Rickettsia-Macrophage Interactions: Host Cell Responses to Rickettsia akari and Rickettsia typhi

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The existence of intracellular rickettsiae requires entry, survival, and replication in the eukaryotic host cells and exit to initiate new infection. While endothelial cells are the preferred target cells for most pathogenic rickettsiae, infection of monocytes/macrophages may also contribute to the establishment of rickettsial infection and resulting pathogenesis. We initiated studies to characterize macrophage-Rickettsia akari and -Rickettsia typhi interactions and to determine how rickettsiae survive within phagocytic cells. Flow cytometry, microscopic analysis, and LDH release demonstrated that R. akari and R. typhi caused negligible cytotoxicity in mouse peritoneal macrophages as well as in macrophage-like cell line, P388D1. Host cells responded to rickettsial infection with increased secretion of proinflammatory cytokines such as interleukin-1β (IL-1β) and IL-6. Furthermore, macrophage infection with R. akari and R. typhi resulted in differential synthesis and expression of IL-β and IL-6, which may correlate with the existence of biological differences among these two closely related bacteria. In contrast, levels of gamma interferon (IFN-γ), IL-10, and IL-12 in supernatants of infected P388D1 cells and mouse peritoneal macrophages did not change significantly during the course of infection and remained below the enzyme-linked immunosorbent assay cytokine detection limits. In addition, differential expression of cytokines was observed between R. akari- and R. typhi-infected macrophages, which may correlate with the biological differences among these closely related bacteria.

As an obligate intracellular pathogen, rickettsial survival is dependent upon entry into, growth, and replication within the eukaryotic host cell cytoplasm and then exit to initiate a new infection cycle (1). Generally, the outcome of infection is the host cell lysis and the release of rickettsial progeny. While the eukaryotic endothelial cells are the preferred target cells for most pathogenic rickettsial species, infection of monocytes/macrophages has also been observed (3, 24, 25). Rickettsial-host cell interaction is complex, and we are just beginning to understand the molecular mechanisms by which rickettsiae initiate infection and cause pathology. Rickettsial infection seldom results in complete shutdown of the host machinery, and in most cases, vigorous host responses to infection clear the rickettsial pathogens. The host’s immune responses to the rickettsial infection lead either to clearance of the pathogen or to the persistence of a subclinical infection. As an example of the latter, Rickettsia prowazekii, the causative agent of louse-borne typhus, can persist in humans years after primary infection and/or appropriate antibiotic treatment (22). Persistence of the rickettsiae in otherwise healthy persons with subsequent reappearance of clinical symptoms (Brill-Zinsser disease) is one mechanism by which rickettsiae perpetuate in the absence of an animal reservoir. How rickettsiae manage to survive in the host cells is via the suppression of the antimicrobial activities of eukaryotic target cells, specifically monocytes/macrophages (27).

This study was initiated to characterize macrophage-rickettsia interactions and to determine how rickettsiae survive within macrophages. Rickettsia akari, the causative agent of rickettsialpox, was used, since it infects mammalian monocytes/macrophages and thus differs significantly from other members of the spotted fever group rickettsiae and particularly Rickettsia rickettsii, the etiologic agent of Rocky Mountain spotted fever. In addition, for comparative analysis, a typhus group rickettsia, R. typhi, capable of surviving in mouse peritoneal macrophages (3) was included in this study. Here we report that R. akari and R. typhi infect the P388D1 cell line as well as mouse peritoneal macrophages. Furthermore, macrophage infection with these rickettsiae resulted in differential cytokine synthesis and expression.

MATERIALS AND METHODS

Rickettsial strains and culture conditions. In this study, we used R. akari (Kaplan) and R. typhi (Wilmington) propagated and maintained in African green monkey kidney cells (Vero) at respective concentrations of 10⁶ and 10⁵ PFU/ml, as previously described (11). After 4 days postinfection, cells were assayed for the presence of rickettsiae by indirect fluorescent antibody assay (IFA) (14, 17) with mouse monoclonal antibodies against the R. akari and R. typhi outer membrane proteins, rOmp (gifts from Centers for Disease Control and Prevention and David H. Walker, UTMB, Galveston, Tex.). In vitro viability of Rickettsia was determined with tissue culture plaque assays (15).

