Relapsing Fever Borreliosis in Interleukin-10-Deficient Mice[∇]

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Relapsing fever (RF) is a spirochetal infection characterized by periods of sickness with fever at time of high bacteremia that alternate with afebrile periods of relative well being during low bacteremia. Patients with epidemic RF who are doing relatively well have extraordinarily high levels of interleukin-10 (IL-10) in the circulation. We investigated the possibility that IL-10 plays an important protective role in this infection using wild-type and IL-10-deficient mice inoculated with virulent serotype 2 of the RF spirochete *Borrelia turicatae*. During peak bacteremia there was increased systemic production of IL-10 that quickly resolved in the postpeak period; in contrast, IL-6 and CXCL13 production increased during the peak but remained elevated during postpeak bacteremia. IL-10 deficiency resulted in lower bacteremia, increased specific antibody production, higher production of CXCL13 and IL-6, and thrombotic and hemorrhagic complications affecting multiple organs with secondary tissue injury. Our results revealed that production of IL-10 is highly regulated during RF and plays an important protective role in the prevention of hemorrhagic and thrombotic complications at the cost of reduced pathogen control.

Relapsing fever (RF) borreliosis is a spirochetal infection caused by different *Borrelia* species in both endemic and epidemic forms (3). Although RF spirochetes can infect a number of organs, they remain predominantly localized in the blood, causing recurrent peaks of bacteremia (3, 8). Each peak is rapidly cleared upon development of specific immunoglobulin M (IgM) antibodies by B cells (1, 4, 16). The pathogenesis of RF is diverse depending on the species, the serotype, and the host (10, 13, 15). During epidemic RF, mortality can reach 70% (32, 37). Patients with epidemic RF have extraordinarily high levels of interleukin-10 (IL-10) in the circulation and still do relatively well clinically (17). We recently found similar high production of IL-10 in mice deficient in B and T cells with persistent high-level bacteremia with *Borrelia turicatae* (19, 20). Treatment with exogenous IL-10 reduced the clinical manifestations of the infection, systemic production of the B-cell chemokine CXCL13, and cerebral microgliosis (20). The absence of IL-10 resulted in rapid death from intracerebral hemorrhage in RAG2-deficient mice infected with virulent serotype 2 of *B. turicatae* (27, 28). The purpose of the current study was to investigate the protective function of IL-10 in RF borreliosis in immunocompetent mice. For this we compared the outcomes of acute infection with *B. turicatae* serotype 2 in wild-type (WT) and congenic IL-10-deficient mice. The results revealed that significant but transient production of IL-10 is a prominent feature of acute RF borreliosis that serves an important protective role against multiorgan hemorrhagic and thrombotic complications.

MATERIALS AND METHODS

Strains and culture conditions. *B. turicatae* serotype 2 has been previously characterized (15, 30, 31). Spirochetes were cultured in BSK-H medium (Sigma) with 12% rabbit serum. Prior to infection, borrelia viability was assessed by motility using phase-contrast microscopy and serotype identity was confirmed by Western blotting with anti-variable surface protein 2 (anti-Vsp2) monoclonal antibody 5F12 (14, 30).

Mouse infections. Female 4- to 5-week-old C57BL/10SgSnAi (WT) and C57BL/10SgSnAi-[KO] IL-10 (IL-10^{-/-}) mice were obtained from Taconic Farms (Germantown, NY). The mice were inoculated intraperitoneally with 5×10^4 *B. turicatae* serotype 2 spirochetes in 200 μ l of phosphate-buffered saline (PBS) or with PBS alone. Groups of four to eight mice each were used for all experiments. Mice were euthanized by inhalation with isoflurane followed by cardiac exsanguination and were extensively perfused with PBS to reduce blood contamination of tissues (11). To confirm infection, necropsy plasma specimens were cultured for 2 weeks and examined for the presence of spirochetes by phase-contrast microscopy. Brain, lungs, spleen, liver, and kidneys were removed at necropsy, fixed in 4% paraformaldehyde overnight at 4°C, paraffin embedded, and stained by hematoxylin and eosin (H&E) and terminal deoxynucleotidyl-transferase-mediated dUTP-biotin nick end labeling (TUNEL) as described previously (26).

