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Matrix Metalloproteinase 9 Plays a Key Role in Lyme Arthritis but Not in Dissemination of *Borrelia burgdorferi*▽

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Borrelia burgdorferi*, the causative agent of Lyme arthritis, does not produce any exported proteases capable of degrading extracellular matrix despite the fact that it is able to disseminate from a skin insertion site to infect multiple organs. Prior studies have shown that *B. burgdorferi* induces the host protease, matrix metalloproteinase 9 (MMP-9), and suggested that the induction of MMP-9 may allow the organism to disseminate and produce local tissue destruction. We examined the role of MMP-9 in dissemination of *B. burgdorferi* and pathogenesis of Lyme arthritis. In a MMP-9$^{-/-}$ mouse model, MMP-9 was not required for the dissemination of the spirochete to distant sites. However, MMP-9$^{-/-}$ exhibited significantly decreased arthritis compared to wild-type mice. The decrease in arthritis was not due to an inability to control infection since the spirochete numbers in the joints were identical. Levels of inflammatory chemokines and cytokines were also similar in MMP-9$^{-/-}$ and wild-type mice. We examined whether decreased inflammation in MMP-9$^{-/-}$ mice may be the result of decreased production of neoattractants by MMP-9-dependent cleavage of collagen. MMP-9 cleavage of type I collagen results in increased monocyte chemoattraction. MMP-9 plays an important role in regulating inflammation in Lyme arthritis, potentially through the cleavage of type I collagen.

Borrelia burgdorferi, the causative agent of Lyme disease, causes a vast array of clinical symptoms in the human host including but not limited to erythema migrans skin lesions, meningitis, myocarditis, and arthritis (5, 12, 50). In order to establish infection, the spirochete migrates from the initial site of inoculation, spreads into the surrounding skin, and subsequently enters the bloodstream, where it can colonize more distal organs. While this dissemination almost certainly requires breakdown of cellular barriers and digestion of extracellular matrix (ECM) proteins, the sequenced *B. burgdorferi* genome does not encode for any spirochetal genes that share homology with known exported proteases that degrade the ECM. One class of host molecules that may aid in the dissemination of the spirochete during infection is the matrix metalloproteinases (MMPs). MMPs are zinc and calcium ion-dependent, secreted proteases that function in the degradation and remodeling of ECM (42).

MMP-9 (also known as gelatinase B) is a 92-kDa enzyme whose substrates include collagen (types I, II, IV, V, and VII), elastin, and gelatin (18, 39, 48). MMP-9 is upregulated in erythema migrans in the skin of patients with acute Lyme disease and in in vitro models of skin infection (54). It has been proposed to play an important role in the dissemination of the spirochete from the initial site of infection (54). Supporting the notion that MMP-9 may promote the dissemination of the spirochete, activated MMP-9 released from human peripheral monocytes and human neutrophils has been shown to promote the translocation of *B. burgdorferi* through matrices of type I and type IV collagen and laminin (27). However, the role of MMP-9 in dissemination has not been confirmed in an animal or human model.

In addition to a potential role in dissemination of *B. burgdorferi*, MMPs may play a role in the development of some of the signature manifestations of Lyme disease, including arthritis and carditis. MMPs have been shown to play a role in many different forms of arthritis and can be responsible for the degradation of bone and cartilage tissue. Increased amounts of MMP-9 have been found in the synovial fluids of some Lyme arthritis patients, as well as in cartilage explants; however, in vitro expression from chondrocytes were not seen after *B. burgdorferi* infection, suggesting that the source of MMP-9 may be from other infiltrating cells (7, 32). Using gene arrays of infected joints, which contain multiple cell types, Crandall et al. (19) found that MMP-9 was significantly increased at 2 weeks but not at 4 weeks postinfection in C3H mice. Changes in the balance of activated to inactive MMP-9 were not examined. *B. burgdorferi* has been shown to induce the MMP-9 activator, MMP-3, in the joint tissues of infected mice (7, 19).

