *In* B. R. Bloom (ed.), Tuberculosis. ASM Press, Washington, D.C.
27. **Silver, R. F., Q. Li, and J. J. Ellner.** 1998. Expression of virulence of *Mycobacterium tuberculosis* within human monocytes: virulence correlates with intracellular growth and induction of tumor necrosis factor alpha but not with evasion of lymphocyte-dependent monocyte effector functions. Infect. Immun. **66:**1190–1199.
28. **Zhang, M., J. Gong, Y. Lin, and P. F. Barnes.** 1998. Growth of virulent and avirulent *Mycobacterium tuberculosis* strains in human macrophages. Infect. Immun. **66:**794–799.

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