Cytokines and chemokines. Concentrations of tumor necrosis factor (TNF), IL-6, IL-10, IL-12 (IL-12p70), granulocyte-macrophage colony-stimulating factor (GM-CSF), gamma interferon, CCL2, CCL3, CCL5, and CXCL1 in necropsy plasma from both infected and uninfected control mice were quantified with the Luminex 100 Multi-Analyte Profiling System (Luminex Corp, Austin, TX) using BioPlex Manager software (Bio-Rad Laboratories, Hercules, CA) and Lincoplex cytokine assay kits (Linco Research, St. Charles, MO) per the manufacturer's instructions. We measured CXCL13 by regular enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN).

DNA extraction and TaqMan PCR. One hundred microliters of necropsy plasma was centrifuged at $8,500 \times g$ for 30 min to precipitate spirochetes, and the pellets were used for DNA extraction with QIAmp DNA Micro kits (catalog no. 56304) according to the manufacturer's instructions. The amount of total DNA was quantified with a Nanodrop and determined by the A_{260}/A_{280} ratio. Quantitative real-time PCR was performed on an ABI Prism 7500 sequence detection system (Applied Biosystems) using 100 ng of DNA in 50 μ l of reaction volume with TaqMan Universal master mix reagents (Applied Biosystems) under standard conditions for 40 cycles. The primers and probes for the quantification of spirochetes targeted the borrelia 16S rRNA gene (2, 9, 35). A standard curve using log₁₀ dilutions from DNA extracted from a known number of cultured *B. turicatae* serotype 2 spirochetes in 100 ng of uninfected host DNA was used for

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FIG. 1. (A) Bacteremia and specific antibody production in WT and IL-10^{-/-} mice infected with *Borrelia turicatae*. Blood was harvested by cardiac puncture from groups of four mice each infected for 4 days. Spirochetes were counted by phase-contrast microscopy. Results are presented as spirochetes/ml plasma (log scale) in box plots. The bacteremia was significantly higher in WT mice than IL-10^{-/-} mice. (B) Spirochetal load per 100 ng of plasma DNA, determined using TaqMan PCR. Total DNA was extracted from 100 μ l of plasma pellets and measured for the *Borrelia* 16S rRNA gene. Results for WT and IL-10^{-/-} mice are presented as box plots. (C and D) Detection of Vsp2-specific IgM (C) and IgG (D). Plasma was collected from WT and IL-10^{-/-} mice on days 4 and 5 after inoculation of *B. turicatae* serotype 2. Vsp2-specific antibody levels were determined by ELISA using Vsp2 extracted from cultured *B. turicatae* serotype 2 as the antigen. Data represent the fold optical density (OD) difference, and bars indicate the mean scores and SD. Results for uninfected controls are shown by a dotted line.

quantification (35). Analysis was done with the AB sequence detection system software version 1.2.3 by extrapolation of the sample's threshold cycle to the standard curve.

Specific antibody production. Plasma obtained by cardiac exsanguination was assayed for the presence of anti-Vsp2 IgG and IgM antibodies by ELISA. Microtiter plates were coated with 5 μ g/ml purified Vsp2 produced by *n*-octyl- β -D-glucopyranoside nonionic detergent extraction (14). Plasma dilutions were added to plates and left for 1 h at 37°C, and bound murine Ig was detected by addition of alkaline phosphatase-conjugated antibody to murine IgG or horseradish peroxidase-conjugated antibody to murine IgM. The relative amounts of plasma anti-Vsp2 antibody were determined by comparing the optical density between groups.

Statistical analysis. Results were expressed as median and range or mean and standard deviation (SD). Two-sided nonparametric tests were used to determine differences between medians (Mann-Whitney *U* test) and the *t* test was used for differences between means. Differences in percentages were analyzed by Fisher's exact test. A *P* value of <0.05 was considered significant. All analysis used GraphPad Prism 4 for Windows.

RESULTS

Bacteremia and specific antibody production in IL-10-deficient mice. First we examined the question of whether IL-10 affects the pathogen load during acute infection. For this, eight WT or IL-10-deficient (IL-10^{-/-}) C57BL/10 mice were inoculated intraperitoneally with 5×10^4 *B. turicatae* serotype 2 spirochetes and their tail vein blood examined for the presence of spirochetes by phase-contrast microscopy at various times. No spirochetes were observed in the blood of WT or IL-10^{-/-} mice in the morning or afternoon of day 3 after inoculation. However, on the morning of day 4 after inoculation, spirochetes were visible in tail vein blood in all eight WT mice, compared with only three of eight IL-10^{-/-} mice (*P* < 0.05).

