Interleukin-10 Stimulates *Coxiella burnetii* Replication in Human Monocytes through Tumor Necrosis Factor Down-Modulation: Role in Microbicidal Defect of Q Fever

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Coxiella burnetii, an obligate intracellular bacterium, is the agent of Q fever. The chronic form of the disease is associated with the overproduction of interleukin-10 and deficient *C. burnetii* killing by monocytes. We hypothesized that the replication of *C. burnetii* inside monocytes requires a macrophage-deactivating cytokine such as interleukin-10. In the absence of interleukin-10, *C. burnetii* survived but did not replicate in monocytes. *C. burnetii* replication (measured 15 days) was induced in interleukin-10-treated monocytes. This effect of interleukin-10 is specific since transforming growth factor β 1 had no effect on bacterial replication. *C. burnetii* replication involves the down-modulation of tumor necrosis factor (TNF) release. First, interleukin-10 suppressed *C. burnetii*-stimulated production of TNF. Second, the addition of recombinant TNF to interleukin-10-treated monocytes inhibited bacterial replication. Third, the incubation of infected monocytes with neutralizing anti-TNF antibodies favored *C. burnetii* replication. On the other hand, deficient *C. burnetii* killing by monocytes from patients with chronic Q fever involves interleukin-10. Indeed, *C. burnetii* replication was observed in monocytes from patients with Q fever endocarditis, but not in those from patients with acute Q fever. Bacterial replication was inhibited by neutralizing anti-interleukin-10 antibodies. As monocytes from patients with endocarditis overproduced interleukin-10, the defective bacterial killing is likely related to endogenous interleukin-10. These results suggest that interleukin-10 enables monocytes to support *C. burnetii* replication and to favor the development of chronic Q fever.

Q fever is caused by *Coxiella burnetii*, an obligate intracellular bacterium. The disease is commonly divided into acute and chronic forms (28). Acute Q fever manifestations are associated with inflammatory and protective immune responses, both indicated by the presence of granuloma (34). Nevertheless, cell-mediated immunity could not lead to the complete eradication of *C. burnetii* from an infected host (28). The chronic form of Q fever, mainly as endocarditis, sets in several months to years after acute infection, essentially in patients with valvulopathy and, to a lesser extent, in immunocompromised patients (28, 33). Chronic Q fever seems to result from an inefficient protective response to *C. burnetii*, as demonstrated by the lack of granuloma, the failure of *C. burnetii* to induce lymphoproliferation, and the presence of high levels of antibodies (Abs) to *C. burnetii* (23). The deficiency in specific cell-mediated immune response has been associated with the suppressive activity of monocytes and macrophages, *in vivo* targets of *C. burnetii* (26). It has been shown that interleukin-10 (IL-10) and transforming growth factor β (TGF- β), two immunoregulatory cytokines, are released by monocytes from patients with Q fever endocarditis and that IL-10 is specifically associated with the occurrence of relapses (6). We and a colleague have also demonstrated that monocytes from patients with Q fever endocarditis exhibit defective killing of *C. burnetii* (10).

Immunoregulatory cytokines such as IL-10 and TGF- β are involved in increased susceptibility to intracellular pathogens

(25). The administration of anti-IL-10 Abs to mice increases their resistance to *Mycobacterium avium* challenge (11). Transgenic mice with IL-10-secreting T cells are unable to clear the infection with Calmette-Guerin bacillus (29). These cytokines may also account for the severity and/or the reactivation of some infectious diseases. Hence, transcripts for IL-10 are associated with lepromatous leprosy (40). The administration of TGF- β 1 exacerbates the progression of experimental pulmonary tuberculosis in guinea pigs (8). In murine tuberculosis, the latency and the control of mycobacterial growth are associated with the production of type 1 cytokines by T cells whereas reactivation results in a shift to IL-10-producing cells (21).