Isolation and culture of macrophages. The macrophage-like cell line P388D1 (10⁶ per ml) was seeded into flat-bottom 96-well culture plates and cultured for 24 h in RPMI 1640 medium (Gibco BRL, Bethesda, Md.) supplemented with 10% fetal bovine serum (FBS). P388D1 cells were infected with R. akari or R. typhi at a multiplicity of infection (MOI) of 50 rickettsiae/cell. The culture medium was changed daily, and cells were then harvested, 24, 48, and 96 h postinfection and analyzed by fluorescence-activated cell sorter (FACS) analysis to measure Rickettsia-induced apoptosis and necrosis. Controls included untreated P388D1 cells as well as 96-h-infected cells that have been treated with Escherichia coli lipopolysaccharide (LPS) (0.5 mg/ml). Peritoneal macrophages were harvested from 3-week-old female C3H/HeN-
CellBr mice purchased from Charles River Laboratories (Wilmington, Mass.). Mice were inoculated intraperitoneally with 10 ml of sterile ice-cold serum-free RPMI medium supplemented with 0.2 mM EDTA (RPMI-EDTA). The abdomen was gently agitated, and the medium was withdrawn. Harvested cells were washed three times (50 × g, 30 min, 4°C) with serum-free RPMI-EDTA supplemented with addition of 100 μg of gentamicin (Gibco BRL) per ml and resuspended to 10⁷ cells/ml in RPMI 1640 supplemented with 10% FBS. The cells were cultured for 2 h at 37°C and washed gently to remove nonadherent cells. Afterward, the medium was replaced with RPMI supplemented with sodium pyruvate (1 mM), gentamicin (100 μg/ml), and 15% FBS. After overnight incubation, the cell medium was discarded and replaced with gentamicin-free RPMI and 10% FBS. The macrophages were then infected with R. akari and R. typhi. Untreated cells and LPS-treated and infected cells were also included in this study. Cells were harvested 24, 48, and 96 h postinfection and analyzed by FACS.

Flow cytometry. Flow cytometry was performed with a FACS-sort flow cytometer having both argon and helium/neon lasers and with CellQuest software on a Macintosh 4x computer (Becton Dickinson, Sunnyvale, Calif.). Analysis of phosphodiesterase on the outer membrane of P388D1 apoptotic cells was performed with the Annexin-V-Fluos staining kit (Roche). Briefly, 10⁶ infected cells per ml (untreated and treated) were washed with phosphate-buffered saline and centrifuged at 200 × g for 5 min. The pellet was resuspended in 100 μl of staining solution (20 μl of Annexin-V-fluorescein and 20 μl of propidium iodide (PI) diluted in 1,000 μl of HEPES buffer) incubated at room temperature for 15 min and analyzed by FACS. We used a 488-nm excitation and a 515-nm band pass filter for fluorescein detection and a >600-nm-size filter for PI detection. Positive controls for apoptosis included P388D1 cells incubated for 4 h at 37°C in the presence or absence of 4 mg of camptothecin per ml (Sigma) or anti-Fas monoclonal antibody (CD95/APO-1; Boehringer Mannheim) at a concentration of 1 μg/ml.

LDH assays. A colorimetric assay (Roche) was used to quantitate cell death and cell lysis. This assay is based upon quantification of lactate dehydrogenase (LDH) released from the cytosol of damaged cells. Supernatants from uninfected control and infected cells were collected in tubes and spun for 5 min at 12,000 rpm. Fifty-microliter portions of cleared supernatants were then used for the LDH assay. The assay was performed at room temperature according to the manufacturer’s instructions (16). To quantitate cytotoxicity, we calculated the average A₅₀ of triplicate samples and compared the values to those of background controls (cells incubated with camptothecin).

