Animal Model

Immunopathology and Ehrlichial Propagation Are Regulated by Interferon-γ and Interleukin-10 in a Murine Model of Human Granulocytic Ehrlichiosis

Mary E. Martin,* Karen Caspersen,* and
J. Stephen Dumler†

From the Division of Comparative Medicine and the
Department of Pathology,† Division of Medical Microbiology,
The Johns Hopkins University School of Medicine,
Baltimore, Maryland*

Previous studies of human granulocytic ehrlichiosis (HGE) suggest a role for host immune response in resolving infection and in causing histopathological lesions. We hypothesize that interferon (IFN)-γ allows tissue injury that is suppressed by interleukin (IL)-10 after initiation by ehrlichia infection. Thus, parental C57BL/6, IL-10−/−, and IFN-γ−/− strains of mice were infected and then assayed for hepatic histopathological lesions, ehrlichial burden, and cytokine responses to ehrlichial antigen in primary splenic cultures during the first 21 days after infection. Histopathological severity in C57/BL6 and IL-10−/− mice rose in parallel through day 7, but then diverged as pathology in IL-10−/− mice continued to increase and remained high throughout the course of the study. The histopathological rank of C57BL/6 of mice decreased at day 10 and returned to baseline levels at days 14 and 21. In contrast, the IFN-γ−/− strain had baseline pathology scores throughout the course of the infection, yet had significantly higher ehrlichial burden both in the blood and tissues than C57BL/6 or IL-10−/− mice. This suggests that histopathological lesions in the HGE murine model do not result from direct ehrlichia-mediated injury but from immunopathological mechanisms initiated by ehrlichial infection. The similarities with lesions in humans suggest an immunopathological basis for HGE. *(Am J Pathol 2001, 158:1881–1888)*

Murine models have proven useful for studying various aspects of the pathogenesis of human granulocytic ehrlichiosis (HGE), an acute, febrile illness caused by *Ehrlichia phagocytophila.* Although mice do not develop clinical signs, they do develop pathological lesions closely resembling those seen in humans and other species with granulocytic ehrlichiosis.[1–4] Sero-epidemiological studies in humans and horses suggest that the disease is often mild or inapparent.[5,6] However, severe infection in humans may be complicated by acute respiratory distress syndrome and opportunistic infections.[7–9] The precise mechanism by which pathological injury occurs in HGE is not known. The temporal, microanatomical, and quantitative disparity between ehrlichiae detected in tissue and the degree of histopathological injury does not support a role for a direct bacterial cytolytic mechanism. Some aspects of the clinical disease and histopathological lesions of HGE suggest the potential for host-mediated immunological injury.[1] Therefore, previous studies in our laboratory have examined host immune response in a murine model, focusing on the role of cytokines in relation to developing pathology and ehrlichial quantity. In C3H/HeJ mice, a strain known to be susceptible to rickettsial infections, levels of plasma interferon (IFN)-γ peak before maximal pathological injury when ehrlichiae are absent in tissues, supporting a role for host immunity in the pathogenesis of HGE.[3] The purpose of this study was to examine the roles of proinflammatory and anti-inflammatory cytokines and ehrlichial quantity on host pathology in a murine model using IFN-γ- and interleukin (IL)-10-deficient mice.

Materials and Methods

Mice

Pathogen-free male mice (3 to 6 weeks of age) were obtained from Jackson Labs (Bar Harbor, ME) and main-

tained in microisolator cages in accordance with institutional guidelines. 48 mice of each of the following strains were obtained: C57BL/6-$^{\text{Ifng-tm1Ts}}$ (IFN-γ−/−), C57BL/6-$^{\text{IL10-tm1Cgn}}$ (IL-10−/−), and parental C57BL/6 (hereafter referred to as C57BL/6). They were allowed to acclimate for 5 days before manipulation.

Inoculum

Webster strain HGE agent *E. phagocytophila* was grown in HL-60 cells, a human promyelocytic cell line, until 100% of the cells contained morulae. On the day of inoculation, infected and uninfected cells were centrifuged, then resuspended in serum-free RPMI 1640 medium to a concentration of 2×10^6 cells/ml . Twenty-four mice of each strain were inoculated intraperitoneally with 0.5 ml (1×10^6) uninfected HL-60 cells (sham-inoculum), or HL-60 cells infected with *E. phagocytophila* (passage 4). Mice were observed daily for evidence of clinical illness, such as fur ruffling, hunched posture, depression, anorexia, or tachypnea.

Necropsy

Eight mice of each strain (four inoculated with *E. phagocytophila*-infected cells and four with uninfected HL-60 cells) were necropsied at six time points: days 0 (4 hours after inoculation), 4, 7, 10, 14, and 21. The mice were sedated with methoxyflurane, then exsanguinated by cardiac puncture, followed by cervical dislocation. The spleen was sterilely harvested, followed by collection of lung, liver, sublumbar lymph nodes, and brain. Bone marrow was obtained by flushing the femur with 1 ml of sterile phosphate-buffered saline (PBS). Half of the spleen was placed in sterile culture medium (RPMI 1640 and 2% fetal bovine serum), a quarter fixed in 10% formalin, and a quarter was frozen at −80°C. The remainder of the tissues were formalin-fixed and paraffin-embedded for hematoxylin and eosin (H&E) staining and immunohistological examination. Tissues were examined for lymphohistiocytic cell aggregates, necrosis, and apoptosis. The degree of liver pathological changes observed on H&E-stained tissue sections was first ranked from 1 to 72 (1 having the least pathology and 72 having the most severe pathology) among the infected mice based on the number, intensity, and size of lesions. The rankings were then grouped consecutively into six 12-member scoring groups, with a score of 0 corresponding to the absence of pathological findings and a score of 5 corresponding to the most severe changes. The sham-inoculated mice were also examined and scored by the same criteria from 0 to 5 with side-by-side comparisons with infected mice for accuracy of scoring. All slides were reviewed by two individuals (JSD, MEM) for consensus. For each group and time point, the mean score of sham-inoculated mice was subtracted from the score of *E. phagocytophila*-inoculated mice to normalize for differences in strain pathological response.