This suggested that the kinetics of the first peak of bacteremia were different in WT and IL-10^{-/-} mice. To investigate this further, we necropsied half of the animals (*n* = 4 per group) on day 4 and the other half (*n* = 4 per group) on day 5 and measured the pathogen load in the blood by phase-contrast microscopy with a Petroff-Hauser chamber; the sensitivity of this method is $\geq 5 \times 10^4$ spirochetes/ml. The results showed there were significantly more spirochetes in WT than in IL-10^{-/-} mice examined in the afternoon of day 4: the median (range) was 2×10^6 (5×10^4 to 4.1×10^6) in WT mice, compared to 2.5×10^4 (0 to 4×10^5) in IL-10^{-/-} mice (*P* < 0.01) (Fig. 1A). Mice examined in the morning of day 5 showed circulating spirochetes by phase-contrast microscopy in 1/4 IL-10^{-/-} and 0/4 WT mice.

Because of the limited sensitivity of microscopic examination with the Petroff-Hauser chamber, we wanted to confirm the previous results with a more sensitive method. For this we used real-time PCR amplification of borrelial 16S rRNA. The sensitivity of this assay is about one spirochete per ng of DNA. The results revealed that all eight WT mice and six of the eight IL-10^{-/-} mice necropsied on days 4 and 5 had detectable spirochetes in plasma. In mice examined in the afternoon of day 4, the median (range) number of spirochetes per 100 ng of DNA was higher in WT than in IL-10^{-/-} mice: 4.8×10^3 (1.2×10^3 to 6.9×10^5) compared with 1.7×10^3 (0.4×10^1 to 5.7×10^4) (*P* = 0.05) (Fig. 1B). In comparison, the median (range) number of circulating spirochetes was much lower in the mice examined in the morning of day 5 in both groups: 9.5×10^2 (3.5×10^2 to 9.9×10^2) in the WT group and 1×10^2 ($1.1 \times$

FIG. 2. Cytokine response to the infection in WT and IL-10-deficient mice. We measured the concentrations of cytokines in necropsy plasma of WT (A) and IL-10^{-/-} (B) mice by ELISA on day 4 ($n = 4$ each) or 5 ($n = 4$ each) after inoculation of serotype 2 of *B. turicatae* or PBS as a control ($n = 4$). Results are presented as mean (and SD) pg/ml and by whether mice were necropsied during peak (day 4) or postpeak (day 5) bacteremia.

10^2 to 1.9×10^3) in IL-10^{-/-} mice. In WT but not in IL-10^{-/-} mice the bacteremia significantly decreased between the afternoon of day 4 and the morning of day 5 ($P = 0.02$ for the WT group and $P = 0.10$ for the IL-10^{-/-} mice). We concluded that the kinetics and intensity of bacteremia were different in IL-10^{-/-} mice.

Since IL-10^{-/-} mice showed lower bacteremia and Vsp-specific antibodies are responsible for resolution of peak bacteremia in RF borreliosis, we next studied whether IL-10^{-/-} mice developed a stronger specific antibody response. To investigate this, we compared the amount of anti-Vsp2 antibodies in necropsy plasma by ELISA on days 4 to 5 after inoculation of *B. turicatae* serotype 2. The results revealed that IL-10^{-/-} mice had significantly larger amounts of IgM (Fig. 1C) and IgG (Fig. 1D) anti-Vsp2 antibodies than did WT mice.

Cytokine and chemokine response to the infection in IL-10-deficient mice. Next we studied the cytokine and chemokine response to the infection in WT and IL-10^{-/-} mice. Mice sham inoculated with PBS were used as controls ($n = 4$ each). For this we measured by ELISA the levels of various cytokines and chemokines in necropsy plasma from the same mice studied in the previous section. The cytokine results showed that at times of peak bacteremia in WT mice, there was elevated production of IL-10, about 20 times more than in uninfected controls ($P < 0.05$) (Fig. 2A). In contrast, very little IL-10 was detected in the plasma of the four infected WT mice necropsied in the morning of day 5 (Fig. 2A). A similar rapid rise and drop were

FIG. 3. Chemokine response to the infection in WT and IL-10-deficient mice. We measured the concentrations of several chemokines in necropsy plasma of WT (A) and IL-10^{-/-} (B) mice by ELISA after inoculation of serotype 2 of *B. turicatae* (PBS as a control). Results are presented as mean (and SD) pg/ml and by whether mice were necropsied during periods of high (day 4) or low (day 5) bacteremia.

