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Recruitment of Macrophages and Polymorphonuclear Leukocytes in Lyme Carditis[▽]

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Lyme arthritis, caused by the spirochete *Borrelia burgdorferi*, can be recurrent or prolonged, whereas Lyme carditis is mostly nonrecurring. A prominent difference between arthritis and carditis is the differential representation of phagocytes in these lesions: polymorphonuclear leukocytes (PMN) are more prevalent in the joint, and macrophages predominate in the heart lesion. We have previously shown differential efficiency of *B. burgdorferi* clearance by PMN and macrophages, and we now investigate whether these functional differences at the cellular level may contribute to the observed differences in organ-specific pathogenesis. When we infected mice lacking the neutrophil chemokine receptor (CXCR2$^{-/-}$ mice) with spirochetes, we detected fewer PMN in joints and less-severe arthritis. Here we have investigated the effects of the absence of the macrophage chemokine receptor CCR2 on the development and resolution of Lyme carditis in resistant (C57BL/6J [B6]) and sensitive (C3H/HeJ [C3H]) strains of mice. In B6 CCR2$^{-/-}$ mice, although inflammation in hearts is mild, we detected an increased burden of *B. burgdorferi* compared to that in wild-type (WT) mice, suggesting reduced clearance in the absence of macrophages. In contrast, C3H CCR2$^{-/-}$ mice have severe inflammation but a decreased *B. burgdorferi* burden compared to that in WT C3H mice both at peak disease and during resolution. Histopathologic examination of infected hearts revealed that infected C3H CCR2$^{-/-}$ animals have an increased presence of PMN, suggesting compensatory mechanisms of *B. burgdorferi* clearance in the hearts of infected C3H CCR2$^{-/-}$ mice. The more efficient clearance of *B. burgdorferi* from hearts by CCR2$^{-/-}$ versus WT C3H mice suggests a natural defect in the recruitment or function of macrophages in C3H mice, which may contribute to the sensitivity of this strain to *B. burgdorferi* infection.

Lyme disease is caused by the spirochete *Borrelia burgdorferi* and is characterized by the hallmark rash erythema migrans and subsequent inflammatory processes that include arthritis, carditis, and neurological symptoms (28). There are critical differences in clinical outcome between Lyme arthritis, which can be recurrent or prolonged, and Lyme carditis, which is mostly nonrecurring. Without treatment, 60% of patients with Lyme disease in the United States develop arthritis, which may recur at intervals and last for months or years (28). Fewer patients (4 to 10%) suffer carditis, which is generally an early and nonrecurring manifestation of infection (28). A prominent difference between arthritis and carditis is the differential representation of phagocytes in these lesions: polymorphonuclear leukocytes (PMN) are more prevalent in the joint, while macrophages predominate in the heart lesion (2, 5, 6, 28). The differential deployment of phagocytes, which we have shown have differential efficiency of *B. burgdorferi* clearance (29), may contribute to these observed differences in organ-specific pathogenesis.

In vitro, spirochetes are eliminated by numerous effective clearance mechanisms, including both phagocytic and extracellular killing by intact macrophages and PMN, as well as by granule components of PMN (15, 26, 27, 29, 32). We have shown that in vitro, PMN kill *B. burgdorferi* efficiently only in the presence of specific antibodies and use a large array of nonoxidative products that are released from their granules (26, 29). In addition, the PMN cytoplasmic protein calprotectin inhibits *B. burgdorferi* growth and confers resistance to killing by certain antibiotics (27, 34). In contrast to PMN, macrophages kill spirochetes very efficiently, and largely internally, in the presence or absence of antibody, with little debris that could damage surrounding tissue (29–33). These functional differences at the cellular level may determine the ultimate clinical outcome of Lyme disease in host organs.

Mice infected with *B. burgdorferi* develop a systemic infection that closely mimics human disease. The acute manifestations in joints and hearts peak at days 14 to 21 of infection and resolve at 30 to 45 days (5). Several lines of evidence support the critical role of PMN in controlling Lyme arthritis. *B. burgdorferi* infection of *beige* mice, which have defects in vesicle trafficking leading to reduced PMN function (42), results in more-severe arthritis without changing macrophage-mediated carditis (4). Treatment of mice to deplete PMN during *B. burgdorferi* infection resulted in robust recruitment of immature forms of PMN, earlier development of arthritis, and higher *B. burgdorferi* loads, perhaps due to the inefficiency of spirochete killing by the recruited cells (9). Similarly, when we examined *B. burgdorferi* infection of chemokine receptor knockout (CXCR2$^{-/-}$) mice, in which the PMN cannot respond to chemotactic signals and do not enter the joint, arthri-

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tis was less severe, further demonstrating the contribution of PMN to joint inflammation (8). Deficiency in CCR2, a monocyte chemokine receptor, had no effect on arthritis, as might be expected, since macrophages constitute a smaller proportion of the inflammatory infiltrate in *B. burgdorferi*-infected joints (8).

In murine Lyme carditis, the predominant infiltrating cell type is the macrophage, which mediates the inflammation directly, as shown by the development of carditis in the absence of B and T lymphocytes (SCID mice) or major histocompatibility complex class II determinants (2, 36, 45). We have previously shown appropriate site-specific activation of infiltrating macrophages in Lyme carditis in vivo (35). CCR2 is the receptor for CC chemokines and mediates the recruitment of monocytes to the site of infection (12). Here we have investigated the effects of the absence of CCR2 on the development and resolution of macrophage-mediated Lyme carditis.

MATERIALS AND METHODS

Cultivation of bacteria. A low-passage-number virulent clonal isolate of *B. burgdorferi* strain N40 was grown to logarithmic phase in Barbour-Stoenner-Kelley II (BSK) medium by incubating for 5 days at 32°C. For experiments, *B. burgdorferi* was enumerated using a Petroff-Hausser hemocytometer (Hausser Scientific Partnership, Horsham, PA) under dark-field microscopy.

Mouse infection and tissue harvest. Chemokine receptor knockout animals on the C3H/HeJ (C3H) and C57BL/6J (B6) genetic backgrounds were generated by backcrossing as described previously (8) and have now been backcrossed for 10 generations. Wild-type (WT) and CCR2$^{-/-}$ littermate mice (5 to 10/group) were syringe-inoculated in both hind footpads with 2.5×10^5 *B. burgdorferi* strain N40 organisms in 0.05 ml of BSK II medium (8). At the harvest date, mice were euthanized, and hearts, blood, and bladder were collected. For RNA analysis, hearts were cut in half longitudinally with a sterile blade, rinsed in sterile phosphate-buffered saline, and frozen in RNase- and DNase-free Eppendorf tubes containing 1 ml RNA*later* (Ambion, Austin, TX). For histology, hearts were cut in half sagittally and each half was separately fixed in 10% buffered zinc-formalin (Anatech LTD, Battle Creek, MI).