Splenic Cultures

The splenic tissues that had been placed in sterile culture medium were immediately pooled into groups of four by mouse strain and inoculum, the tissue finely minced with a sterile scissors, and dispersed into a single cell suspension. Cells were washed twice in sterile culture medium, red blood cells were lysed in a hypotonic salt solution, and remaining cells were washed and resuspended in RPMI 1640 medium with 10% fetal bovine serum and gentamicin (50 μg/ml) at a concentration of 2×10^6 cells/ml. One ml of the cell suspension was added to each well of a 24-well culture plate. After 24 hours of incubation the cell suspension was stimulated in duplicate wells by adding either sterile medium (control), or the following (final concentrations): ConA (1 μg/ml), lipopolysaccharide (5 μg/ml), or three different concentrations of renografin-purified *E. phagocytophila* Webster strain (50, 5, 0.5 μg/ml). Seventy-two hours later (determined optimal for *E. phagocytophila* stimulation in pilot studies) the supernatant was harvested from each well and frozen at −80°C until analyzed.

Cytokine Assays

Levels of IFN-γ and IL-10 were assayed in the supernatant of the splenic cultures by sandwich enzyme-linked immunosorbent assay. Capture and detection monoclonal antibodies and recombinant antigen for IFN-γ and IL-10 were obtained from Pharmingen (San Diego, CA). Briefly, 96-well plates were coated with capture antibody and incubated overnight at 4°C. The wells were blocked with PBS with 3% bovine serum albumin for 2 hours, and then washed with PBS/Tween. Doubling dilutions of recombinant antigen (diluted in PBS with 3% bovine serum albumin) were used for a standard curve. Then recombinant antigen standards or supernatants were added to duplicate wells and incubated overnight at 4°C. Plates were washed and reacted with biotinylated secondary antibody, and developed at room temperature for 45 minutes. After washing, plates were reacted with streptavidin alkaline phosphatase (DAKO, Carpinteria, CA) at room temperature for 30 minutes, and washed again with PBS/Tween. Color was developed with the TMB peroxidase system (KPL, Gaithersburg, MD) and the optical density measured at 630 nm on an enzyme-linked immunosorbent assay plate-reader.

Serology

Plasma was assayed for *E. phagocytophila* group antibodies by indirect immunofluorescence assay, as described elsewhere,[2,3] using *E. phagocytophila*-infected HL-60 cells (Webster strain) as antigen. Plasma samples reactive at a 1:80 dilution were titrated to endpoint.

Measurement of Ehrlichial DNA Quantity

Plasma was separated from packed blood cells, and nucleic acids were prepared from the cellular fraction

(~ 200 to 400 μl) using the Gentra PureGene kit (Gentra Systems, Minneapolis, MN). The samples obtained from mice inoculated with uninfected HL-60 cells were tested for *E. phagocytophila* DNA by polymerase chain reaction (PCR) using the primers ge9f and ge10r.[2] DNA extracted from the blood of mice inoculated with infected cells was tested with a quantitative PCR method using an ABI TaqMan 7700 instrument (PE Biosystems, Foster City, CA). The TaqMan reporter probe and primer sequences were modified from Pusterla and colleagues.[10] The fluorescent reporter dye at the 5′ end of the TaqMan probe (ehr 80p, CCTATGCATTACTCACCCGTCTGCCACT) was 6-carboxy-fluorescein (FAM); the quencher at the 3′ end was 6-carboxy-tetramethyl-rhodamine (TAMRA). Primers were amplified from a 106-bp fragment of the 16S rRNA gene (ehr 50r, 5′-TTCGAACGGATTATTCTTTATAGCTTG-3′, and ehr 145f, 5′-CCATTTCTAGTGGCTATCCCCATAC-TAC-3′). The standard curve was developed using DNA prepared from a known quantity of *E. phagocytophila*-infected HL-60 cells added to ethylenediaminetetraacetic acid-anti-coagulated mouse blood from which 10-fold dilutions were made in sterile water. The 50-μl PCR mixture contained 900 nmol/L each of forward and reverse primers, 150 nmol/L probe, 25 μl TaqMan Universal PCR Master Mix (Roche, Branchburg, NJ), and ~50 ng (range, 25 to 65 ng) of DNA. After target denaturation for 3 minutes at 95°C, amplification conditions were five cycles of 30 seconds at 95°C and 20 seconds at 62°C, followed by 45 cycles of 40 seconds at 85°C and 60 seconds at 62°C. Results were expressed as quantity of infected cells per μl host DNA template where 1 μl was determined to represent ~10^5 host cells.