ELISA-based cytokine detection assays. Macrophages were infected with R. akari or R. typhi at an MOI of 50 rickettsiae/cell. The rickettsiae were allowed to adhere to cells for 1 h at room temperature and maintained at 34°C in 5% CO₂. Afterward, the cell culture medium was changed daily, and the infection rate was monitored at selected time points. The cell culture medium was collected and processed for cytokine analysis. The cytokines interleukin-1β (IL-1β), IL-6, IL-10, IL-12, gamma interferon (IFN-γ), transforming growth factor β (TGF-β), and tumor necrosis factor alpha (TNF-α) were measured as secreted protein products of infected and uninfected macrophages at various time points by using cytokine-specific enzyme-linked immunosorbent assays (ELISAs). Assays were performed according to the manufacturer’s guidelines at the Cytokine Core Laboratory located at the University of Maryland, Baltimore. Briefly, mouse cytokines were measured by two-antibody ELISA with a biotin-streptavidin-peroxidase detection kit and compared against a standard curve with SoftPro (Molecular Dynamics). Results were expressed as picograms of cytokine per milliliter. The lower limits of detection for the assays were as follows: IL-1β, 15 pg/ml; IL-6, 12 pg/ml; IL-10 and IL-12, 31.2 pg/ml; IFN-γ and TGF-β, 31 pg/ml; and TNF-α, 15.6 pg/ml. All data shown are from reproducible experiments. Values are expressed as means with standard deviations from triplicate samples.

RNA isolation and RT-PCR assays. For reverse transcription-PCR (RT-PCR), 9 × 10⁶ P388D1 cells and mouse peritoneal macrophages (C3H/HeNCrBr) were inoculated with R. akari and R. typhi at an MOI of 50 rickettsiae/cell and monitored by Gimenez staining 24, 48, and 96 h postinfection. Cells were detached from the 150-cm² flasks with a cell scraper, transferred to a 50-ml tube, and centrifuged at 13,000 × g for 10 min (26). The supernatant was discarded, and the cells were treated with 1 ml of Trizol reagent (Life Technologies, Gaithersburg, Md.). For subsequent RNA extractions, we followed the manufacturer’s protocol (Gibco BRL). The RNA pellets were dissolved in diethyl pyrocarbonate (DEPC) water, quantified spectrophotometrically, and stored at −80°C. cDNAs derived from infected P388D1 cells, peritoneal macrophages, and uninfected controls were examined by formaldehyde agarose gel electrophoresis to confirm that RNA had not become degraded during the extraction procedure. Prior to RT-PCR, it was essential to test all RNA samples with DNase, by using 1 U of DNase/μg of RNA. Following DNase treatment, RNAs were precipitated with 100% isopropanol, and pellets were washed with 70% ethanol and resuspended in DEPC water. For RT, the SuperScript RT protocol for random hexamer-primed cDNA synthesis was used.

We chose to amplify the 17-kDa cell surface common antigen gene as our target (11). The RT-PCRs were performed with 6 μl of cDNAs as the template, 8 μM (each) primer, 1× PCR buffer (20 mM Tris-HCl [pH 8.3], 50 mM KCl), 1.5 mM MgCl₂, 200 mM deoxynucleoside triphosphate (dNTP) mix, and 1 U of Taq polymerase. After denaturation of the initial cDNA-RNA hybrid (95°C for 4 min), an extra 1 U of Taq polymerase was added to each sample to improve the yield of amplicons. Amplification conditions were 94°C for 30 s, 50°C for 45 s, and 72°C for 45 s over 30 cycles. An aliquot of 10 μl of each RT-PCR product was electrophoresed on a 1% agarose gel and stained with ethidium bromide.