Many mechanisms are used by immunoregulatory cytokines to allow bacterial survival, such as interference with T-cell-mediated adaptative immune responses (12, 27) and/or down-modulation of the microbicidal functions of monocytes/macrophages (12). Indeed, IL-10 down-modulates the anti-*M. avium* activity of human monocytes and murine macrophages (3) and enables *M. avium* survival by preventing the apoptosis of infected human macrophages (2). The treatment of monocytes with TGF- β accelerates the intracellular replication of *Mycobacterium tuberculosis* (19). The specificity or the redundancy of immunoregulatory cytokines is also influenced by the nature of their targets. IL-10 favors the growth of *M. avium* in murine macrophages but not in human monocytes (36). In the murine model, the inhibition of reactive nitrogen intermediate generation by immunoregulatory cytokines clearly decreases the resistance to infections by intracellular pathogens, but the results are more ambiguous in human cells (14).

Because the overproduction of IL-10 (6) and the defect in *C. burnetii* killing by monocytes (10) are critically associated

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with chronic Q fever, we investigated the role of IL-10 in *C. burnetii* replication. We found that IL-10 stimulated the replication of *C. burnetii* through the inhibition of tumor necrosis factor (TNF) production. On the other hand, monocytes from patients with chronic Q fever allowed *C. burnetii* replication but those from patients with acute Q fever did not. IL-10 neutralization by specific Abs inhibited *C. burnetii* replication in monocytes from patients with chronic Q fever, suggesting that IL-10 plays a major role in Q fever pathophysiology.

MATERIALS AND METHODS

Cells. Blood from healthy volunteers was collected into EDTA anticoagulant tubes. Monocytes were isolated from peripheral blood mononuclear cells by adherence on glass slides (Lab-Tek chamber; Nalge Nunc Int., Naperville, Ill.) in RPMI 1640 medium containing 25 mM HEPES, 10% fetal calf serum, and 2 mM L-glutamine (Life Technologies, Eragry, France). More than 90% of adherent cells were monocytes (7). Cells were then cultured for 15 days at 37°C in RPMI 1640 supplemented with 10% human AB serum (Sigma Chemical Co., St. Louis, Mo.). L929 cells and HEL cells, provided by the European Collection of Animal Cell Cultures (Sophia Antipolis, France), were cultured in RPMI 1640 and in minimum essential medium containing 10% fetal calf serum and 2 mM L-glutamine, respectively. All culture media were treated with polysulfone filters (10) and checked for the absence of endotoxins by *Limulus* amoebocyte lysate assay (BioWhittaker, Emerainville, France). In some experiments, we studied monocytes from Q fever patients. Informed consent was obtained for each individual, and the study was approved by the Ethics Committee of the Université de la Méditerranée (Marseille, France). The study included 10 patients with ongoing Q fever endocarditis, consisting of 7 men and 3 women (mean age, 43 years; range, 22 to 64 years) and of 8 patients with acute Q fever (6 men and 2 women; mean age, 39 years; range, 32 to 63 years). The diagnosis of Q fever endocarditis was based on modified Duke University criteria, i.e., pathological evidence of endocarditis, positive echocardiogram, positive blood culture, and high titers of immunoglobulin G (IgG) (mean, 10,000; range, 1,600 to 51,200) directed against phase I *C. burnetii* (15). All these patients had been subjected to valve replacement and medical treatment consisting of doxycycline and chloroquine. Acute Q fever was diagnosed by detection of IgM (mean, 200; range, 100 to 800) and IgG (mean, 1,000; range, 200 to 1,600) specific for phase II *C. burnetii* (29). These patients were in the early phase (between 1 and 3 months after onset) of the disease, as demonstrated by the high level of specific IgM.

Bacteria. BALB/c mice were injected intraperitoneally with 10^8 *C. burnetii* organisms (Nine Mile strain), as previously described (5). One week later, mice were killed and their spleens were homogenized. Spleen homogenates were added to L929 cell monolayers, and cultures were maintained for five passages. Infected L929 cells were then detached with glass beads and sonicated. Sonicates were spun down at $300 \times g$ for 10 min to remove unbroken cells, and supernatants containing bacteria were centrifuged at $8,000 \times g$ for 10 min. 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. The concentration of *C. burnetii* was determined by Gimenez staining. *C. burnetii* organisms were aliquoted at 10^9 organisms/ml and stored at -80°C .