Immunohistochemistry

Five-μm thick paraffin-embedded tissue sections were prepared and examined by immunohistological techniques with rabbit anti-*E. phagocytophila* Webster strain as the primary antibody, as described elsewhere.[2] Slides were examined by light microscopy for the presence of intracytoplasmic morulae stained red in color. The total number of infected cells per volume of tissue was calculated.

Results

Animals and Pathology

Consistent with previous studies, none of the mice exhibited any clinical signs of illness. C57BL/6 mice had mild to moderate histopathological lesions (lymphohistiocytic aggregates, apoptotic cells, mild hepatitis) evident at 7 to 14 days, which were resolving by day 21. In the IL-10−/− mice, hepatic histopathological lesions were much more severe at each time point and included marked lymphohistiocytic accumulations, severe hepatitis with numerous apoptoses, and necrosis. The IFN-γ−/− mice had minimal hepatic pathology throughout the course of the experiment (Figure 1). The quantitative and kinetic results of histopathological examinations are summarized in Figure

2. Mice of the C57BL/6 and IL-10−/− strains had mildly elevated histopathology scores as compared to the IFN-γ−/− strain at day 0 ($P = 0.011$, $P = 0.21$, respectively, *t*-test). Histopathological severity in C57/BL6 and IL-10−/− mice rose in parallel through day 7, but then diverged as histopathological lesions in IL-10−/− mice continued to increase and remained high throughout the course of the study ($P = 0.023$, *t*-test). The histopathology rank of C57BL/6 strain of mice decreased at day 10 and returned to baseline levels at days 14 and 21. In contrast, the IFN-γ−/− strain had baseline pathology scores throughout the course of the infection.

Serology

All sham-inoculated mice remained seronegative throughout the course of the study, whereas all mice inoculated with *E. phagocytophila*-infected HL-60 cells seroconverted. Serum titers of the *E. phagocytophila*-infected mice are shown in Figure 3, and confirm results obtained in pilot studies. At day 7, the serological titer of the IFN-γ−/− mice was significantly, or near significantly, higher than the C57BL/6 ($P = 0.063$) or IL-10−/− ($P = 0.024$) mice. At day 10, the serum titer of the IL-10−/− mice continued to increase, so that by days 14 and 21 it was not significantly different from the IFN-γ−/− mice. In contrast, the C57BL/6 mice had a significantly lower serum titer than either of the other two strains at days 10 and 14 ($P = 0.002$, $P = 0.024$). By day 21, the C57BL/6 mice had a marked increase in serological titer, approaching that of the other two strains ($P = 0.060$).

Cytokine Measurements

To control for endogenous cytokine production by the cells in the splenic cultures, the cytokine measurements from the sham-inoculated mice were subtracted from that of the *E. phagocytophila*-inoculated mice. This adjusted value was plotted by averaging the duplicate cytokine measurements of each mouse strain per day in duplicate cultures (Figure 4). Splenic cultures stimulated with 50 μg/ml and 5 μg/ml of *E. phagocytophila* antigen produced nearly identical quantities and kinetics of IFN-γ and IL-10 whereas those stimulated with 0.5 μg/ml had little cytokine production greater than baseline levels. Day 7 results were eliminated from the calculations as the splenic cultures from that day did not respond to either ConA or lipopolysaccharide (control) stimulation. The reason for the lack of splenic culture stimulation on that day is unclear; however, the results suggest a technical problem with culture on that day alone. For the IFN-γ−/− mice, no IFN-γ was produced during the 21-day trial. The IL-10−/− and C57BL/6 strains both had IFN-γ production by days 4 to 10, which later became undetectable. IL-10 was undetectable in IL-10−/− mice throughout the study. In both IFN-γ−/− and C57BL/6 mice, IL-10 was detected early in the infection (day 0 to 4) and in the IFN-γ−/− mice again at day 21.
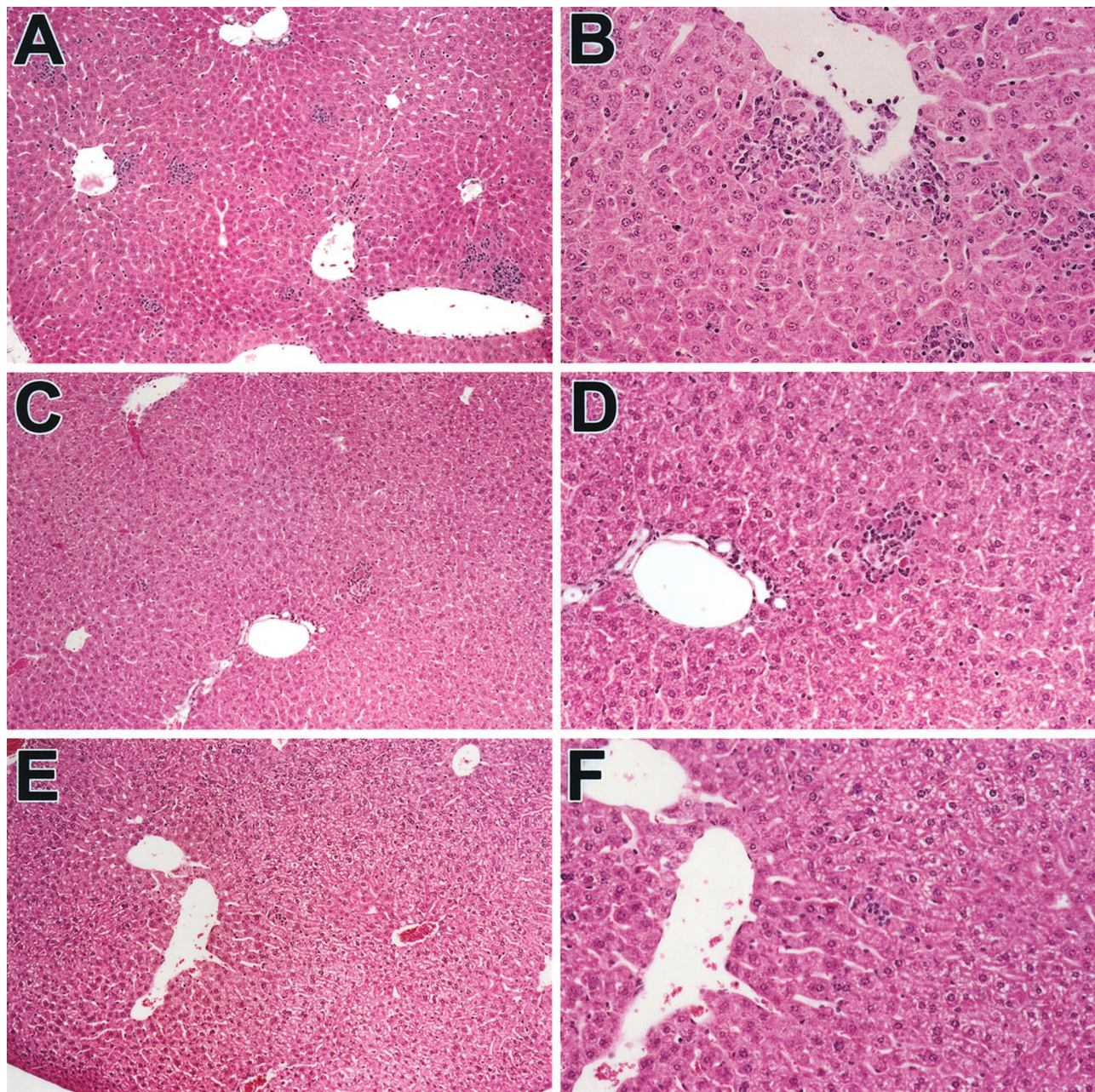
Figure 1. Hepatic pathology with HGE agent infection in IL-10−/− mice at day 14 after inoculation (**A** and **B**); C57BL/6 mice at day 21 after inoculation (**C** and **D**); and IFN-γ−/− mice at day 14 after inoculation (**E** and **F**). Note the large number of lesions and extensive necrosis/apoptosis in the IL-10−/− mice that is ameliorated in the control C57BL/6 mice, and absent in the IFN-γ−/− mice. H&E; original magnifications: ×40 (**A**, **C**, and **E**) and ×160 (**B**, **D**, and **F**).