Semi-quantitative RT-PCR of ltxB mRNA. Total cellular RNA was isolated from R. akari- and R. typhi-infected P388D1 cells, infected peritoneal macrophages, and uninfected controls as described above. For detection of ltxB, 15 μl of the RT reaction mixture was amplified in 100 μl of volume. The primers used were as follows: ltxB forward primer, 5′-GCCTGGAGCTGGAAAGC-3′; ltxB reverse primer, 5′-GCCCTGTAGTTAACCCTC-3′ (666-bp product). The PCR cycle consisted of an initial incubation at 95°C for 10 s, followed by cycling at 95°C for 30 s, 65°C for 30 s, and 72°C for 60 s, with a final incubation at 72°C for 7 min (19). Ten-microliter samples were subjected to electrophoresis on a 2% NuSieve 3:1 agarose gel (FMC Bioproducts, Rockland, Maine) in 1× TAE buffer.

Statistical analysis. Appropriate transformations were performed for statisti- cal comparison of means for cytokine data by the Student t test. Nonparametric statistics were also used with similar data. The significance differences between the means at P < 0.05 are marked in the figures where appropriate.

RESULTS

The underlying rationale for this study was R. akari survival within macrophages that may contribute to the establishment of rickettsial infection and resulting pathogenesis. Initially, we focused our study on determining whether R. akari survives and replicates within the P388D1 cell line and mouse peritoneal macrophages. Both R. akari and R. typhi were able to infect and replicate inside P388D1 cells. The macrophage infection rates were determined by IFA and FACS with monoclonal antibodies against R. akari and R. typhi outer membrane protein (rOmpB). At 24 h postinfection, IFA indicated that 24% of P388D1 cells were infected with R. akari, while only 15% of the cells were infected with R. typhi. Infection rates for R. akari increased to 52% by 48 h and 85% at 96 h postinfection, while those for R. typhi were 32 and 52% at the same time points. FACS analysis revealed slightly higher infection rates of P388D1 cells for R. akari (25% at 24 h, 55% at 48 h, and 92% at 96 h) and R. typhi (17% at 24 h, 35% at 48 h, and 56% at 96 h). Infection rates for C3H/HeNCrBr peritoneal macrophages as determined by IFA ranged from 18 to 78% for R. akari and 10 to 49% for R. typhi during the 96 h of observations. FACS analysis revealed the same general pattern for mouse peritoneal macrophage infection rates ranging from 19 to 78% for R. akari and 13 to 51% for R. typhi (Fig. 1). As shown in Fig. 1, the addition of 0.5 mg of E. coli LPS per ml to 96-h-infected cells drastically reduced the infection rates. The presence and progress of infection with R. akari and R. typhi were additionally confirmed by RT-PCR (Fig. 2).

To determine if rickettsial infection resulted in macrophage killing, LDH release was measured as an indicator of R. akari- and R. typhi-induced cytotoxicity. Overall, low-level cytotoxic- ity was observed, ranging from 2.9 to 8.4% cell lysis for P388D1 cells and 3.5 to 13% cell lysis for the peritoneal macrophages infected with rickettsiae during the 96-h observation period (Fig. 3). The background lysis of uninfected P388D1 and mouse peritoneal macrophages ranged from 1.4 to 2.8%.
cell lysis rates for *R. akari* varied from 4.8% at 24 h to nearly 9% over the next 72 h postinfection. In contrast, *R. typhi* exhibited even lower cytotoxicity, with only 2.8 to 4.5% of the P388D1 cells lysed during the 96 h of the infection cycle. Overall, infection with both rickettsia species resulted in comparable low cell lysis. In order to determine whether lysis of infected macrophages would have been increased if the macrophages were activated, *E. coli* LPS was added. Overnight treatment of 96-h-infected and uninfected P388D1 cells with *E. coli* LPS (0.5 mg/ml) resulted in a significant increase in cell lysis (Fig. 3). The addition of LPS increased the cell lysis over threefold in both *R. typhi* (13.6%)- and *R. akari* (24.4%)-infected macrophages compared to the level in their untreated controls (no LPS). However, the level of increase due to LPS in infected macrophages was substantially lower than those observed in uninfected macrophages (62.8%).