Infection procedure. Monocytes at 5×10^4 cells/ml were pretreated with various doses of human recombinant IL-10 (rIL-10) or rTGF- β 1 (R&D Systems, Abingdon, United Kingdom) for 24 h at 37°C and then incubated with *C. burnetii* at a bacterium-to-cell ratio of 200:1 for 24 h at 37°C. Cells were then washed to remove free bacteria; this time was designated day 0. Infected cells were again cultured with newly added cytokines for 15 days. In some experiments, human rTNF or neutralizing anti-TNF or anti-IL-10 goat Abs (R&D Systems) were included in monocyte cultures. Infection was quantified by indirect immunofluorescence (5). Monocytes were fixed with 1% formaldehyde and permeabilized by 0.1 mg of lysophosphatidylcholine (Sigma)/ml. Cells were then incubated with rabbit anti-*C. burnetii* serum at a 1/250 dilution in phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin (Sigma) for 30 min. Fluorescein isothiocyanate-conjugated F(ab')₂ anti-rabbit Ab (Immunotech, Marseille, France) was added to monocytes, which were counterstained with Evans blue. Immunofluorescence results were expressed as an infection index, which is the product of the mean number of bacteria per infected cell and the percentage of infected cells $\times 100$ (10).

Bacterial and cellular viability. Monocytes, treated or not with cytokines and subsequently infected with *C. burnetii*, were briefly sonicated, and the homogenates were added to HEL cell monolayers in shell vials (Sterilin, Feltham, United

Kingdom) as previously described (9). After 10 days at 37°C, the culture medium was removed and cells were fixed with methanol. Vacuoles containing *C. burnetii* were revealed by indirect immunofluorescence. Results were expressed as the number of fluorescent vacuoles per shell vial.

The number of adherent monocytes was assessed as described elsewhere (30). Briefly, monocytes were washed with PBS to remove dead cells and 0.2 ml of naphthol blue black (Sigma) was added to each well and incubated for 15 min at room temperature. The number of monocyte nuclei was determined.

Cytokine determination. (i) **Immunoassays.** Adherent monocytes (10^5 cells/assay) were treated with 5 ng of IL-10 or TGF- β 1/ml for 24 h and then stimulated by *C. burnetii* at a bacterium-to-cell ratio of 200:1 in flat-bottom 48-well plates for 24 h. Supernatants were assayed for the presence of TNF, IL-10, IL-1 receptor antagonist (IL-1ra), and TNF-RII. Cytokines were measured by enzyme-linked immunosorbent assay (ELISA) for TNF, IL-10 (Immunotech), IL-1ra, and TNF-RII (R&D Systems), as described by the manufacturers. The limits of detection were 1 pg/ml for TNF and TNF-RII, 4 pg/ml for IL-10, and 15 pg/ml for IL-1ra. The intra- and interassay coefficients of variation of the ELISA kits ranged between 5 and 10%.

(ii) **RNA extraction and PCR amplification.** Monocytes (5×10^5 cells/assay) were treated with cytokines for 24 h, and they were stimulated with *C. burnetii* (bacterium-to-cell ratio, 200:1) for 4 h at 37°C. RNA was extracted using the Trizol method (Life Technologies), and 1 μ g was incubated with reverse transcriptase (Superscript; Life Technologies) for 1 h at 37°C. The reaction was stopped by heat inactivation (10 min, 95°C), thereby permitting cDNA generation. cDNA was added to a mixture containing *Taq* polymerase and specific primers for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or TNF (7). The mixtures were subjected to 24 cycles of denaturation, annealing (60°C for GAPDH; 65°C for TNF), and extension. PCR products were electrophoresed through 2% agarose gel containing ethidium bromide. TNF transcripts were also quantified with the CytoXpress detection kit (BioSource, Fleurus, Belgium) as previously described (10). Results were expressed as the number of TNF cDNA copies/ μ g of total RNA.

Statistical analysis. Results were expressed as the means $\pm$ the standard errors (SE) and were compared using analysis of variance. Differences were significant at $P < 0.05$.