PCR

All sham-inoculated mice were negative by qualitative PCR. The *E. phagocytophila*-inoculated mice were tested in triplicate by quantitative PCR (Figure 5). Both C57BL/6 and IL-10−/− strains had very low quantities of *E. phagocytophila* DNA detected. The IFN-γ−/− mice on average had ehrlichial DNA detected at every time point except days 10 and 21, but not all inoculated mice had *E. phagocytophila* DNA detected at each time point. When IFN-γ−/− strain values were compared to the combined grouping of C57BL/6 and IL-10−/− strains, there were significant, or near significant, differences at days 0, 4,

and 7 ($P < 0.025$, <0.100, and 0.010, respectively; Wilcoxon rank sum).

Immunohistochemistry

Ehrlichial tissue infection detected by immunohistochemistry is summarized in Table 1. For both the C57BL/6 and IL-10−/− mice, small numbers of infected cells were detected only in the lung, and at only one time point. The IFN-γ−/− mice had ehrlichia-infected cells detected in the lungs at days 4 and 7 and in the spleen at days 7 and
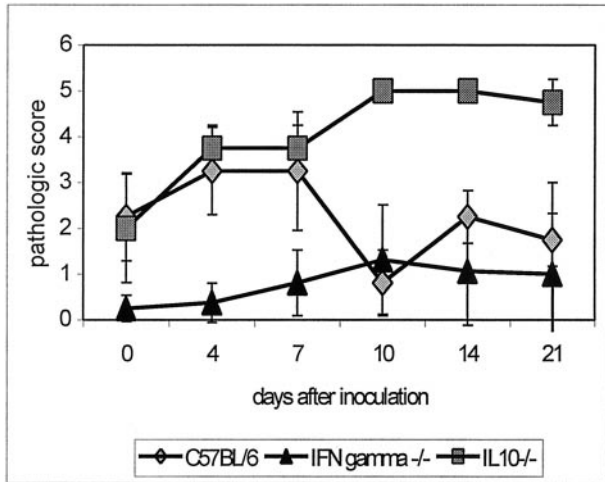
Figure 2. Comparison of quantitative hepatic histopathology among C57BL/6, IL-10−/−, and IFN-γ−/− mice in the murine HGE model. The histopathological scores for IL-10−/− mice were high at every time point, whereas IFN-γ−/− mice had near-normal scores throughout the course of the experiment. Error bars represent one SEM.