Since rickettsial infections have been associated with control of apoptosis (4, 18, 27), we tested whether the cell lysis induced by *R. akari* and *R. typhi* was due to apoptosis or necrosis. We addressed this question by using FACS analysis of Annexin-V- and PI-stained *R. akari*- and *R. typhi*-infected P388D1 cells and mouse peritoneal macrophages at 96 h postinfection. While positive staining for PI alone identified necrotic cells, staining with both PI and Annexin-V was counted as host cell apoptosis. Analyses of data for *R. akari*-infected P388D1 revealed that 7.4% of the cells were apoptotic and 1.4% of the cells were necrotic. Similarly, 8.3% of *R. akari*-infected mouse peritoneal macrophages were apoptotic, while 4.5% were necrotic. In contrast, all of the *R. typhi*-infected cells that lysed were positive by PI and by Annexin-V, indicating apoptotic events.

Prevention of apoptosis prolongs rickettsial endothelial infection and thereby allows rickettsiae to multiply and reinfect...
neighboring cells. It has been shown for \textit{R. rickettsii}, the causative agent of Rocky Mountain spotted fever, that activation of host NF-\kappa B prevents apoptosis of endothelial cells (20). In a pilot study, we also looked for NF-\kappa B p65 expression in \textit{R. akari}- and \textit{R. typhi}-infected macrophages during the rickettsial growth cycle. Rickettsial infections of macrophages resulted in an increased level of I\kappa B\textsubscript{mRNA} as determined by RT-PCR of I\kappa B\textsubscript{mRNA} (Fig. 4).

LPS-activated macrophages are capable of producing a substantial array of cytokines, chemokines, and toxic mediators (12, 13). To determine whether interaction between pathogenic rickettsiae and macrophages resulted in increased cytokine synthesis and release by infected cells, we initially studied \textit{R. akari}- and \textit{R. typhi}-induced cytokine profiles in P388D1 cells in vitro. Cytokine profiles for \textit{R. akari}- and \textit{R. typhi}-infected as well as uninfected P388D1 cells are presented in Fig. 5 and 6. Nonactivated P388D1 cells infected with either \textit{R. akari} or \textit{R. typhi} demonstrated increased levels of proinflammatory cytokines, such as TNF-\alpha, IL-1\beta, and IL-6 (Fig. 5A, B, and C, respectively) compared to uninfected controls. In general, TNF-\alpha production by \textit{R. akari}-infected P388D1 cells displayed uniform elevation by 24 h and remained unchanged throughout the observed infection compared to that of uninfected controls (\textit{P} < 0.05; Fig. 5A). However, \textit{R. typhi}-infected cells displayed a significant increase in TNF-\alpha at 24 h postinfection and declined fourfold afterward (\textit{P} < 0.05). In contrast, there were no changes in TNF-\alpha levels between infected and uninfected peritoneal macrophages during the 96-h sampling.

We noted a significant difference in IL-1\beta production between \textit{R. akari}- and \textit{R. typhi}-infected cells (Fig. 5B). At 96 h postinfection, cells infected with \textit{R. typhi} secreted fourfold more IL-1\beta (68 pg/ml) compared to cells infected with \textit{R. akari}.
Levels of other cytokines, such as IFN-γ, were also measured. Background levels (30 pg/ml for uninfected cells) were observed for IL-12 and IL-10. In contrast, TGF-β secretion was significantly higher than that from uninfected controls (2580 RADULOVIC ET AL. INFECT. IMMUN.).

Overall, there were no statistical differences between R. akari- and R. typhi-infected mouse macrophages, but the patterns of the IL-6 production during the time course of infection were different. IL-6 production in infected cells was significantly different from that in uninfected controls (<0.001) with the exception of P388D1 cells infected with R. akari at 96 h, which did not differ from the baseline data.

The production of IL-1β by R. typhi-infected cells continued to increase over the culture period, while R. akari-infected cells failed to produce more than baseline levels of IL-1β (Fig. 5C). Peritoneal macrophages secreted much greater levels of IL-1β than did P388D1 cells. In cells infected with R. akari, elevated IL-1β increased further at 96 h postinfection (745 pg/ml at 24 h, 736 pg/ml at 48 h, and 1,247 pg/ml at 96 h). In contrast, R. typhi-infected cells displayed IL-1β secretion that peaked by 48 h postinfection and then began to decline. IL-1β production by R. akari and R. typhi during 24 to 96 h postinfection was significantly different from that of the uninfected controls (P < 0.001).