observed for GM-CSF. The only other cytokine that increased as a result of infection was IL-6. However, unlike for IL-10 and GM-CSF, the circulating levels of IL-6 did not drop in the postpeak period (Fig. 2A). We did not detect any change in the concentrations of TNF, gamma interferon, or IL-12 in WT mice as a result of the infection. As expected, infected IL-10^{-/-} mice did not have any detectable IL-10 in the blood (Fig. 2B). The only cytokine that increased with infection in IL-10^{-/-} mice was IL-6, with marked variability from mouse to mouse ($P < 0.05$) (Fig. 2B). Similar to WT mice, IL-6 did not decrease and in fact was higher in mice examined in the morning of day 5 than in mice examined the day before. The median (range) levels of IL-6 in infected WT and IL-10^{-/-} mice were similar, at 60 (2 to 222) versus 132 (23 to 644) pg/ml ($P = 0.27$) (Fig. 2A and B).

The comparison of the chemokine response to the infection revealed that in WT mice there was high production of the B-cell chemokine CXCL13 that, unlike IL-10 or GM-CSF but similar to IL-6, remained elevated in the postpeak period (Fig. 3A). Small but significant increases in CCL2 and CXCL1 were also observed in WT mice during peak bacteremia, although these were of smaller magnitude than that of CXCL13 (Fig. 3A). CXCL13 was the only chemokine that increased as a result of the infection in IL-10^{-/-} mice, with values more than 10 times higher than in uninfected control mice ($P < 0.05$, Fig. 3B). Compared with infected WT mice, the median (range) CXCL13 levels in infected IL-10^{-/-} mice were also more than 10 times higher: 6.96×10^3 (7×10^2 to 8.3×10^3) pg/ml versus

TABLE 1. Hemorrhage at necropsy in C57BL/10 mice without or with IL-10 deficiency during acute RF borreliosis

Mice	Inoculum	No. of mice with hemorrhage in organ/ total no. of mice examined ^a			
		Brain	Kidney	Liver	Lung
WT	PBS	0/4	0/4	0/4	0/4
	<i>B. turicatae</i> serotype 2	0/8	0/8	0/8	0/8
IL-10 ^{-/-}	PBS	0/4	0/4	0/4	0/4
	<i>B. turicatae</i> serotype 2	3/8	5/8	5/8	3/8

^a Hemorrhages were studied macroscopically at necropsy 4 or 5 days after intraperitoneal inoculation of 5×10^4 *B. turicatae* spirochetes or PBS as a control.

6.1×10^2 (3.3×10^2 to 9.2×10^2) pg/ml ($P < 0.001$) (Fig. 3A and B). We concluded that the predominant cytokine and chemokine produced in acute RF borreliosis are IL-10 and CXCL13, respectively. The absence of IL-10 results in much greater production of CXCL13. Unlike that of CXCL13, the production of IL-10 is transient.

Disease in IL-10-deficient mice. Since infection in WT mice resulted in production of elevated amounts of IL-10 during peak bacteremia (Fig. 2A), it was possible that IL-10 was helping control disease, as previously observed in RAG1- and Igh6-deficient mice (19, 20). To investigate this possibility, one of us (D.L.) had performed masked clinical examinations of WT and IL-10^{-/-} mice daily after inoculation with *B. turicatae* serotype 2 or PBS for 5 days in the mice previously discussed. Analyses of the clinical disease severity scores showed that unlike RAG1- or Igh6-deficient mice or *scid* mice (15, 19, 20), none of the infected WT or IL-10^{-/-} immunocompetent mice showed any signs of clinical disease or had lost weight compared with uninfected controls (not shown). Examination of the spleens removed at necropsy showed splenomegaly of a similar degree in all infected WT and IL-10^{-/-} mice compared with uninfected controls (not shown). Next we studied whether IL-10 deficiency resulted in any pathological abnormalities as a result of the infection. Macroscopic examination of mice necropsied on day 4 or 5 after inoculation revealed the presence of hemorrhages in several organs in IL-10^{-/-} mice but not in WT mice or any of the uninfected controls: all eight infected IL-10^{-/-} mice had hemorrhage in at least one organ, compared with none of the eight infected WT mice (Table 1) ($P < 0.01$). These hemorrhages were more common in the kidneys and the liver (62%) than in the brain or the lungs (37%) (Table 1). Microscopic examination of H&E-stained tissue sections confirmed that hemorrhages occurred only in infected IL-10^{-/-} mice. The most prominent hemorrhages were found in the subcapsular area of the kidneys (Fig. 4A). In addition to hemorrhage, the livers of IL-10^{-/-} mice also showed thrombotic occlusion of multiple vessels surrounded by lobular necrosis (Fig. 4C). Although the livers of infected WT mice did not show hemorrhage or necrosis, they did show scattered foci of inflammatory cells and hepatocellular degeneration (Fig. 4B and D). We also observed areas with hemorrhage and leukocyte accumulation in and around microvessels in the lungs, sparse foci of inflammatory cells in the leptomeninges, and