RESULTS

IL-10 induces the replication of *C. burnetii* in monocytes. Monocytes were pretreated for 24 h with IL-10 at 5 ng/ml, the concentration that inhibited more than 80% of TNF release by lipopolysaccharide-stimulated monocytes (data not shown). Then, monocytes were infected with *C. burnetii* at a bacterium-to-cell ratio of 200:1 for 24 h (day 0), leading to substantial and reproducible infection of monocytes (10). In the absence of IL-10, 75% of cells were infected by about two bacteria per cell (Fig. 1A). Infection slowly decreased from day 0 to day 6 (40% inhibition) and then steadily increased; it reached the initial value after 15 days. In IL-10-treated monocytes, the infection index at day 0 was significantly higher than in untreated cells ($P < 0.007$). It increased from day 0 to day 6 and reached a plateau between day 9 and day 15: almost all monocytes were infected with about six bacteria per cell. The differences between IL-10-treated monocytes and control monocytes were significant ($P < 0.002$) for each incubation time. The increase in *C. burnetii* infection of monocytes depended on IL-10 doses. It was observed with 1.25 ng of IL-10/ml and reached a plateau with 5 to 10 ng of IL-10/ml (Fig. 1B).

The increase in the infection index resulted from *C. burnetii* replication. Bacterial viability was assessed by measuring *C. burnetii*-containing vacuoles in HEL cells. In control monocytes, the number of vacuoles per shell vial decreased up to 6 days and then increased; it reached a value similar to the initial value after 12 days (Fig. 1C). In IL-10-treated monocytes, the number of vacuoles at day 0 was higher than in control cells; it was markedly enhanced after 6 days and remained high after

FIG. 1. Effect of IL-10 on *C. burnetii* replication. (A) Monocytes were pretreated with IL-10 or TGF- β 1 (5 ng/ml) for 24 h and then infected with *C. burnetii* at a bacterium-to-cell ratio of 200:1 for 24 h (designated day 0). Bacteria were revealed with rabbit immune serum and fluorescein isothiocyanate-conjugated anti-rabbit F(ab')₂ Ab. The infection index was measured every 3 days; the results are expressed as the mean $\pm$ SE of five experiments. (B) Monocytes were pretreated with different doses of IL-10 and infected with *C. burnetii* as described above. The infection index was measured after 12 days; the results are expressed as the mean $\pm$ SE of three experiments. (C) Infected monocytes were sonicated and dilutions of homogenates containing bacteria were added to HEL cell monolayers. Bacteria were revealed by indirect immunofluorescence. Results are expressed as the mean number $\pm$ SE of fluorescent vacuoles per shell vial and represent three experiments conducted in triplicate.

12 days. Because the measurements of infection index and bacterial viability were correlated, only the determination of infection index was used in subsequent experiments.

The replication of *C. burnetii* mediated by IL-10 was not affected by the magnitude of bacterial uptake. First, monocytes were incubated with *C. burnetii* at a bacterium-to-cell ratio of

200:1 for 24 h and treated with IL-10 (at 5 ng/ml) and then the infection index was assessed (Fig. 2A). IL-10 elicited an increase (3.5 times) in infection index, which was maximum after 6 days, as for IL-10-pretreated monocytes. Second, IL-10-pretreated monocytes were incubated with *C. burnetii* at different bacterium-to-cell ratios and then treated with IL-10 for 12 days (Fig. 2B). The effect of IL-10 was observed in monocytes infected with *C. burnetii* at a bacterium-to-cell ratio of 100:1 and became maximum for bacterium-to-cell ratios of 200:1 and 400:1. IL-10 had to be added after infection to act on *C. burnetii* replication, since its removal after 24 h diminished bacterial replication by 40%. In all subsequent experiments, cytokines were added to the culture medium after monocyte infection. These results show that *C. burnetii* survives in resting monocytes and that IL-10 induces bacterial replication.

TGF- β 1 is not active on *C. burnetii* replication. Because TGF- β 1 has been reported to depress the microbicidal activity of monocytes and macrophages as IL-10 does (37), we wondered if it favors the replication of *C. burnetii* in monocytes. TGF- β 1 (at 5 ng/ml) did not affect the infection index at day 0 and did not stimulate *C. burnetii* replication (Fig. 1A). However, TGF- β prevented the transient decrease in bacterial number and viability observed after 6 days of monocyte cul-

FIG. 2. Effect of *C. burnetii* uptake on bacterial replication. (A) Monocytes were infected with *C. burnetii* at a bacterium-to-cell ratio of 200:1 for 24 h (designated day 0) and then treated with IL-10 at 5 ng/ml. Infection was determined as described in the Fig. 1A legend; the results are the mean $\pm$ SE of four experiments. (B) IL-10-pretreated monocytes were infected with *C. burnetii* at different bacterium-to-cell ratios for 24 h. The infection index was determined after 12 days; the results represent the mean $\pm$ SE of three experiments.