Infection with both R. typhi and R. akari induced dramatic increases in IL-6 production by P388D1 cells (Fig. 6). IL-6 secretion by R. akari-infected cells peaked at 24 h postinfection (1,500 pg/ml), followed by a steady decline to baseline 96 h postinfection. In contrast, R. typhi-infected cells produced uniformly high levels of IL-6 through 96 h postinfection. Interestingly, primary mouse peritoneal macrophages infected with R. akari and R. typhi showed a very different, although still robust, pattern of IL-6 secretion. At 24 h postinfection, both infected cultures had elevated levels of IL-6 (R. akari, 1,125 pg/ml; R. typhi, 1,755 pg/ml). However, IL-6 secretion by R. akari-infected macrophages continued to increase throughout the course of the experiment, while secretion from R. typhi-infected cells decreased slightly, but remained substantially higher than that from uninfected controls.

Also of interest was the production of TGF-β by infected mouse peritoneal macrophages, although none was detected from infected P388D1 cells. Secretion of TGF-β by R. typhi-infected mouse peritoneal macrophages dramatically increased after 48 h in culture and remained high through the 96 h of observation (P < 0.05) compared to that in controls. In contrast, TGF-β in R. akari-infected macrophages remained at background levels (<30 pg/ml for uninfected cells) (Fig. 7). Levels of other cytokines, such as IFN-γ, IL-10, and IL-12 in supernatants of R. akari- and R. typhi-infected P388D1 and mouse peritoneal macrophages, were below the ELISA detection limits.

**DISCUSSION**

We have shown by RT-PCR that both R. akari and R. typhi were capable of infecting and surviving within P388D1 cells and mouse peritoneal macrophages. R. akari attained a greater rickettsial load in infected P388D1 cells and mouse macrophages during 96 h than did R. typhi. This observation is consistent with rickettsial growth observed previously in endothelial cells, which are the main mammalian target for most rickettsial species (5–8). R. typhi, after entry into the host endothelial cells, multiplies to achieve and maintains a high rickettsial load before lysing the host cells. R. akari and R. rickettsii, on the other hand, maintain a low-level infection, but eventually lyse their target cells. Both R. typhi, and R. akari can freely leave the endothelial cells before host cell lysis occurs (3, 23). However, the utilization of host cell actin assembly varies between R. akari and R. typhi (10). Presumably, this escape mechanism allows the rickettsiae to leave the host cell’s hostile environment before being destroyed (9).

A combination of intracellular growth and lysis is considered to be the basis for rickettsial pathogenesis (1, 7, 8). While the molecular mechanism of rickettsia-induced endothelial cell lysis is not known, the lysis mechanism or mechanisms allow rickettsiae to be released and to infect the neighboring cells (8, 9, 21, 22). Our data indicated that infection of unactivated macrophages with R. akari and R. typhi did not result in efficient cytokytosis through the 96-h time point of evaluation.

Consideration of the NF-κB data permits an alternative explanation. Activation of host cell NF-κB has been demonstrated in response to infection by numerous human pathogens, and an increase in the level of IκBα is a sensitive indicator of NF-κB activation (4). In the present study, we observed the presence of NF-κB and an increased level of IκBα mRNA in R. akari- and R. typhi-infected P388D1 cells. IκBα mRNA was detected in R. typhi but not in R. akari-infected peritoneal macrophages. Sporn et al. have demonstrated that R. rickettsii induced activation of the transcrip-

![FIG. 6. IL-6 response of P388D1 cells and peritoneal macrophages infected with R. akari and R. typhi. *, statistical significance at P < 0.05 for IL-6 production between R. akari- and R. typhi-infected P388D1 cells. Overall, there were no statistical differences between R. akari- and R. typhi-infected mouse macrophages, but the patterns of the IL-6 production during the time course of infection were different. IL-6 production in infected cells was significantly different from that in uninfected controls (<0.001) with the exception of P388D1 cells infected with R. akari at 96 h, which did not differ from the baseline data.](http://iai.asm.org/)