Activated MMP-9 may play a role in modulation of inflammation through its actions on inflammatory mediators. MMPs can have multiple effects on inflammation through their action in cleaving and activating or inactivating chemokines, cytokines, and their receptors. In addition, certain MMPs, including MMP-8, MMP-12, and MMP-14, have been shown to cleave ECM proteins to release peptides with chemoattractive and/or inflammatory properties (24, 31, 41). Because of the potential for opposing effects on inflammation, it is difficult to predict the inflammatory responses in MMP-9 deficient mice to various challenges (43). To date, different types of patho-

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gens tested in MMP-9 deficient mice have shown different responses in terms of control of infection and level of inflammation with *Staphylococcus aureus* and *Escherichia coli* in an abdominal sepsis model causing greater inflammation, while *Francisella tularensis*, *Mycobacterium tuberculosis*, and *Chlamydia muridarum* infection showed decreased inflammation in MMP-9$^{-/-}$ mice (13, 34, 40, 45, 51). These findings suggest that the effect of MMP-9 may be both pathogen and tissue specific.

We were interested in determining the effects of MMP-9 deletion in an animal model of Lyme disease. We report here the impact of MMP-9 on the ability of the organism to disseminate to distant sites and on the development of murine arthritis and carditis.

MATERIALS AND METHODS

Mice and *B. burgdorferi* infection. MMP-9-deficient mice on the C57BL/6 background were backcrossed for 10 generations onto a C3H/HeN background. Homozygous MMP-9$^{-/-}$ mice were bred to maintain the colony and used for all subsequent experiments. Wild-type (WT) C3H/HeN mice that were used for backcrossing and as controls were purchased from Charles River Laboratories (Wilmington, MA). The MMP-9-deficient mice were all genotyped as previously described (53). All mouse protocols were approved by the Tufts University Institutional Animal Care and Use Committee. Four-week-old mice were infected intradermally with *B. burgdorferi* (5×10^4, strain N40) by needle inoculation and sacrificed at 3.5 weeks postinfection. Successful infection of each mouse was confirmed by culturing ear tissue in Barbour-Stoenner-Kelly H at various times after needle inoculation of *B. burgdorferi*. Cultures were monitored by dark-field microscopy as previously described (7). Ankle thickness measurements were obtained at various intervals after infection using previously described methods (38).

Quantitative PCR of *B. burgdorferi* and inflammatory markers. DNA from the ankle joint and heart tissue was prepared by using the DNeasy blood and tissue kit (Qiagen, Valencia, CA), and real-time PCR was performed to assess the number of copies of the *recA* gene using known standards as previously described (8). The copy numbers of *recA* were normalized to the copy number of the mouse housekeeping gene, *nidogen*, as determined by real-time PCR using known standards in order to control for the total amount of tissue isolated.

For analysis of mRNA gene expression from tissues, ankle joints were dissected and homogenized in TRIzol (Invitrogen, Carlsbad, CA) by using 5-mm steel beads (Qiagen) in a bead beater. The samples were then centrifuged to remove the debris from the homogenization, and total RNA was purified by using TRIzol according to the manufacturer's instructions. The isolated RNA was then treated with DNase to remove any contamination with genomic DNA. The DNA-free RNA was then converted to cDNA using random hexamer primers in a reverse transcription reaction (Improm II reverse transcriptase; Promega, Madison, WI). Quantification of cDNA from specific mRNA transcripts was determined by real-time quantitative reverse transcription-PCR (iCycler; Bio-Rad, Hercules, CA) with SYBR green reagents (Quantitect SYBR green PCR; Qiagen) as previously described (7). The specific primers and their annealing temperatures were used as previously described (6). Contamination of cDNA with DNA was checked by using primers that spanned an intron.