The infection status of mice was confirmed by serum reactivity against a *B. burgdorferi* lysate by immunoblotting, using sera at a 1:100 dilution. Specific binding was detected by using an alkaline phosphatase-conjugated goat antibody specific for mouse IgG (Vector Laboratories) and was visualized with 5-bromo-4-chloro-3-indoylphosphate and nitroblue tetrazolium substrate (Kirkegaard & Perry Laboratories, Gaithersburg, MD) as described previously (35).

Q-PCR. DNA was first extracted from the urinary bladders and hearts of individual mice using the DNeasy tissue kit (QIAGEN, Valencia, CA) according to the manufacturer's instructions and then eluted in 60 μl double-distilled H_2O. DNA from each tissue was assessed by quantitative PCR (Q-PCR) in two separate determinations, and the average data are presented. Real-time PCRs for *B. burgdorferi flaB* and for the eukaryotic *b-actin* gene in murine tissue were performed using the primers and probes we have described previously (25). *flaB* was amplified using the following primers and probe: forward primer, 5′-AGC TGA AGA GCT TGG AAT GC-3′; reverse primer, 5′-AAC AGC AAT TGC CTC ATC CT-3′; probe, 6-carboxyfluorescein (6FAM)–CTT GAA CCG GTG CAG CCT GAG CA–6-carboxytetramethylrhodamine (TAMRA). For *b-actin*, the forward primer was 5′-ATC CTG GCC TCA CTG TCC AC-3′, the reverse primer was 5′-GGG CCG GAC TCA TCG TAC G-3′, and the probe was 6FAM-TCC AGC AGA TGT GGA TCA GCA AGC ATA-TAMRA. Two microliters of isolated DNA was amplified in a 50-μl final volume containing High-Fidelity platinum buffer, 5 mM $MgSO_4$, 200 μM deoxynucleoside triphosphates, 0.2 μM each primer, and 1 U High-Fidelity polymerase (Life Technologies, Gaithersburg, MD). Amplification was performed using an iCycler (Bio-Rad Laboratories, Hercules, CA) for 60 cycles at an annealing temperature of 60°C. Copy numbers for mouse *b-actin* and *B. burgdorferi flaB* were calculated using iCycler software (Applied Biosystems). Values for *B. burgdorferi flaB* were normalized to murine β-actin levels.

Reverse transcriptase Q-PCR. RNA was extracted from hearts by using the RNAeasy kit (QIAGEN, Valencia, CA) according to the manufacturer's instructions. cDNA was synthesized using reverse transcriptase (DNASTAR, Stratagene, La Jolla, CA), and Q-PCR was performed as we have described previously (25, 35). Primers and probes for mouse cytokines were as follows. For interleukin-1 (IL-1), the forward primer was 5′-GCA CCT TCT TTT CCT TCA TCT TTG-3′, the reverse primer was 5′-CCA GCA GGT TAT CAT CAT CAT CC-3′, and the probe was 6FAM-AGC CCA TCC TCT GTG ACT CAT GGG A-TAMRA. For macrophage inflammatory protein 2 (MIP-2), the forward primer was 5′-CAT CCA GAG CTT GAG TGT GAC-3′, the reverse primer was 5′-CTT TTT GAC CGC CCT TGA GAG T-3′, and the probe was 6FAM-ACG CCC CCA GGA CCC CAC TGC GCC CAG ACA GAA GTC A-TAMRA. For monocyte chemoattractant protein 1 (MCP-1), the forward primer was 5′-GTT GGC TCA GCC AGA TGC A-3′, the reverse primer was 5′-AGC CTA CTC ATT GGG ATC ATC TTG-3′, and the probe was 6FAM-TTA ACG CCC CAC TCA CCT GCT GCT ACT-TAMRA.

Semiquantitative assessment of tissue histopathology. Mice were sacrificed at peak infection (21 days), and hearts were harvested for histopathologic examination. Hearts (5 per group) were bisected sagittally through both atria and ventricles and were immersed in 10% buffered zinc-formalin (Anatech LTD). All tissues were processed to slides by dehydration in graded ethanol, cleared in xylene, and embedded in paraffin (Blue Ribbon; Surgipath Medical Industries, Inc., Richmond, IL). Each heart was serially sectioned at a thickness of 5 μm, and the 5th, 10th, 15th, and 20th sections (80 total) were stained with hematoxylin and eosin, followed by placement of coverslips. Observers were blinded to the study conditions until after assessment of the histopathologic features.

Each histopathologic parameter was assessed and scored separately using a semiquantitative criterion-based methodology adapted from a published method (41). Heart tissues were assessed for inflammation by microscopic examination at low (×2.5)-, intermediate (×10)-, and high (×40, ×60)-power magnification and were scored for severity of inflammation (carditis, vasculitis) according to the percentage of inflammation at the heart base upon examination at low power (×2.5). Scores of 0 (none), 1 (minimal; less than 5%), 2 (mild; between 5% and 20%), 3 (moderate; between 20% and 35%), 4 (marked; between 35% and 50%), and 5 (severe; greater than 50%) were assigned for the severity of inflammation. The character of the inflammatory infiltrate (neutrophil, macrophage, multinucleated giant cell, lymphocyte) was assessed by examination of a minimum of 5 random fields at the level of the heart base at ×40 and ×60. Digital light microscopic images were recorded using a Zeiss Axioskop microscope, an AxoCam MRC camera, and AxioVision 4.4 imaging software (Carl Zeiss Micro Imaging, Inc., Thornwood, NY).

Statistics. Statistical significance was assessed by the Mann-Whitney test (two-tailed) using GraphPad Prism software. The significance level was set at a P value of <0.05.

RESULTS

We have examined carditis in mice deficient in the CCR2 type chemokine receptor (CCR2$^{-/-}$ mice) in both a *Borrelia*-resistant (B6) and a *Borrelia*-sensitive (C3H) mouse background. Using quantitative PCR and histologic assays, we have assessed the spirochete burden, expression of cytokines and chemokines, and inflammation in infected hearts.