14. No infected cells were detected in the liver in any strain, at any time point.

Discussion

Our results indicate that IFN-γ plays a crucial role in the clearance of the organism, and is a major determinant of histopathology associated with HGE. Mice that were deficient in IFN-γ developed minimal hepatic pathology, but demonstrated a significantly higher quantity of ehrlichiae in the blood and tissues than either of the other two strains examined. In contrast, IL-10−/− and C57BL/6
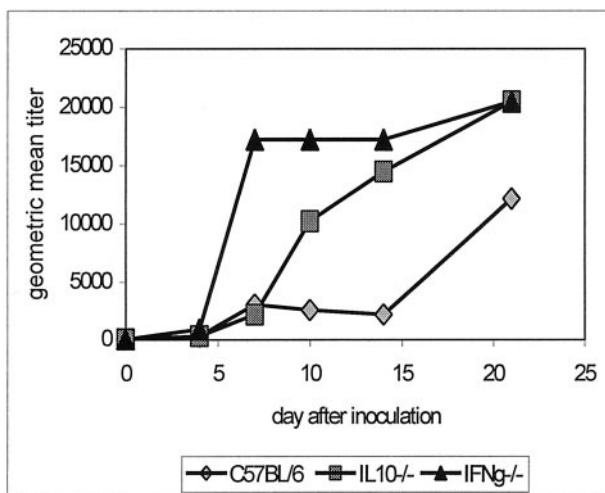

Figure 3. Serological responses in C57BL/6, IL-10−/−, and IFN-γ−/− mice after inoculation with *E. phagocytophila*-infected HL-60 cells. At day 7, the serological titer of the IFN-γ−/− mice was significantly, or near significantly, higher than the C57BL/6 ($P = 0.063$) or IL-10−/− ($P = 0.024$) mice. In contrast, the C57BL/6 mice had a significantly lower serum titer than either of the other two strains at days 10 and 14 ($P = 0.002$, $P = 0.0240$). By day 21, the serological titer of C57BL/6 mice increased to approach that of the other two strains ($P = 0.060$). Standard errors for each geometric mean titer value were <3.8.
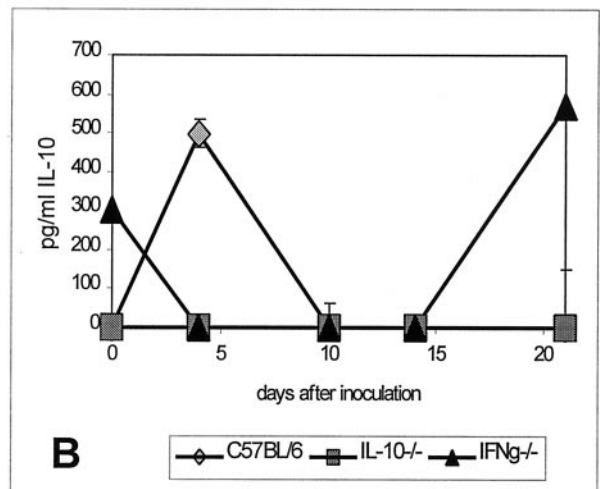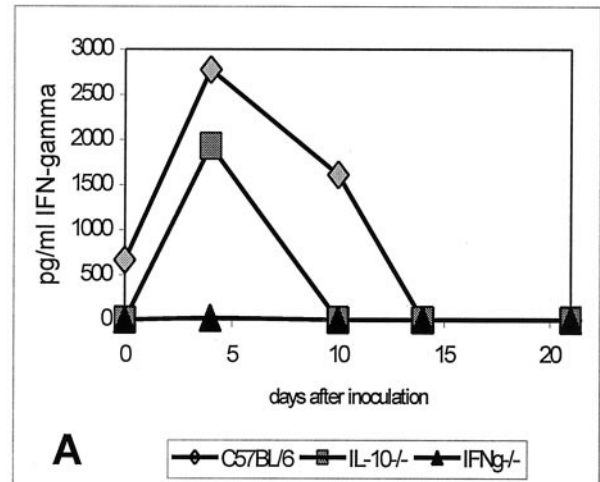

Figure 4. IFN-γ (**A**) and IL-10 (**B**) levels in supernatants from splenic cultures of mice stimulated with 5 μg/ml of *E. phagocytophila* antigen. **A:** The IL-10−/− and C57BL/6 strains both had IFN-γ detected in splenic culture supernatants only on days 4 or 10. **B:** IL-10 was detected in supernatants from splenic cultures of the IFN-γ−/− mice only at days 0 (4 hours after inoculation) and 21. In contrast, C57BL/6 mice had IL-10 detected in supernatants only at day 4.

mice had a significantly greater degree of hepatic pathology, despite often undetectable levels of ehrlichiae in the blood at the same time point.