TABLE 1. Effect of cytokine combinations on *C. burnetii* replication

Cytokine pretreatment ^a	Infection index (mean $\pm$ SE) ^b
None.....	140 $\pm$ 25
IL-10.....	543 $\pm$ 55
TGF- β 1.....	119 $\pm$ 23
IL-10 + TGF- β 1.....	781 $\pm$ 60

^a Monocytes were pretreated with IL-10, TGF- β 1 (5 ng/ml), or their combination for 24 h and incubated with *C. burnetii* at a bacterium-to-cell ratio of 200:1 for 24 h.

^b The infection index was determined at day 12. Results are expressed as the mean $\pm$ SE of three experiments.

ture. Raising the TGF- β 1 doses to 10 ng/ml did not affect *C. burnetii* replication (data not shown). In another series of experiments, we tested the combination of IL-10 and TGF- β 1 on *C. burnetii* replication (Table 1). The combination of IL-10 and TGF- β 1 was more efficient than IL-10 alone in stimulating *C. burnetii* replication. The synergistic effect of IL-10 and TGF- β 1 on *C. burnetii* replication was not related to increased bacterial uptake at day 0 since their addition to infected monocytes induced the same synergistic effect. Taken together, these results show that IL-10 acts on *C. burnetii* replication through a specific mechanism.

IL-10 down-modulates TNF production in *C. burnetii*-infected cells. As IL-10 is known to depress the production of inflammatory cytokines such as TNF in monocytes (12), we investigated the effect of IL-10 on *C. burnetii*-stimulated production of TNF. TNF transcripts were just detectable in unstimulated monocytes and were absent in cells treated with IL-10 or TGF- β 1 used as control (Fig. 3A). *C. burnetii* stimulated the transcription of the TNF gene (Fig. 3B). IL-10 largely down-modulated it whereas TGF- β 1 had no effect. These results were confirmed by a quantitative approach. IL-10 decreased the amounts of TNF mRNA by 85% $\pm$ 10% (2,300 $\pm$ 220 copies per ng of RNA in untreated cells versus 353 $\pm$ 75 copies per ng of RNA in IL-10-treated monocytes), whereas the inhibition induced by TGF- β 1 did not exceed 20% (1,888 $\pm$ 250 copies per ng of RNA). The release of immunoreactive TNF by monocytes was also assessed (Fig. 3C). *C. burnetii* elicited maximum TNF release after 8 h; it steadily decreased to the value of unstimulated cells after 48 h. IL-10 completely prevented the release of TNF as early as after 8 h of incubation. In contrast, TGF- β 1 inhibited TNF release by only 25% $\pm$ 7% after 8 h, when TNF was no longer detectable in the presence of IL-10 (Fig. 3C). After 24 h of incubation with *C. burnetii*, the TNF release was depressed by TGF- β 1 by 41% $\pm$ 5%. In another set of experiments, monocytes were washed 24 h after *C. burnetii* stimulation to remove secreted cytokines and were incubated in new culture medium for 48 h. TNF release steadily increased within 48 h but remained lower than that observed without washing. In monocytes pretreated with regulatory cytokines, the recovery of TNF release was lower in IL-10-treated monocytes than in TGF- β 1-treated monocytes (data not shown). These results suggest that IL-10 is more potent than TGF- β 1 at down-modulating TNF production.

IL-10 upregulates the release of TNF-RII. Because immunoregulatory cytokines may affect monocyte functions through the upregulation of IL-1ra and/or soluble receptors for cytokines (13), we studied their production. Monocytes were pre-

treated with IL-10 or TGF- β 1 and stimulated with *C. burnetii*, and the release of IL-1ra and TNF-RII was assessed. The release of IL-1ra was increased in response to *C. burnetii* (Fig. 4A). Pretreatment of monocytes with IL-10 or TGF- β 1 did not affect *C. burnetii*-stimulated IL-1ra release. In contrast, the release of TNF-RII was differently affected by IL-10 and TGF- β 1. In the absence of regulatory cytokines, *C. burnetii* elicited a steady increase in TNF-RII release within 48 h of incubation (Fig. 4B), confirming recent results (16). IL-10 amplified *C. burnetii*-stimulated release of TNF-RII at 0 and 8 h postinfection ($P < 0.05$). TGF- β 1 did not affect TNF-RII release (Fig. 4B). Clearly, IL-10 upregulated the release of TNF-RII.