Disseminated spirochete burden does not reflect heart infection. To assess the importance of macrophage-lineage cells in the host defense against *B. burgdorferi*, we infected CCR2$^{-/-}$ mice with spirochetes. We quantified disseminated infection in WT and CCR2$^{-/-}$ animals by Q-PCR assessment of spirochete loads in urinary bladders (Fig. 1A). Bladders of several strains of mice become infected with *B. burgdorferi*, can remain infected for 6 to 12 months, and are especially representative of disseminated burden, because they can be harvested intact and do not have an inflammatory infiltrate (6, 25, 44). The level of disseminated infection reflected in bladders is lower for CCR2$^{-/-}$ than for WT B6 animals, although levels are similar for C3H WT and CCR2$^{-/-}$mice. The *B. burgdorferi* burdens in CCR2$^{-/-}$ animals of both strains show that the overall efficient clearance of *B. burgdorferi* is maintained in these animals despite the reduction in macrophage recruitment, suggesting that additional mechanisms may be employed.

The importance of macrophages makes it of particular in-

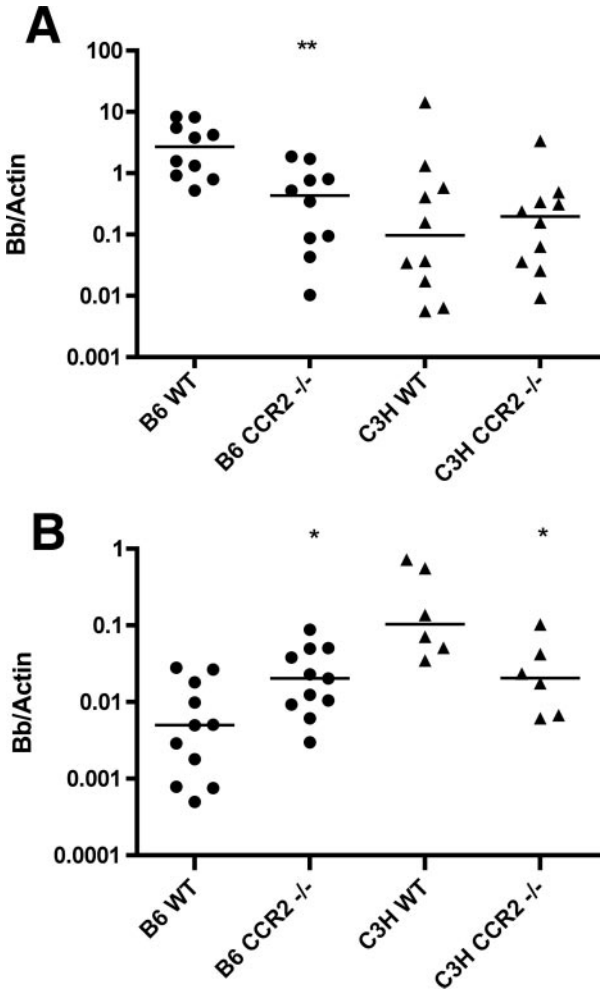


FIG. 1. *B. burgdorferi* burdens in tissues of CCR2$^{-/-}$ mice. Mice were infected via footpad inoculation, and organs were harvested at 21 days. The *B. burgdorferi* loads in urinary bladders (A) and hearts (B) were determined by Q-PCR using *flaB* for spirochetes and normalizing to murine β-actin. Data are representative of one of two separate experiments, each using 6 to 10 mice/group, Bars indicate median values. Data trends are similar in replicate experiments and reach statistical significance for CCR2$^{-/-}$ versus WT animals for bladders ($P = 0.004$) and hearts ($P = 0.02$) of B6 mice and for C3H mice ($P = 0.03$).

terest to examine Lyme carditis in CCR2$^{-/-}$ animals. When we examined *B. burgdorferi* burdens in these mice, we found that levels of *B. burgdorferi* were higher in the hearts of CCR2$^{-/-}$ B6 mice than in those of WT B6 mice (Fig. 1B) and lower in the hearts of CCR2$^{-/-}$ C3H mice than in those of WT C3H mice (Fig. 1B). These data suggest that for B6 mice, in the absence of recruitment of macrophages, *B. burgdorferi* clearance from the heart is impaired or delayed. However, our studies are from a single harvest time point and thus cannot reflect the kinetics of disease progression (Fig. 1B). Interestingly, at 21 days of infection, WT B6 mice have a lower *B. burgdorferi* burden in hearts than WT C3H mice ($P = 0.005$), which may contribute to the overall resistance of B6 mice to the development of Lyme carditis (3). This may reflect either a difference in the kinetics of macrophage-mediated clearance or