The mechanisms of tissue injury with *E. phagocytophila* infection are poorly understood. Unlike the situation with vasculotropic rickettsioses,[11,12] vasculitis because of rickettsia-mediated endothelial injury does not occur with the ehrlichioses. Although *in vitro* propagation of *E. phagocytophila* strains clearly leads to significant host cell cytolysis, necrosis, and changes in apoptotic activity,[13–15] it is unclear whether these bacteria-driven mechanisms have a role *in vivo*. Previous studies in humans and other animals with granulocytic ehrlichiosis have shown a stark disparity in tissue localization and quantities of ehrlichiae as opposed to the degree of pathological injury.[1] Moreover, certain aspects of the clinical course including toxic shock-like manifestations and acute respiratory distress syndrome[9,16–18] and pathological findings such as hepatic apoptoses and erythro-
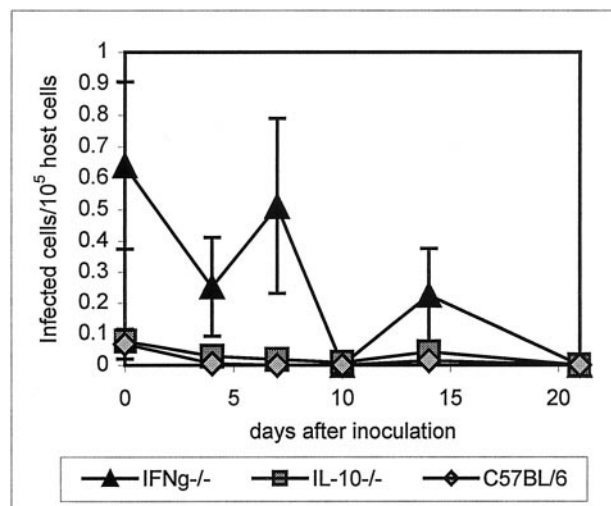
Figure 5. Comparison of the quantity of *E. phagocytophila* DNA in the blood of infected C57BL/6, IFN-γ−/−, and IL-10−/− mice. C57BL/6 and IL-10−/− mice had very low quantities of *E. phagocytophila* DNA detected. The IFN-γ−/− mice had significantly increased ehrlichial DNA quantities as compared with the combined group of C57BL/6 and IL-10−/− mice at days 0 and 7 ($P < 0.025$ and $P = 0.010$, respectively, Wilcoxon rank sum) and approached significance ($P = 0.100$) at day 4.

phagocytosis[1] suggest a role for host and immune factors in the pathogenesis. The studies here provide strong evidence of a host-mediated mechanism for pathological injury with HGE. Although pathology closely follows the rising and falling levels of IFN-γ in mice, it is clear that such responses are initiated by the ehrlichial infection. In fact, Bunnell and colleagues[2] showed that pathological lesions in C3H-SCID mice may co-localize with ehrlichiae-infected neutrophils, further confirming the role of the bacterium as the trigger for the ensuing inflammatory response. However, as in humans, the mouse model shows a disparity between ehrlichial quantity and tissue location with regard to pathology, focusing attention on the host immune response and not the bacterium as the critical effector of pathological lesions.

The niche occupied by obligate intracellular bacteria presents a significant challenge to host immunity. Typically these infections elicit a cell-mediated immune response that results in the production of IFN-γ and up-regulation of other proinflammatory and cytotoxic responses effective at intracellular killing.[3,19–22] In contrast, IL-10 is an anti-inflammatory cytokine known to down-regulate IFN-γ, potentially allowing decreased clearance or increased propagation of the pathogen, as

is seen with *Brucella abortus* and *Salmonella typhimurium*.[23,24] IL-10 may further modulate pathology by counterbalancing the effects of proinflammatory cytokines, especially IFN-γ, as demonstrated in studies with *Toxoplasma gondii*[25] and *Schistosoma mansoni*.[26] In humans with HGE, IFN-γ-dominated cell-mediated immunity with moderate IL-10 levels is temporally associated with clinical manifestations and recovery from infection.[27]

Despite the lack of IFN-γ, mice deficient in this cytokine did clear the infection by 21 days. The nature of the IFN-γ-independent mechanism is uncertain, but may involve other cellular components (cytotoxic T cells, natural killer cells), other cytokine-driven effectors, or humoral immunity, which peaks at this time. These results concur with earlier studies looking at the importance of IFN-γ, and other possible mechanisms of host response.[3,19] Although humoral immunity may be important in protecting against infection or in clearance of organisms,[19,28] there is no correlation with histopathological injury. Serum titers were low at days 4 and 7 in IL-10−/− and C57BL/6 mice when pathological scores were already increasing; furthermore, at peak serological response (days 14 to 21), the pathological score had decreased to baseline levels. In addition, the IFN-γ−/− mice had minimal pathological scores throughout the study, yet seroconverted earlier in the course of infection and maintained high titers compared with the other two strains. It is possible that the increased antigenic load in these mice during the acute phase of infection triggered a more robust, immediate antibody response, or the response may have been compensatory for the lack of typical Th1-type immunity.

Unique in this study was the relationship between pro- and anti-inflammatory cytokines and the effect on histopathological lesions. IFN-γ was essential to the development of pathology, even while suppressing ehrlichial replication. In contrast, loss of IL-10-mediated anti-inflammatory mechanisms were associated with severe inflammatory reactions and tissue injury, even in the absence of a significant ehrlichial burden. However, differences in IFN-γ levels between C57BL/6 and IL-10−/− mice were not readily demonstrated. Thus, the mechanisms that control ehrlichial propagation *in vivo* must be more complex than can be explained by these data alone. It was observed that C57BL/6 mice had relatively high levels of IFN-γ and restricted ehrlichial quantities at day 0 (4 hours after inoculation), whereas IL-10−/− mice had a similar level of ehrlichial growth restriction, but no IFN-γ detected at the same time point. This suggests that ehrlichial infection induces nonspe-

Table 1. Quantity of *Ehrlichia phagocytophila* Morulae Detected by Immunohistochemistry in C57BL/6, IFNγ−/−, and IL-10−/− Mice

Mouse strain (tissue)	Day after inoculation					
	0	4	7	10	14	21
C57BL/6 (lung)	0	0	3 (38.5)	0	0	0
IL-10−/− (lung)	0	1 (25)	0	0	0	0
IFNγ−/− (lung)	0	5 (34.5)	8 (42.1)	0	0	0
IFNγ−/− (spleen)	0	0	11 (139.4)	0	1 (18.1)	0

Results are expressed as number infected cells (No. infected cells/μl tissue).

cific mechanisms responding to ehrlichial components early in infection. To what degree these responses result from biological variations and sampling intervals is uncertain. Further investigation is needed to elucidate which other components of early intrinsic Th1 immunity are important in restricting ehrlichial growth.