TNF is involved in the IL-10-mediated increase in *C. burnetii* replication. To correlate the increase in *C. burnetii* replication and the inhibition of TNF production, we used two approaches. First, monocytes were infected with *C. burnetii* in the presence of neutralizing Abs directed to TNF for 12 days. The anti-TNF Abs were active, since they neutralized TNF present

FIG. 3. Effect of IL-10 and TGF- β 1 on TNF production. (A and B) Monocytes were pretreated with IL-10 or TGF- β 1 (5 ng/ml) and then incubated in the absence (A) or the presence (B) of *C. burnetii* (bacterium-to-cell ratio, 200:1) for 4 h. Total RNA was extracted and transcribed in cDNA. After amplification, PCR products were analyzed by agarose gel electrophoresis and ethidium bromide staining. The figure is representative of three experiments. (C) Monocytes were pretreated with IL-10 or TGF- β 1 for 24 h and then infected with *C. burnetii*. Supernatants were assayed for the presence of TNF by ELISA after different times. The results are expressed as the mean $\pm$ SE of three experiments.

FIG. 4. Effect of immunoregulatory cytokines on the production of IL-1ra and TNF-RII. Monocytes were pretreated with IL-10 or TGF-β1 (5 ng/ml) for 24 h and then stimulated with *C. burnetii* (bacterium-to-cell ratio, 200:1) for 24 h. Supernatants were assayed for the presence of IL-1ra (A) and soluble TNF-RII (B) by ELISA after different times. The results are expressed as the mean $\pm$ SE of three experiments.

in supernatants from *C. burnetii*-stimulated monocytes on cytotoxic bioassay (data not shown). Anti-TNF Abs increased the replication of *C. burnetii* in a dose-dependent manner (Fig. 5A). Bacterial replication was measurable in the presence of 5 μ g of Abs/ml and it became maximum with 10 μ g of Abs/ml. Note that *C. burnetii* replication remained lower than that induced by IL-10. Second, IL-10-pretreated monocytes were infected with *C. burnetii* in the presence of rTNF for 12 days. As shown in Fig. 5B, IL-10 increased monocyte infection 3.5-fold and the addition of rTNF inhibited the effect of IL-10 in a dose-dependent manner. TNF at 500 pg/ml significantly ($P < 0.02$) inhibited the IL-10 effect; its inhibitory effect was maximum with 2,000 pg of TNF/ml ($P < 0.005$), leading to an infection index similar to that of untreated cells. These results show that the modulation of TNF is clearly involved in the replication of *C. burnetii* mediated by IL-10.

Monocyte defect of *C. burnetii* killing in Q fever involves IL-10. We and a colleague recently showed that monocytes from patients with ongoing Q fever endocarditis are unable to kill *C. burnetii* (10) and produce more IL-10 than do those from healthy controls (6). As IL-10 stimulates *C. burnetii* replication, we wondered whether IL-10 is responsible for the defective microbicidal killing of patient monocytes. Of the 18 patients with Q fever investigated, 10 exhibited ongoing endocarditis according to clinical and serological criteria and 8 had acute Q fever. First, we assessed IL-10 release by monocytes (Table 2). It was low in unstimulated monocytes from healthy donors and increased in patients with acute Q fever. IL-10 was

FIG. 5. Effect of anti-TNF Abs and exogenous TNF on monocyte infection. (A) Monocytes were infected with *C. burnetii* (bacterium-to-cell ratio, 200:1) and cultured for 12 days in the presence of neutralizing anti-TNF Abs. (B) Monocytes were treated with IL-10 for 24 h, incubated with *C. burnetii* for 24 h, and cultured for 12 days in the presence of TNF. The infection index at day 12 was expressed relative to day 0. Results are the mean $\pm$ SE of four experiments.