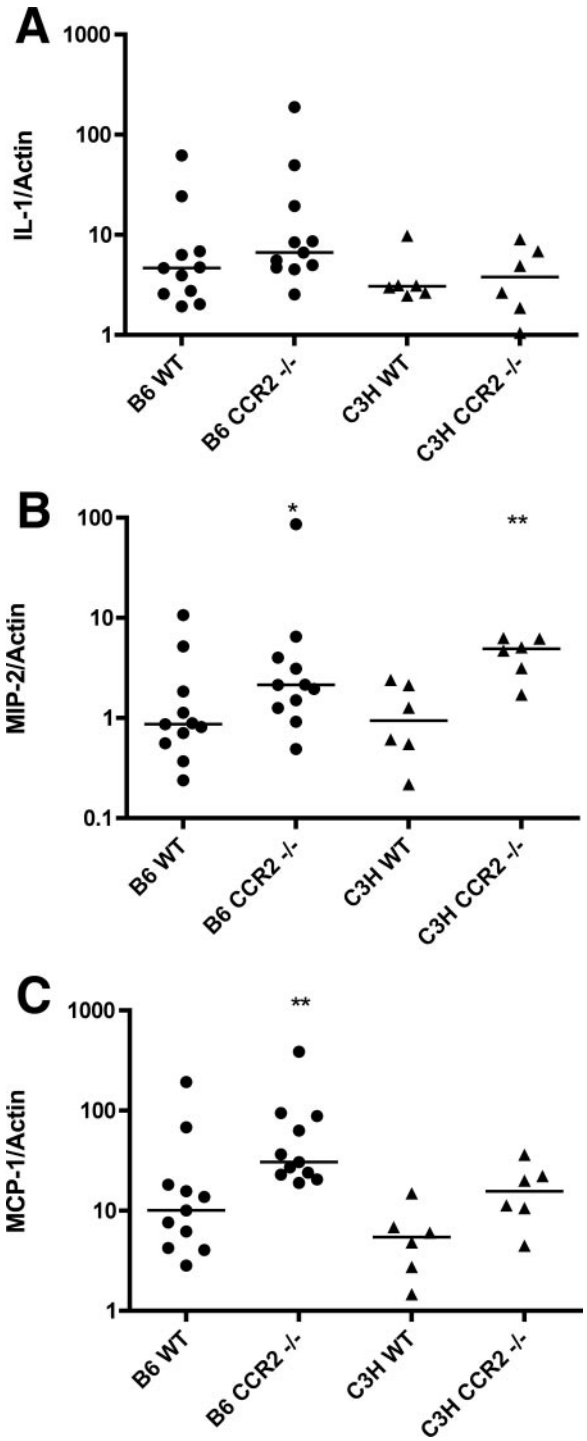
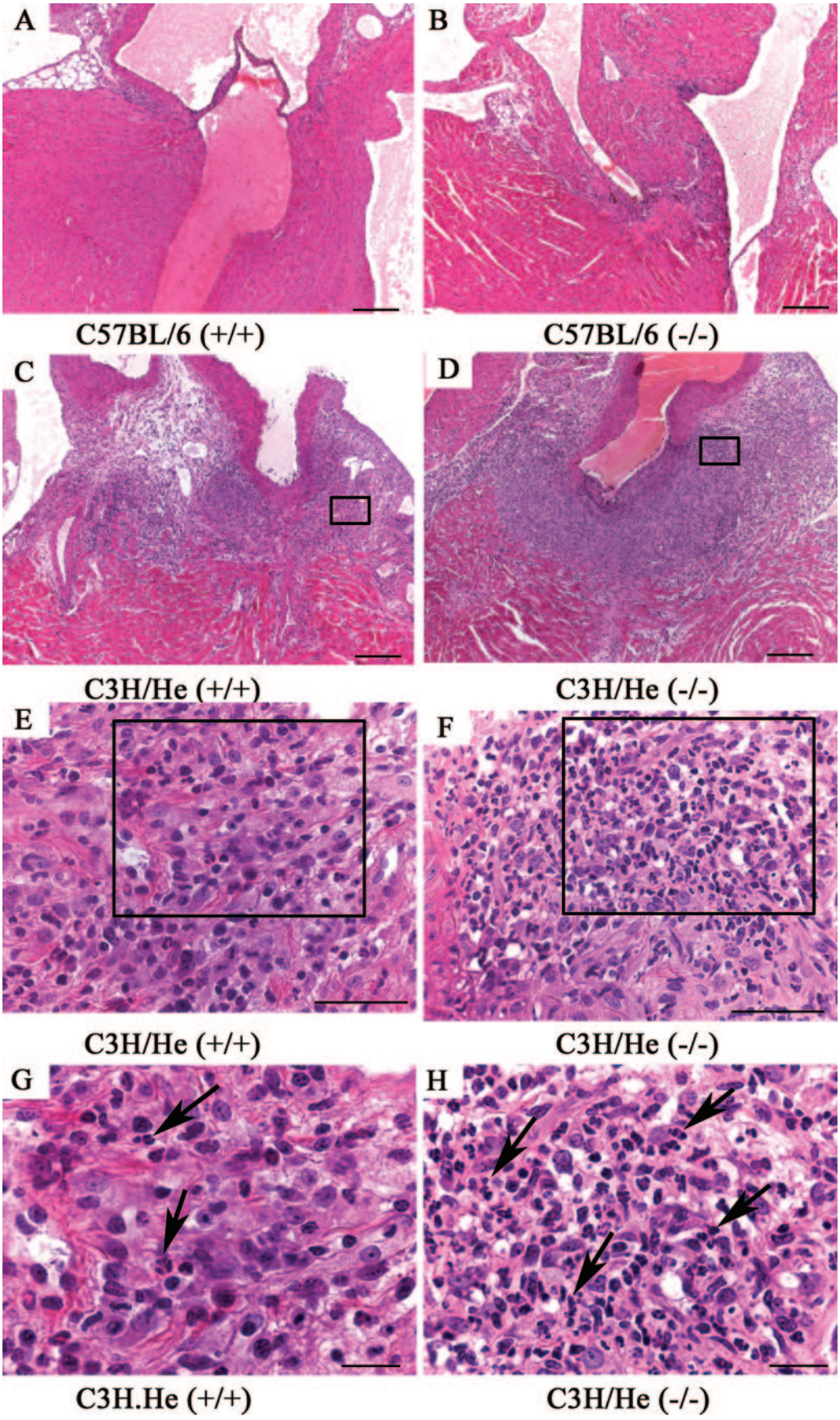


FIG. 2. Cytokine production in hearts. Animals were infected with *B. burgdorferi* and sacrificed at 21 days postinfection. Levels of cytokines and chemokines in infected hearts were assessed by Q-PCR analysis. (A) IL-1; (B) MIP-2; (C) MCP-1. Two separate experiments were performed with 5 to 11 mice/group. Data trends are similar in replicate experiments. Differences between CCR2$^{-/-}$ and WT animals reach statistical significance for MIP-2 in B6 ($P = 0.049$) and C3H ($P = 0.009$) mice and for MCP-1 in B6 mice ($P = 0.006$). Bars indicate median values.


A
B
C57BL/6 (+/+)
C57BL/6 (-/-)
C
D
C3H/He (+/+)
C3H/He (-/-)
E
F
C3H/He (+/+)
C3H/He (-/-)
G
H
C3H.He (+/+)
C3H/He (-/-)

possibly a natural defect in the recruitment or function of macrophages in C3H mice. In addition, the differences in *B. burgdorferi* burden between bladders and hearts suggest the importance of tissue-specific control of spirochetes.

Cytokine and chemokine expression in *B. burgdorferi*-infected hearts. The lack of signaling through CCR2 is likely to have a significant effect on the recruitment and activation of inflammatory cells following *B. burgdorferi* infection. To examine this effect, we first quantified the expression of the proinflammatory cytokine IL-1 in the hearts of infected mice by reverse transcriptase Q-PCR at 21 days postinfection. WT and CCR2$^{-/-}$ animals of both strains expressed IL-1 in infected hearts. Slight increases were noted in CCR2$^{-/-}$ groups from both strains, but these were not statistically significant (Fig. 2A). The similarity of these levels in WT and CCR2$^{-/-}$ hearts suggest that the production of IL-1 may depend largely on resident cells of the heart. Of note, somewhat lower levels of IL-1 were detected in both groups (WT and CCR2$^{-/-}$) of C3H animals than in B6 animals. The higher level of IL-1 produced by B6 mice may contribute to the more efficient resistance of the B6 strain to *B. burgdorferi* infection (3).

To assess the role of inflammatory cell recruitment to the chemokine milieu of the infected heart, we investigated the levels of chemokines in infected tissues in the absence of CCR2 expression. When we examined MIP-2 (CXC chemokine) and MCP-1 (CC chemokine) levels in infected hearts, we observed increases in the levels of both chemokines in the hearts of infected CCR2$^{-/-}$ mice compared to those in WT mice (Fig. 2B and C) for both disease-resistant (B6) and disease-susceptible (C3H) strains of mice. Thus, in the absence of CCR2 signaling, there was a dramatic elevation in the production of chemokines, which may reflect the presence of a feedback mechanism for cell recruitment to infected hearts.