![FIG. 7. TGF-β response of peritoneal macrophages infected with R. akari and R. typhi at 24, 48, and 96 h postinfection. *, statistical significance at P < 0.05 for TGF-β production between R. akari- and R. typhi-infected macrophages 48 h postinfection.](http://iai.asm.org/)
tional factor NF-κB in vitro and in vivo (18, 19). R. rickettsii infection also was associated with an increased level of IkBα mRNA. Furthermore, they suggested the involvement of the TNF-α receptor, but not TNF-α or apoptosis, in R. rickettsii-induced signal transduction, leading to NF-κB activation (20). Recently, Rikihisha et al. provided convincing evidence that human granulocytic Ehrlichia delayed apoptosis of infected granulocytes (27). These few studies suggested that delayed host cell apoptosis is a general phenomenon used among pathogenic rickettsiae to prolong their survival within the eukaryotic cells. However, it would be premature to assume a universal feature among rickettsiae in terms of intracellular survival, considering biological and genetic differences among rickettsiae in terms of host cell preference, growth, and intracellular location. The inhibition of apoptosis at two levels, both in primary infection and after induction of proapoptotic immunological processes of acquired immunity, could set the conditions for persistent rickettsial infection. Surviving rickettsiae could then replicate within the macrophages remaining at a site of rickettsia-infected endothelium. The activation of NF-κB induced by R. akari and R. typhi thus could prevent apoptotic signals triggered by the host defense mechanism, such as interaction with immune cells or inflammatory cytokines. Our ongoing experiments may address these questions in the near future.

Among the roles macrophages play in the immune system is the production of an array of cytokines that are important in directing the immune response to host cell damage and pathogen removal (27). The inactivation of IL-6 release in infected macrophages by pathogen bacteria such as E. coli and Porphyromonas gingivalis has been reported by bacterial secretion of specific proteases (2). The response of macrophages to rickettsial infection included a differential increase in proinflammatory cytokines, such as TNF-α, IL-1β, and IL-6, while the production of other cytokines (IFN-γ, IL-10, and IL-12) remained undetectable. P388D1 cells and mouse peritoneal macrophages infected with R. akari failed to induce remarkable TNF-α or TGF-β secretion. IL-1β secretion was robust from peritoneal macrophages, but not from the P388D1 cell line, and IL-6 secretion was remarkable from both cultures. While the increased production of IL-1β and IL-6 by P388D1 cells and mouse peritoneal macrophages infected with R. typhi was noticeable, the expression of TNF-α and TGF-β differed between these two host cells. The relationship between TNF-α, the nitric oxide synthase pathway, and LPS to the killing of IFN-γ-treated macrophagelike RAW264.7 cells by R. prowazekii was studied previously (21). Furthermore, depletion of IFN-γ and TNF in mice infected with Rickettsia conorii resulted in fatal and overwhelming rickettsial infection (7). Differences observed in cytokine profiles between R. akari and R. typhi may contribute to their survival within the confines of their eukaryotic target cells, despite their relationship to host defense mechanisms.

The studies reported here were carried out in vitro in the absence of many cellular components of host immune responses. In vivo, vigorous host responses occur that are dependent upon the recruitment and activation of macrophages and neutrophils, which are required to effectively clear rickettsial infection. In most cases, infection with pathogenic rickettsiae in natural hosts (e.g., house mice and rats for R. akari and R. typhi, respectively) is resolved with rickettsial clearance. In contrast, human infection is characterized by severe pathology and some mortality in untreated cases. Low rickettsial cytotoxicity toward the host cells observed in this study not only provides ample opportunity for rickettsial spread to neighboring cells, but may also result in rickettsial persistence. Indeed, we have been successful in isolating R. typhi from spleen and kidneys of wild-caught rats exhibiting high antirickettsial antibodies (unpublished data), which further confirms rickettsial persistence.

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