It is interesting to note that no ehrlichial DNA or morulae were detected at day 10 in the IFN-γ−/− mice by quantitative PCR or immunohistochemistry, yet at day 14 low levels of ehrlichiae were detected by both techniques. Whether this is within biological variation, or is the result of biologically meaningful change is not known. *E. phagocytophila* strains are known to be antigenically diverse[29–31] and it is possible that the second peak in ehrlichial quantity on day 14 represents the emergence of *E. phagocytophila* antigenic variants. In other rickettsial species, such as *Anaplasma marginale, Ehrlichia risticii, Ehrlichia canis,* and *Ehrlichia chaffeensis*[32–35] antigenic variation results from the differential expression of one or more major surface proteins encoded by a family of paralogous genes. It is speculated that the ability of ehrlichiae to modify major surface protein profiles may allow immune evasion and persistence of infection. This observation needs further investigation, but may explain some disparity in ehrlichial quantity at different time points.

In summary, IFN-γ-related mechanisms are important in clearance of the organism in the early phases of HGE. However, IFN-γ also plays an essential role in pathology associated with the infection. IL-10 moderates the pathology, perhaps through down-regulatory effects on IFN-γ or through other anti-inflammatory mechanisms. Further studies are needed to determine what components of the ehrlichial organism stimulate the inflammatory response and drive the IFN-γ production, as well as the mechanisms of disease resolution and control of immunopathology. Whether results derived from murine models have relevance to human and veterinary granulocytic ehrlichiosis has yet to be proven. However, the mouse model that implicates immunopathological responses in the pathogenesis of HGE does provide a good pathological and experimental mimic of infection and should provide insights that can be extrapolated to humans and tested in other infection models.

References

1. Lepidi H, Bunnell JE, Martin ME, Madigan JE, Stuen S, Dumler JS: Comparative pathology, and immunohistology associated with clinical illness after Ehrlichia phagocytophila-group infections. Am J Trop Med Hyg 2000, 62:29–37
2. Bunnell JE, Trigiani ER, Srinivas SR, Dumler JS: Development and distribution of pathologic lesions are related to immune status and tissue deposition of human granulocytic ehrlichiosis agent-infected cells in a murine model system. J Infect Dis 1999, 180:546–550
3. Martin ME, Bunnell JE, Dumler JS: Pathology, immunohistology, and cytokine responses in early phases of human granulocytic ehrlichiosis in a murine model. J Infect Dis 2000, 181:374–378
4. Madigan JE, Gribble D: Equine ehrlichiosis in northern California: 49 cases (1968–1981). J Am Vet Med Assoc 1987,190: 445–448
5. Bakken JS, Goellner P, Van Etten M, Boyle DZ, Swonger OL, Mattson S, Krueth J, Tilden RL, Asanovich K, Walls J, Dumler JS: Seropreva-