small hemorrhages in the brain parenchyma and cerebellum of infected IL-10^{-/-} mice (not shown). To confirm this observation, we similarly infected new groups of WT and IL-10^{-/-} mice with *B. turicatae* serotype 2 or PBS ($n = 4$ each) and necropsied them 7 or 12 days later. Macroscopic examination at necropsy confirmed the presence of hemorrhages in most of the IL-10^{-/-} mice (7/8) compared with none of the WT mice (0/4) ($P < 0.05$). As before, none of the uninfected control mice had any detectable hemorrhage.

To confirm the development of tissue injury in IL-10^{-/-} mice, we stained paraffin-embedded liver sections from infected and uninfected WT and IL-10^{-/-} mice with the TUNEL assay, which labels both apoptotic and necrotic cells (21). The results revealed the presence of multiple large areas of TUNEL staining in the liver in the vicinity of thrombosed microvessels only in IL-10^{-/-} mice that corresponded to the necrotic areas previously observed with H&E staining (Fig. 4E). Examination of the livers of infected WT mice showed

FIG. 4. Effect of IL-10 deficiency on the pathology of acute RF borreliosis. Representative histological H&E staining (A to D) and TUNEL staining (E and F) from kidney and livers of IL-10^{-/-} (A, C, and E) and WT (B, D, and F) mice necropsied 4 or 5 days after inoculation of serotype 2 of *B. turicatae* are shown. The kidneys in IL-10^{-/-} mice show large subcapsular hemorrhages (*) that were not seen in infected WT mice (B). The livers in IL-10^{-/-} mice showed necrotic areas (*) next to thrombosed microvessels and a higher influx of inflammatory cells in vessels (arrow in panel D) not seen in WT mice. TUNEL shows large areas of positive staining in necrotic areas in the IL-10^{-/-} mice (E) and scattered TUNEL-positive cells in the WT mice (F) (magnification, $\times 20$).

scattered TUNEL-positive cells (Fig. 4F). We concluded that IL-10 prevented hemorrhagic, thrombotic complications, and tissue injury during acute RF borreliosis.

DISCUSSION

The first indication that IL-10 plays an important protective role in RF borreliosis came from a clinical study by Cooper et al., which found extraordinarily high levels of IL-10 in the blood of patients with epidemic RF borreliosis who were doing relatively well at the time of admission (17). Further evidence of an important protective role for IL-10 in this infection came from our previous study with RAG1- and Igh6-deficient mice persistently infected with *B. turicatae*, which showed a strong negative correlation between circulating levels of IL-10 and clinical disease and improvement of clinical disease by treatment with exogenous IL-10 (19, 20). In a more recent study with RAG2-deficient mice, we demonstrated a critical role for IL-10 in survival during acute severe infection with *B. turicatae* and showed that the protective role of IL-10 is mediated in large part by neutralization of TNF (27, 28). In the present study we investigated for the first time the role of IL-10 in acute RF borreliosis in immunocompetent mice. The main findings were the following. (i) Immunocompetent mice produce elevated amounts of IL-10 during acute RF borreliosis but only during times of high bacteremia. (ii) IL-10 deficiency altered the kinetics of bacteremia and lowered the pathogen load in the blood via increased specific antibody production. (iii) The B-cell chemokine CXCL13 is the major chemokine produced in response to the infection and significantly increases in the absence of IL-10. (iv) In the absence of IL-10, acute RF borreliosis results in multiorgan thrombotic and hemorrhagic complications with secondary tissue injury.