Differential representation of inflammatory cells in the absence of CCR2. To determine the functional role of CCR2-recruited cells in the resolution of Lyme carditis, we compared the severity of cardiac inflammation by histopathologic analysis of hearts from infected WT and CCR2$^{-/-}$ mice of resistant (B6) and sensitive (C3H) strains. We noted that B6 animals had very minimal inflammatory infiltrates following *B. burgdorferi* infection (Fig. 3). The severity of inflammation tended to be higher for WT than for CCR2$^{-/-}$ mice of each strain but was mild for the B6 mice, reaching an inflammation score of only 0.6 on a scale of 1 to 5 (Table 1). In contrast, C3H animals had dramatically higher inflammatory responses, and C3H WT mice were even more severely inflamed than C3H CCR2$^{-/-}$ mice (Fig. 3).

In addition, the nature of the inflammatory infiltrate differed by strain and genotype. All mice had a mixture of lymphocytes and macrophages within the adventitia where the carditis was observed (Fig. 3E to H). Although the severity of inflammatory infiltrates was lower for B6 than for C3H mice, macrophages tended to predominate over lymphocytes in the WT mice and macrophages and lymphocytes were equivalent in the CCR2$^{-/-}$ mice of both strains. There was a significant difference in the level of PMN infiltration by genotype. PMN were essentially absent from the inflammatory response for B6 mice: 9/10 had no PMN infiltration. For the single WT B6 mouse with infiltrating PMN, they comprised less than 10% of the inflammatory infiltrate. The susceptible C3H mice overall had a markedly higher percentage of PMN in the inflammatory infiltrate: 3/4 of the WT C3H mice had infiltrates with as much as 30% PMN (Fig. 3E and G; Table 1). For the C3H CCR2$^{-/-}$ mice, the percentage of PMN was even higher: 3/4 of the mice had 40% or more (as much as 60%) PMN (Fig. 3F and H). One WT C3H mouse had numerous multinucleated giant cells as a component of the inflammatory response, and two WT C3H mice had small numbers of lymphocytes in the ventricular epicardium.

TABLE 1. Inflammatory infiltrates in infected hearts[a]

Mouse strain	Severity score	Avg % PMN
B6		
WT	1.3 ± 0.1	0.6 ± 0.6
CCR2$^{-/-}$	1.2 ± 0.4	0.0 ± 0
C3H		
WT	4.5 ± 0.4	21.4 ± 6.6
CCR2$^{-/-}$	3.9 ± 0.4	28.9 ± 6.8

[a] Mice were infected with *Borrelia burgdorferi* as described in the text and were sacrificed at 21 days (peak disease) for examination of carditis. Severity was scored in hematoxylin and eosin-stained sections using a scale of 1 to 5, where a score of 5 is the most severe. Five mice per group were examined, and 4 sections per heart were assessed for inflammatory infiltrates. Data are averages from four determinations per heart for each group of five animals.

Differential clearance of spirochetes in the absence of CCR2. We examined the course of *B. burgdorferi* infection during the resolution phase for WT and CCR2$^{-/-}$ mice. *B. burgdorferi* burdens for all groups were dramatically lower at day 45 than at peak infection, as expected (Fig. 4). Among C3H mice, however, CCR2$^{-/-}$ mice had higher *B. burgdorferi* burdens in bladders than did WT mice (Fig. 4A) ($P = 0.0037$), suggesting that levels of disseminated spirochetes remain elevated in the absence of CCR2. However, *B. burgdorferi* loads in bladders of the resistant B6 mice were equivalent for WT and CCR2$^{-/-}$ animals, in keeping with the efficient clearance of spirochetes noted at peak infection for that strain (Fig. 1). No increase in *B. burgdorferi* loads was apparent in the hearts of CCR2$^{-/-}$ mice of either strain at day 45 (Fig. 4B), suggesting that clearance of spirochetes and resolution of carditis proceed via alternate mechanisms in the absence of CCR2. *B. burgdorferi* burdens in bladders did not correlate with levels in hearts for C3H or B6 mice during resolution, as was noted previously at peak disease.

FIG. 3. Inflammation in hearts of mice with Lyme borreliosis. Animals (5/group) were infected with *B. burgdorferi* and sacrificed at 21 days as described in the text. Histological analysis of tissue sections of hearts showed minimal inflammation for B6 mice (A and B) and extensive inflammatory infiltrates for C3H mice (C and D). At higher magnifications, macrophages are apparent as the predominant cell type in C3H WT mice (E and G), while more PMN (indicated by arrows) are present in CCR2$^{-/-}$ animals (F and H). Bars, 200 μm for panels A to D, 50 μm for panels E and F, and 20 μm for panels G and H.

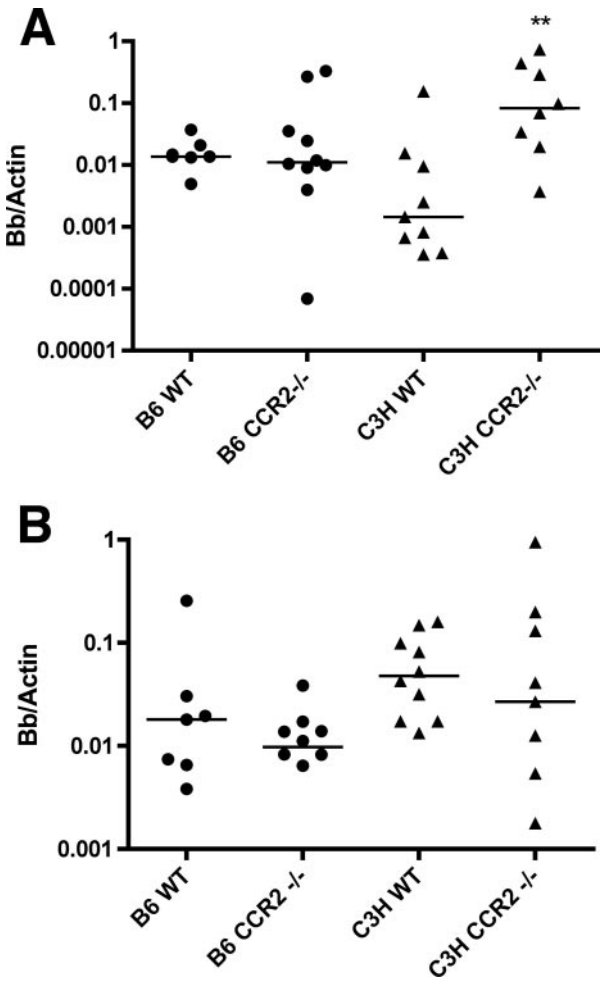


FIG. 4. *B. burgdorferi* burdens in hearts during resolving infection. Animals were infected with *B. burgdorferi* as described in the text and were sacrificed at 45 days. (A) Bladders; (B) hearts. Q-PCR analysis of spirochete numbers shows a higher burden of *B. burgdorferi* in bladders of C3H CCR2$^{-/-}$ mice ($P = 0.004$) but equivalent clearance of *B. burgdorferi* from B6 bladders and from WT and CCR2$^{-/-}$ hearts for both strains. Data, in semilogarithmic plots, are from one experiment using 7 to 10 mice per group. Bars indicate median values.