6. lence of human granulocytic ehrlichiosis among permanent residents of northwestern Wisconsin. Clin Infect Dis 1998, 27:1491–1496
6. Madigan JE, Hietala S, Chalmers S, DeRock E: Seroepidemiologic survey of antibodies to Ehrlichia equi in horses of northern California. J Am Vet Med Assoc 1990, 196:1962–1964
7. Bakken JS, Krueth J, Wilson-Nordskog C, Tilden RL, Asanovich K, Dumler JS: Clinical and laboratory characteristics of human granulocytic ehrlichiosis. J Am Med Assoc 1996, 275:199–205
8. Aguero-Rosenfeld ME, Horowitz HW, Wormser GP, McKenna DF, Nowakowski J, Munoz J, Dumler JS: Human granulocytic ehrlichiosis: a case series from a medical center in New York State. Ann Intern Med 1996, 125:904–908
9. Wong S, Grady LJ: Ehrlichia infection as a cause of severe respiratory distress. N Engl J Med 1996, 334:273
10. Pusterla N, Leutenegger CM, Chae JS, Lutz H, Kimsey RB, Dumler JS, Madigan JE: Quantitative evaluation of ehrlichial burden in horses after experimental transmission of human granulocytic Ehrlichia agent by intravenous inoculation with infected leukocytes and by infected ticks. J Clin Microbiol 1999, 37:4042–4044
11. Walker DH, Popov VL, Wen J, Feng HM: Rickettsia conorii infection of C3H/HeN mice. A model of endothelial-target rickettsiosis. Lab Invest 1994, 70:358–368
12. Feng HM, Wen J, Walker DH: Rickettsia australis infection: a murine model of a highly invasive vasculopathic rickettsiosis. Am J Pathol 1993, 142:1471–1482
13. Goodman JL, Nelson C, Vitale B, Madigan JE, Dumler JS, Kurtti TJ, Munderloh UG: Direct cultivation of the causative agent of human granulocytic ehrlichiosis. N Engl J Med 1996, 334:209–215
14. Hsieh TC, Aguero-Rosenfeld ME, Wu JM, Ng C, Papanikolauo NA, Varde SA, Schwartz I, Pizzolo JG, Melamed M, Horowitz HW, Nadelman RB, Wormser GP: Cellular changes and induction of apoptosis in human promyelocytic HL-60 cells infected with the agent of human granulocytic ehrlichiosis (HGE). Biochem Biophys Res Commun 1997, 232:298–303
15. Yoshiie K, Kim HY, Mott J, Rikihisa Y: Intracellular infection by the human granulocytic ehrlichiosis agent inhibits human neutrophil apoptosis. Infect Immun 2000, 68:1125–1133
16. Hardalo CJ, Quagliarello V, Dumler JS: Human granulocytic ehrlichiosis in Connecticut: report of a fatal case. Clin Infect Dis 1995, 21: 910–914
17. Bakken JS, Dumler JS, Chen SM, Eckman MR, Van Etta LL, Walker DH: Human granulocytic ehrlichiosis in the upper Midwest United States. A new species emerging? J Am Med Assoc 1994, 272:212–218
18. Dumler JS, Bakken JS: Ehrlichial diseases of humans: emerging tick-borne infections. Clin Infect Dis 1995, 20:1102–1110
19. Akkoyunlu M, Fikrig E: Gamma interferon dominates the murine cytokine response to the agent of human granulocytic ehrlichiosis and helps to control the degree of early rickettsemia. Infect Immun 2000, 68:1827–1833
20. Fresno M, Kopf M, Rivas L: Cytokines and infectious diseases. Immunol Today 1997, 18:56–58
21. Mansueto S, Vitale G, Cillari E, Mocciaro C, Gambino G, Piccione E, Buscemi S, Rotondo G: High levels of interferon-gamma in boutonneuse fever. J Infect Dis 1994, 170:1637–1638
22. Feng HM, Popov VL, Walker DH: Depletion of gamma interferon and tumor necrosis factor alpha in mice with Rickettsia conorii-infected endothelium: impairment of rickettsicidal nitric oxide production resulting in fatal, overwhelming rickettsial disease. Infect Immun 1994, 62:1952–1960
23. Fernandes DM, Baldwin CL: Interleukin-10 downregulates protective immunity to Brucella abortus. Infect Immun 1995, 63:1130–1133
24. Pie S, Matsiota-Bernard P, Truffa-Bachi P, Nauciel C: Gamma interferon and interleukin-10 gene expression in innately susceptible and resistant mice during the early phase of Salmonella typhimurium infection. Infect Immun 1996, 64:849–854
25. Gazzinelli RT, Wysocka M, Hieny S, Scharton-Kersten T, Cheever A, Kühn R, Muller W, Trincheri G, Sher A: In the absence of endogenous IL-10, mice acutely infected with Toxoplasma gondii succumb to a lethal immune response dependent on CD4+ T cells and accompanied by overproduction of IL-12, IFN-gamma and TNF-alpha. J Immunol 1996, 157:798–805
26. Wynn TA, Cheever AW, Williams ME, Hieny S, Caspar P, Kühn R, Müller W, Sher A: IL-10 regulates liver pathology in acute murine

Schistosomiasis mansoni but is not required for immune down-modulation of chronic disease. J Immunol 1998, 160:4473–4480

27. Dumler JS, Trigiani ER, Bakken JS, Aguero-Rosenfeld ME, Wormser GP: Serum cytokine responses during acute human granulocytic ehrlichiosis. Clin Diagn Lab Immunol 2000, 7:6–8

28. Sun W, JW IJ, Telford SR III, Hodzic E, Zhang Y, Barthold SW, Fikrig E: Immunization against the agent of human granulocytic ehrlichiosis in a murine model. J Clin Invest 1997, 100:3014–3018

29. Murphy CI, Storey JR, Recchia J, Doros-Richert LA, Gingrich-Baker C, Munroe K, Bakken JS, Coughlin RT, Beltz GA: Major antigenic proteins of the agent of human granulocytic ehrlichiosis are encoded by members of a multigene family. Infect Immun 1998, 66:3711–3718

30. Zhi N, Ohashi N, Rikihisa Y: Multiple p44 genes encoding major outer membrane proteins are expressed in the human granulocytic ehrlichiosis agent. J Biol Chem 1999, 274:17828–17836

31. Asanovich KM, Bakken JS, Madigan JE, Aguero-Rosenfeld M, Wormser GP, Dumler JS: Antigenic diversity of granulocytic Ehrlichia isolates from humans in Wisconsin and New York and a horse in California. J Infect Dis 1997, 176:1029–1034

32. Brown WC, Zhu D, Shkap V, McGuire TC, Blouin EF, Kocan KM, Palmer GH: The repertoire of Anaplasma marginale antigens recognized by CD4(+) T-lymphocyte clones from protectively immunized cattle is diverse and includes major surface protein 2 (MSP-2) and MSP-3. Infect Immun 1998, 66:5414–5422

33. French DM, Brown WC, Palmer GH: Emergence of Anaplasma marginale antigenic variants during persistent rickettsemia. Infect Immun 1999, 67:5834–5840

34. Biswas B, Vemulapalli R, Dutta SK: Molecular basis for antigenic variation of a protective strain-specific antigen of Ehrlichia risticii. Infect Immun 1998, 66:3682–3688

35. Reddy GR, Streck CP: Variability in the 28-kDa surface antigen protein multigene locus of isolates of the emerging disease agent Ehrlichia chaffeensis suggests that it plays a role in immune evasion. Mol Cell Biol Res Commun 1999, 1:167–175