significantly ($P < 0.03$) higher in patients with Q fever endocarditis than in patients with acute Q fever. In response to *C. burnetii*, the release of IL-10 was similar in healthy donors and in patients with acute Q fever. In contrast, release was increased in patients with Q fever endocarditis. Second, monocytes were incubated with *C. burnetii* in the presence or the absence of neutralizing anti-IL-10 Abs, and monocyte infection was monitored for 12 days. In patients with acute Q fever, the infection index steadily decreased from day 0 to day 6 and slightly increased at day 12 (Fig. 6A). This pattern was similar to that of infected cells from controls (see Fig. 1A). In patients with Q fever endocarditis, the infection index at day 6 and day 12 was significantly higher than that at day 0 ($P < 0.01$ and $P <$

TABLE 2. IL-10 secretion by monocytes from Q fever patients^a

Subjects	IL-10 secretion (pg/ml) with:	
	Unstimulated cells	<i>C. burnetii</i> -stimulated cells
Healthy individuals	47 $\pm$ 32	699 $\pm$ 194
Patients with acute Q fever	250 $\pm$ 164	574 $\pm$ 362
Patients with Q fever endocarditis	1,054 $\pm$ 219 ^b	1,552 $\pm$ 222 ^b

^a Monocytes from 12 controls, 8 patients with acute Q fever, and 10 patients with ongoing Q fever endocarditis were incubated with *C. burnetii* at a bacterium-to-cell ratio of 200:1 for 24 h. Supernatants were assayed by ELISA for the presence of IL-10.

^b P of < 0.03 represents the comparison between patients with acute Q fever and patients with Q fever endocarditis.

FIG. 6. Effect of IL-10 on *C. burnetii* replication in Q fever endocarditis. (A) Monocytes from patients with acute Q fever or ongoing Q fever endocarditis were incubated with *C. burnetii* at a bacterium-to-cell ratio of 200:1, and the infection index was assessed at days 0, 6, and 12 as described in the legend to Fig. 1A. (B and C) Infected monocytes from patients with acute Q fever (B) or from patients with Q fever endocarditis (C) were cultured in the presence of neutralizing anti-IL-10 Abs or control serum (10 μ g/ml), and the infection index was determined after 6 and 12 days relative to day 0.

0.002, respectively). When infected monocytes from patients with acute Q fever were cultured with anti-IL-10 Abs (at 10 μ g/ml), the infection index at day 6 and day 12 remained unchanged compared to that of untreated cells (Fig. 6B). In contrast, when anti-IL-10 Abs were added to infected monocytes from patients with Q fever endocarditis, the infection index was significantly depressed at day 6 ($P < 0.008$) and at day 12 ($P < 0.001$) (Fig. 6C). Thus, the neutralization of IL-10 enabled monocytes from patients with Q fever endocarditis to restrict *C. burnetii* replication.

DISCUSSION

We show here that exogenous IL-10 promotes *C. burnetii* replication in human monocytes in a specific way since TGF-

β 1, another potent immunoregulatory cytokine, had no effect on bacterial replication. As *C. burnetii* organisms do not replicate in resting monocytes, our results provide new insights into mechanisms of microbicidal defect in Q fever endocarditis. An IL-10-mediated increase in *C. burnetii* uptake is consistent with the ability of IL-10 to increase the expression of monocyte receptors involved in the phagocytosis of microorganisms (12). Nevertheless, the increased bacterial uptake induced by IL-10 was not required for *C. burnetii* to replicate. Indeed, varying the magnitude of *C. burnetii* uptake (by varying the bacterium-to-cell ratio) did not affect bacterial replication. In addition, IL-10 induced similar bacterial replication when it was added to monocytes before or after *C. burnetii* infection. This may be related to the ability of IL-10 added to monocytes before or after infection to enhance the multiplication of *Legionella pneumophila* (32). On the other hand, the experiments combining IL-10 and TGF- β 1 show that they markedly amplified the replication of *C. burnetii*, confirming the previously reported synergism of IL-10 and TGF- β 1 in the prevention of microbicidal activation of macrophages (31).