At peak disease, increased levels of chemokines were detected in CCR2$^{-/-}$ animals of both strains (Fig. 2). At day 45, levels of IL-1, MIP-2, and MCP-1 were similar for WT and CCR2$^{-/-}$ animals of both strains (data not shown). Thus, the absence of effective macrophage recruitment does not dramatically alter the clearance of *B. burgdorferi* or delay the resolution of carditis in those animals, suggesting that the resolution of carditis proceeds via alternate mechanisms in the absence of macrophage recruitment.

DISCUSSION

In Lyme disease, infection of joints and the heart with the same organism in the same host results in quite distinct courses of disease: macrophages predominate in the heart lesion and PMN in infected joints (2, 5, 6, 28). We have shown that macrophages infiltrating the heart are appropriately activated (35) and clear spirochetes efficiently through largely intracellular killing mechanisms (29, 30, 32). Myocarditis resolves at day 30 to 45, and spirochetes are rare at late intervals (2).

The differential recruitment of phagocytic cells to the site of infection is likely to be mediated by specific chemokines. We have previously shown for *B. burgdorferi* infection of CXCR2$^{-/-}$ mice that PMN do not enter the joint and arthritis development is attenuated (8). In the current study, we have examined the development of macrophage-mediated Lyme carditis in CCR2-deficient mice, for whom recruitment of macrophages is impaired (23, 24). We show here that *B. burgdorferi* burdens are significantly increased in infected hearts of B6 CCR2$^{-/-}$ animals at peak disease, confirming the anticipated role for macrophages in the clearance of *B. burgdorferi* in Lyme carditis. However, the elevation of *B. burgdorferi* levels in hearts did not significantly change the degree of inflammation or delay the resolution of carditis for B6 mice. Expression of the chemokines MIP-2 and MCP-1 was increased in CCR2$^{-/-}$ mice of both sensitive and resistant strains, suggesting that local chemokine levels in WT mice may be modulated in a feedback loop by infiltrating cells.

Surprisingly, we found no significant differences in the severity of cardiac pathology between WT and CCR2$^{-/-}$ mice on either a genetically resistant or a susceptible background, although histopathologic examination revealed differences between resistant and sensitive mouse strains. Inflammation was minimal in B6 mice (WT and CCR2$^{-/-}$), and thus, few effects of the absence of CCR2 could be appreciated. However, for C3H mice, which have more-pronounced inflammation, the cellular makeup of the inflammatory infiltrate was changed in CCR2$^{-/-}$ animals. While the overall levels of inflammation remained similar for C3H WT and CCR2$^{-/-}$ animals, there was an increased presence of PMN in C3H CCR2$^{-/-}$ mice, suggesting that PMN recruitment is a compensatory mechanism in the absence of CCR2. The dramatic increase in the recruitment of PMN in CCR2$^{-/-}$ C3H mice was accompanied by an unexpected decrease in *B. burgdorferi* loads. Thus, infected C3H mice have more-efficient clearance of *B. burgdorferi* in the absence of macrophages (CCR2$^{-/-}$) than in their presence (WT). This brings to light a possible natural defect in the recruitment or function of macrophages in WT C3H mice, which may contribute to the increased sensitivity of C3H animals to infection with *B. burgdorferi*.

Although a partial reduction in macrophage functions as a result of silica treatment did not significantly affect murine Lyme carditis as assessed by histology (4), other macrophage deficiencies do contribute to the pathology of infected hearts. Lyme carditis was more severe in the absence of β_2 integrins, which mediate the adhesion of leukocytes to the endothelium and thus entry into infected organs (17, 18). Increased carditis in β_2 integrin-deficient animals may be due to either an increased pathogen burden or increased expression of MCP-1, which has been shown to increase myocarditis (14, 22). Infection of MyD88-deficient mice, which have a defect in macrophage activation via toll-like receptors, results in significantly higher spirochete burdens in the heart but no increase in cardiac inflammatory responses (7, 25). These studies of inhibited or absent macrophages document that the presence of macrophages is critical for the resolution of Lyme carditis in vivo.

New insights into the role of macrophages in carditis are emerging from studies of CCR2$^{-/-}$ mice. Monocytes from

CCR2$^{-/-}$ mice exhibit defects in both activation and recruitment to sites of infection (24), and CCR2$^{-/-}$ mice are more susceptible to infections with *Listeria monocytogenes* (23), *Leishmania major* (37), and *Cryptococcus neoformans* (39). CCR2 may act through mediating the release of monocytes from the bone marrow and not through their extravasation into sites of infection (38). Infection of CCR2$^{-/-}$ mice with *Trypanosoma cruzi* resulted in increased cardiac parasitism but no increase in cardiac inflammation (20). Taken together, these studies and the current results suggest that macrophages are more critical for the efficient control of pathogen burden than for the development of inflammation.

In summary, we have demonstrated that the loss of CCR2 does not alter resistance or susceptibility to Lyme carditis, although it does have an effect on *B. burgdorferi* clearance by B6 animals and on the cellular makeup of the inflammatory infiltrate in C3H mice. The development of carditis by susceptible C3H CCR2$^{-/-}$ mice consists of an increased proportion of PMN in the cellular infiltrate and decreased cardiac *B. burgdorferi* loads compared with those for WT mice, highlighting a possible defect in macrophages of the C3H strain. Multiple factors, including tissue-specific components of the extracellular matrix and a mixture of cytokines and chemokines in the extracellular milieu, provide combinatorial signals for migrating leukocytes, which are augmented by chemokine production from newly recruited cells (11, 19, 40). *B. burgdorferi* disseminates through the bloodstream in vivo, where lipoprotein components interact with endothelial cells via CD14 and TLR2 to activate NF-κB and stimulate the release of IL-8 (1, 10, 13, 16, 21, 43). IL-8 favors the recruitment of PMN, yet in Lyme carditis, macrophages dominate the inflammatory infiltrate (2, 5, 36). Understanding how infection with a single pathogen within a single host results in differential recruitment of phagocytes to infected organs will likely depend on organ-specific microenvironments in vivo and may uncover new avenues for manipulating cell recruitment in inflammatory diseases.