The results from this study revealed that in immunocompetent mice, increased production of IL-10 reduces the host's ability to control peak bacteremia (Fig. 1). This is the opposite of what we observed previously in RAG2-deficient mice persistently infected with *B. turicatae*, in which IL-10 deficiency compromised pathogen control via increased leukocyte apoptosis mediated to a great extent by TNF (27, 28). Others have shown that elimination of pathogens from immunocompetent hosts is more effective in the absence of IL-10, including studies with the related Lyme disease spirochete *Borrelia burgdorferi* (22–24). In murine Lyme borreliosis in immunocompetent mice, IL-10 deficiency resulted in a 10-fold decrease in the number of *B. burgdorferi* spirochetes present in ankle tissues (6). It has been hypothesized that IL-10 may suppress the antimicrobial activity of the innate immune system so that in its absence there is more-efficient bacterial killing (18). Similar to our results, although IL-10 deficiency helped control the pathogen load in the murine model of Lyme borreliosis, it also resulted in worsening pathology (6). It is possible that improved pathogen control in IL-10-deficient mice occurs because phagocytosis by the innate immune system improves in the absence of IL-10 (5, 24). However, since specific antibody is primarily responsible for the control and resolution of peak bacteremia in RF borreliosis (1, 4, 11, 29), it is more likely that the lower peak bacteremia in IL-10^{−/−} mice in our study was due to stronger and more rapid specific antibody production (Fig. 1C and D). One way this could occur is via increased

production of the B-cell chemokine CXCL13 in response to infection (Fig. 3B). In murine Lyme borreliosis, the absence of IL-10 also results in increased antibody production (6).

The cytokine network plays a pivotal role in the orchestration of inflammatory responses to bacterial infection. Unlike RAG1^{−/−} (20) or RAG2^{−/−} (27) mice, which showed persistent high-level bacteremia and continuous high production of IL-10, in immunocompetent mice this elevation occurred transiently, only at times of peak bacteremia (Fig. 2A). It is likely that this transient production of IL-10 attenuated the inflammatory syndrome that would otherwise occur due to the presence of high numbers of bacteria in the blood and that it protects against tissue injury. Recently we confirmed that infection with RF spirochetes results in increased production of IL-10 not only systemically but also in tissues (H. Gelderblom and D. Cadavid, unpublished observation). A likely function of this IL-10 surge is suppression of TNF production that can be fatal, as shown in RAG2/IL-10^{−/−} mice infected with *B. turicatae* serotype 2 (27, 28). The *Borrelia recurrentis*-infected patients studied by Cooper et al. who had high levels of IL-10 also had relatively low levels of TNF (17). In infected RAG^{−/−} mice, we have shown that IL-10 downregulates both systemic and local production of TNF (19, 20). Although we did not measure TNF production in tissues in this study, it is possible that increased local production of TNF in IL-10^{−/−} mice was implicated in the observed microvascular and parenchymal tissue injury. One possible reason for the lack of increased TNF levels in the blood of infected IL-10^{−/−} mice was their lower bacteremia. In RAG1^{−/−} mice, there was a strong positive correlation between pathogen load and TNF production systemically and in tissues (20).

Since RF spirochetes remain predominantly localized to the blood, it is not surprising that the main complication of IL-10 deficiency was damage to the microcirculation of multiple organs. However, we were surprised that tissue injury occurred in infected IL-10^{−/−} mice despite their lower peak bacteremia (Fig. 1 and 4). One alternative explanation is that in IL-10^{−/−} mice, peak bacteremia may have occurred earlier than day 3 after inoculation, when they were first examined by tail vein puncture, or in the night between days 3 and 4; this could be explained by the higher-than-usual inoculum we used in this study. The possible explanation is that IL-10 plays a critical role in preventing vascular injury even at the lower peak bacteremia observed in IL-10^{−/−} mice. Vascular injury is a well-known and important complication of infection with most pathogenic spirochetes, notably *Treponema pallidum* (12) and RF (10) and Lyme disease (7) borrelias. The mechanism of vascular injury in spirochetal infections is not known. Outer membrane lipoproteins from spirochetes have been shown to activate endothelial cells to produce proinflammatory mediators (25, 33, 34, 36, 38). Recently we showed that *B. turicatae* serotype 2 and Vsp2 cause apoptosis of human brain microvascular endothelial cells (D. Londono and D. Cadavid, unpublished results) and demonstrated apoptosis in brain endothelial cells from *B. turicatae* serotype 2-infected RAG2/IL-10^{−/−} mice (27).

This study confirms at the experimental level an important protective role for IL-10 in acute RF borreliosis in immunocompetent mice via prevention of hemorrhagic and thrombotic complications. Since injury occurred in IL-10^{−/−} mice despite

lower bacteremia, this indicates that the improved pathogen control conferred by the absence of IL-10 is ultimately not advantageous to the host because of the resultant vascular injury that took place. These findings add to the body of evidence on the important and complex protective role that IL-10 plays in bacterial infections.

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