The effect of IL-10 on *C. burnetii* replication is likely related to its ability to disarm monocytes. First, IL-10 is known to inhibit the production of reactive oxygen intermediates and reactive nitrogen intermediates (12). However, *C. burnetii* was unable to induce hydrogen peroxide and peroxynitrite in human monocytes (data not shown). Monocytes from chronic granulomatous disease patients, which do not produce reactive oxygen intermediates, do not allow the replication of *C. burnetii* (9). Thus, IL-10 cannot induce the replication of *C. burnetii* in monocytes by interfering with the generation of toxic intermediates. Second, IL-10 has been described as an inducer of IL-1ra, which should be involved in decreased resistance to intracellular organisms (1). Mice lacking endogenous IL-1ra are less susceptible to infection with *Listeria monocytogenes* (20). The increased IL-1ra gene expression leads to reduced survival in primary listerial infection (22). Extrapulmonary tuberculosis is associated with a high production of IL-1ra by monocytes in response to *M. tuberculosis* whereas tuberculous pleurisy is associated with a low production (39). We show here that IL-10 did not affect *C. burnetii*-stimulated production of IL-1ra. This may be related to the lack of IL-10 effect on *M. tuberculosis*-stimulated production of IL-1ra (35). IL-1ra has a weak effect on the intracellular replication of *M. tuberculosis* in vitro and on disease susceptibility (39). Hence, the effect of IL-10 on *C. burnetii* replication does not depend on IL-1ra production by infected monocytes.

Third, IL-10 might down-modulate TNF production in *C. burnetii*-stimulated monocytes. IL-10 prevented *C. burnetii*-stimulated expression of TNF mRNA and suppressed TNF release. IL-10 is potent at suppressing TNF release (24) and transcriptional activation of the TNF gene (38). On the other hand, IL-10 upregulated the release of TNF-RII by *C. burnetii*-infected monocytes. This finding agrees with previous reports that IL-10 increases the release of TNF-RII (24). It may be related to recent reports that chronic Q fever is associated with IL-10 overproduction (6), a defect in *C. burnetii* killing (10), and an increase in TNF-RII release (16). As soluble TNF-RII is known to antagonize the activity of TNF, it is likely that its upregulation amplifies the monocyte deactivation mediated by IL-10. We provide here evidence that TNF modulation ac-

counts for the effect of IL-10 on *C. burnetii* replication. Indeed, the addition of exogenous TNF to IL-10-treated monocytes inhibited *C. burnetii* replication. This differs from *L. pneumophila* replication, for which the addition of TNF had no effect on bacterial replication (32).

The notion that IL-10 is required for *C. burnetii* replication in monocytes provides new insights into the pathophysiology of Q fever. Indeed, monocytes from patients with ongoing Q fever endocarditis are unable to kill *C. burnetii*, contrary to those from healthy subjects (10) and patients with acute Q fever. They also overproduced IL-10 spontaneously and in response to *C. burnetii* whereas monocytes from patients with acute Q fever were low producers of IL-10. Incubating monocytes from patients with Q fever endocarditis with *C. burnetii* in the presence of anti-IL-10 Abs inhibited the replication of *C. burnetii*. In contrast, neutralizing IL-10 Abs did not interfere with *C. burnetii* survival by monocytes from patients with acute Q fever. This finding is distinct from the overproduction of IL-10 and TGF- β in tuberculosis, in which only neutralizing anti-TGF- β Abs reduce the intracellular growth of *M. tuberculosis* (19) and increase lymphocyte responses (18). On the other hand, in human immunodeficiency virus infection, which increases the risk of tuberculosis reactivation, the down-modulation of gamma interferon production induced by *M. tuberculosis* is partly corrected by neutralizing anti-IL-10 Abs (17). Recently, it has been reported that IL-10 plays an important role in the anergy of patients with pulmonary tuberculosis, which may result in sustained survival of *M. tuberculosis* (4). Hence, IL-10 produced by monocytes from patients with Q fever endocarditis in an autocrine manner may contribute to the impairment of their microbicidal functions and allow *C. burnetii* replication. Such a mechanism may account for chronic Q fever relapses, which are associated with sustained high levels of IL-10 (6).

In this report, we show that IL-10 stimulates the replication of *C. burnetii* in human monocytes. Its effect is associated with the suppression of TNF production. IL-10 may provide a sustained deactivation of monocytes by interfering with the expression of TNF transcripts and by upregulating TNF-RII release. Moreover, IL-10 is involved in the defective killing of *C. burnetii* by monocytes in Q fever endocarditis. It is likely that IL-10 produced by monocytes during *C. burnetii* infection allows the replication of *C. burnetii* via an autocrine loop involving TNF and causes a chronic outcome of the disease.

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