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REFERENCES

1. **Aliprantis, A. O., R. B. Yang, M. R. Mark, S. Suggett, B. Devaux, J. D. Radolf, G. R. Klimpel, P. Godowski, and A. Zychlinsky.** 1999. Cell activation and apoptosis by bacterial lipoproteins through Toll-like receptor-2. Science **285:**736–739.
2. **Armstrong, A. L., S. W. Barthold, D. H. Persing, and D. S. Beck.** 1992. Carditis in Lyme disease susceptible and resistant strains of laboratory mice infected with *Borrelia burgdorferi*. Am. J. Trop. Med. Hyg. **47:**249–258.
3. **Barthold, S. W.** 1991. Infectivity of *Borrelia burgdorferi* relative to route of inoculation and genotype in laboratory mice. J. Infect. Dis. **163:**419–420.
4. **Barthold, S. W., and M. de Souza.** 1995. Exacerbation of Lyme arthritis in Beige mice. J. Infect. Dis. **172:**778–784.
5. **Barthold, S. W., M. de Souza, E. Fikrig, and D. H. Persing.** 1992. Lyme borreliosis in the laboratory mouse, p. 223–242. *In* S. E. Schutzer (ed.), Lyme disease: molecular and immunologic approaches. Cold Spring Harbor Press, Cold Spring Harbor, N.Y.
6. **Barthold, S. W., M. S. de Souza, J. L. Janotka, A. L. Smith, and D. H. Persing.** 1993. Chronic Lyme borreliosis in the laboratory mouse. Am. J. Pathol. **143:**959–972.
7. **Behera, A. K., E. Hildebrand, R. T. Bronson, G. Perides, S. Uematsu, S. Akira, and L. T. Hu.** 2006. MyD88 deficiency results in tissue-specific changes in cytokine induction and inflammation in interleukin-18-independent mice infected with *Borrelia burgdorferi*. Infect. Immun. **74:**1462–1470.
8. **Brown, C. R., V. A. Blaho, and C. M. Loiacono.** 2003. Susceptibility to experimental Lyme arthritis correlates with KC and monocyte chemoattractant protein-1 production in joints and requires neutrophil recruitment via CXCR2. J. Immunol. **171:**893–901.
9. **Brown, C. R., V. A. Blaho, and C. M. Loiacono.** 2004. Treatment of mice with the neutrophil-depleting antibody RB6-8C5 results in early development of experimental Lyme arthritis via the recruitment of Gr-1-polymorphonuclear leukocyte-like cells. Infect. Immun. **72:**4956–4965.
10. **Burns, M. J., T. J. Sellati, E. I. Teng, and M. B. Furie.** 1997. Production of interleukin-8 (IL-8) by cultured endothelial cells in response to *Borrelia burgdorferi* occurs independently of secreted IL-1 and tumor necrosis factor alpha and is required for subsequent transendothelial migration of neutrophils. Infect. Immun. **65:**1217–1222.
11. **Campbell, J. J., and E. C. Butcher.** 2000. Chemokines in tissue-specific and microenvironment-specific lymphocyte homing. Curr. Opin. Immunol. **12:** 336–341.
12. **Charo, I. F., and R. M. Ransohoff.** 2006. The many roles of chemokines and chemokine receptors in inflammation. N. Engl. J. Med. **354:**610–621.
13. **Ebnet, K., K. D. Brown, U. K. Siebenlist, M. M. Simon, and S. Shaw.** 1997. *Borrelia burgdorferi* activates nuclear factor-κB and is a potent inducer of chemokine and adhesion molecule gene expression in endothelial cells and fibroblasts. J. Immunol. **158:**3285–3292.
14. **Fuse, K., M. Kodama, H. Hanawa, Y. Okura, M. Ito, T. Shiono, S. Maruyama, S. Hirono, K. Kato, K. Watanabe, and Y. Aizawa.** 2001. Enhanced expression and production of monocyte chemoattractant protein-1 in myocarditis. Clin. Exp. Immunol. **124:**346–352.
15. **Garcia, R., L. Gusmani, R. Murgia, C. Guarnaccia, M. Cinco, and G. Rottini.** 1998. Elastase is the only human neutrophil granule protein that alone is responsible for in vitro killing of *Borrelia burgdorferi*. Infect. Immun. **66:** 1408–1412.
16. **Georganas, C., H. Liu, H. Perlman, A. Hoffmann, B. Thimmapaya, and R. M. Pope.** 2000. Regulation of IL-6 and IL-8 expression in rheumatoid arthritis synovial fibroblasts: the dominant role for NF-κB but not C/EBP β or c-Jun. J. Immunol. **165:**7199–7206.
17. **Guerau-de-Arellano, M., J. Alroy, D. Bullard, and B. T. Huber.** 2005. Aggravated Lyme carditis in CD11a$^{-/-}$ and CD11c$^{-/-}$ mice. Infect. Immun. **73:**7637–7643.
18. **Guerau-de-Arellano, M., J. Alroy, and B. T. Huber.** 2005. β2 integrins control the severity of murine Lyme carditis. Infect. Immun. **73:**3242–3250.
19. **Guerau-de-Arellano, M., and B. T. Huber.** 2005. Chemokines and Toll-like receptors in Lyme disease pathogenesis. Trends Mol. Med. **11:**114–120.
20. **Hardison, J. L., W. A. Kuziel, J. E. Manning, and T. E. Lane.** 2006. Chemokine CC receptor 2 is important for acute control of cardiac parasitism but does not contribute to cardiac inflammation after infection with *Trypanosoma cruzi*. J. Infect. Dis. **193:**1584–1588.
21. **Hirschfeld, M., C. J. Kirschning, R. Schwandner, H. Wesche, J. H. Weis, R. M. Wooten, and J. J. Weis.** 1999. Inflammatory signaling by *Borrelia burgdorferi* lipoproteins is mediated by Toll-like receptor 2. J. Immunol. **163:**2382–2386.
22. **Kolattukudy, P. E., T. Quach, S. Bergese, S. Breckenridge, J. Hensley, R. Altschuld, G. Gordillo, S. Klenotic, C. Orosz, and J. Parker-Thornburg.** 1998. Myocarditis induced by targeted expression of the MCP-1 gene in murine cardiac muscle. Am. J. Pathol. **152:**101–111.
23. **Kurihara, T., G. Warr, J. Loy, and R. Bravo.** 1997. Defects in macrophage recruitment and host defense in mice lacking the CCR2 chemokine receptor. J. Exp. Med. **186:**1757–1762.
24. **Kuziel, W. A., S. J. Morgan, T. C. Dawson, S. Griffin, O. Smithies, K. Ley, and N. Maeda.** 1997. Severe reduction in leukocyte adhesion and monocyte extravasation in mice deficient in CC chemokine receptor 2. Proc. Natl. Acad. Sci. USA **94:**12053–12058.
25. **Liu, N., R. R. Montgomery, S. W. Barthold, and L. K. Bockenstedt.** 2004. Myeloid differentiation antigen MyD88 deficiency impairs pathogen clearance but does not alter inflammation in *Borrelia burgdorferi*-infected mice. Infect. Immun. **72:**3195–3203.
26. **Lusitani, D., S. E. Malawista, and R. R. Montgomery.** 2002. *Borrelia burgdorferi* are susceptible to killing by a variety of PMN components. J. Infect. Dis. **185:**797–804.
27. **Lusitani, D. L., S. E. Malawista, and R. R. Montgomery.** 2003. Calprotectin, an abundant cytosolic protein from human polymorphonuclear leukocytes, inhibits the growth of *Borrelia burgdorferi*. Infect. Immun. **71:**4711–4716.
28. **Malawista, S. E.** 2005. Lyme disease, p. 2645–2664. *In* W. J. Koopman and L. W. Moreland (ed.), Arthritis and allied conditions: a textbook of rheumatology, 15th ed. Lippincott Williams & Wilkins, Philadelphia, PA.
29. **Montgomery, R. R., D. L. Lusitani, A. de Boisfleury Chevance, and S. E. Malawista.** 2002. Human phagocytic cells in the early innate immune response to *Borrelia burgdorferi*. J. Infect. Dis. **185:**1773–1779.
30. **Montgomery, R. R., and S. E. Malawista.** 1996. Entry of *Borrelia burgdorferi* into macrophages is end-on and leads to degradation in lysosomes. Infect. Immun. **64:**2867–2872.
31. **Montgomery, R. R., M. H. Nathanson, and S. E. Malawista.** 1994. Fc and non Fc-mediated phagocytosis of *Borrelia burgdorferi* by macrophages. J. Infect. Dis. **170:**890–893.

32. **Montgomery, R. R., M. H. Nathanson, and S. E. Malawista.** 1993. The fate of *Borrelia burgdorferi*, the agent for Lyme disease, in mouse macrophages: destruction, survival, recovery. J. Immunol. **150:**909–915.
33. **Montgomery, R. R., R. E. Palmarozza, D. S. Beck, E. Ngo, K. A. Joiner, and S. E. Malawista.** 2000. Functional competence of peritoneal macrophages in murine Lyme borreliosis. Inflammation **24:**277–288.
34. **Montgomery, R. R., K. Schreck, X. Wang, and S. E. Malawista.** 2006. Human neutrophil calprotectin reduces the susceptibility of *Borrelia burgdorferi* to penicillin. Infect. Immun. **74:**2468–2472.
35. **Montgomery, R. R., X. Wang, and S. E. Malawista.** 2001. Murine Lyme disease: no evidence for active immune downregulation in resolving or subclinical infection. J. Infect. Dis. **183:**1631–1637.
36. **Ruderman, E. M., J. S. Kerr, S. R. Telford III, A. Spielman, L. H. Glimcher, and E. M. Gravallese.** 1995. Early murine Lyme carditis has a macrophage predominance and is independent of major histocompatibility complex class II-CD4$^+$ T cell interactions. J. Infect. Dis. **171:**362–370.
37. **Sato, N., S. K. Ahuja, M. Quinones, V. Kostecki, R. L. Reddick, P. C. Melby, W. A. Kuziel, and S. S. Ahuja.** 2000. CC chemokine receptor (CCR)2 is required for Langerhans cell migration and localization of T helper cell type 1 (Th1)-inducing dendritic cells. Absence of CCR2 shifts the *Leishmania major*-resistant phenotype to a susceptible state dominated by Th2 cytokines, B cell outgrowth, and sustained neutrophilic inflammation. J. Exp. Med. **192:**205–218.
38. **Serbina, N. V., and E. G. Pamer.** 2006. Monocyte emigration from bone marrow during bacterial infection requires signals mediated by chemokine receptor CCR2. Nat. Immunol. **7:**311–317.
39. **Traynor, T. R., W. A. Kuziel, G. B. Toews, and G. B. Huffnagle.** 2000. CCR2 expression determines T1 versus T2 polarization during pulmonary *Cryptococcus neoformans* infection. J. Immunol. **164:**2021–2027.
40. **Vaday, G. G., S. Franitza, H. Schor, I. Hecht, A. Brill, L. Cahalon, R. Hershkoviz, and O. Lider.** 2001. Combinatorial signals by inflammatory cytokines and chemokines mediate leukocyte interactions with extracellular matrix. J. Leukoc. Biol. **69:**885–892.
41. **Wang, G., C. Ojaimi, R. Iyer, V. Saksenberg, S. A. McClain, G. P. Wormser, and I. Schwartz.** 2001. Impact of genotypic variation of *Borrelia burgdorferi* sensu stricto on kinetics of dissemination and severity of disease in C3H/HeJ mice. Infect. Immun. **69:**4303–4312.
42. **Ward, D. M., G. M. Griffiths, J. C. Stinchcombe, and J. Kaplan.** 2000. Analysis of the lysosomal storage disease Chediak-Higashi syndrome. Traffic **1:**816–822.
43. **Wooten, R. M., V. R. Modur, T. M. McIntyre, and J. J. Weis.** 1996. *Borrelia burgdorferi* outer membrane protein A induces nuclear translocation of nuclear factor-κB and inflammatory activation in human endothelial cells. J. Immunol. **157:**4584–4590.
44. **Yang, L., J. H. Weis, E. Eichwald, C. P. Kolbert, D. H. Persing, and J. J. Weis.** 1994. Heritable susceptibility to severe *Borrelia burgdorferi*-induced arthritis is dominant and is associated with persistence of large numbers of spirochetes in tissues. Infect. Immun. **62:**492–500.
45. **Zimmer, G., U. E. Schaible, M. D. Kramer, G. Mall, C. Museteanu, and M. M. Simon.** 1990. Lyme carditis in immunodeficient mice during experimental infection of *Borrelia burgdorferi*. Virchows Archiv. A **417:**129–135.

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