Histological analysis of infected tissues. Each ankle was measured three times, and the average of each measurement was determined. For histological staining, tissues were fixed in Bouin's solution for 1 week, and then the hind left leg was skinned, cut in half longitudinally, decalcified, and embedded in paraffin. Sections were then obtained and the slides were stained with hematoxylin and eosin (H&E) by the Rodent Histopathology Core at Harvard Medical School. The slides were then blindly scored for inflammation on a scale of 1 to 4, with a score of 1 representing the least amount of inflammation and a score of 4 representing the greatest amount of inflammation.

Chemotaxis assays. Purified activated MMP-9 (Calbiochem, San Diego, CA) was incubated with either type I, II, IV, or V collagen (1 mg/ml; Sigma, St. Louis, MO) for 4 h at 37°C. Successful cleavage of collagen was confirmed by Coomassie blue staining of sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels. Human peripheral blood mononuclear cells (PBMC) were isolated from whole blood by using a Histopaque column according to the manufacturer's instructions. The cleaved substrates were subsequently mixed with Dulbecco modified Eagle medium and placed in the lower chamber of the modified Boyden chamber (Neuroprobe, Gaithersburg, MD). *N*-Formyl-methionyl-leucyl-phenylalanine at 10^{-8} M was used as a positive control for the chemotaxis of isolated PBMC. An 8 μM polycarbonate filter (Neuroprobe, Gaithersburg, MD) was placed over the lower chamber, and the chamber was assembled. PBMC were added to the top chamber. The chamber was subsequently incubated at 37°C with 5% CO_2 for 1.5 h. The chamber was disassembled, and the membrane was removed. Unbound PBMC were removed by washing the membrane with phosphate-buffered saline and scraping it with a rubber scraper according to the manufacturer's instructions. The membrane was fixed with methanol and stained with Wright stain. Samples were performed in quadruplicate wells, and the migration of PBMC into the membrane was analyzed under ×100 magnification using Metamorph cell counting software (Molecular Devices, Sunnyvale, CA).

TABLE 1. Number of mice that were ear culture positive during the 4-week time course after *B. burgdorferi* infection[a]

Mouse strain	No. of ear punch-positive mice/total no. of mice tested at:			
	Wk 1	Wk 2	Wk 3	Wk 4
WT	0/7	7/7	7/7	7/7
MMP-9 KO	0/7	4/7	7/7	7/7

[a] Ear punches were obtained at 1-week intervals, cultured in BSK media, and spotted on a slide for scoring by dark-field microscopy. Five random fields of view were examined for the presence of spirochetes in the culture.

RESULTS

MMP-9 is not required for the dissemination of *B. burgdorferi*. MMP-9 is increased in human erythema migrans lesions and shown to be involved in the ability of the organism to penetrate through layers of an artificial ECM. To determine whether MMP-9 was required for dissemination of *B. burgdorferi* in vivo, we infected MMP-9$^{-/-}$ mice and their WT controls with *B. burgdorferi* by needle inoculation in the middle of the lower back. Dissemination to a distant skin site was measured by the culturing of ear punch biopsies at 1-week intervals. As shown in Table 1, no cultures were positive at 1 week. At 2 weeks seven of seven wild-type and four of seven MMP-9$^{-/-}$ were positive for *B. burgdorferi*, and by 3 weeks all mice were positive by ear culture. The difference at 2 weeks was not statistically significant ($P < 0.1$, Fisher exact score).

We also examined the quantification of the organism at two sites where it has a predilection: the heart and the ankle joint. To quantify the amount of spirochetes present at these two sites, mice were sacrificed at 3.5 weeks after infection. We isolated DNA from the homogenized ankle joint and heart tissue and performed quantitative real-time PCR for the *B. burgdorferi* gene *recA*. As shown in Fig. 1, the spirochetal burden in both the ankle joint and the heart was nearly identical and showed no statistical difference between the MMP-9-deficient and WT control mice.

MMP-9 deficiency results in decreased arthritis. C3H/HeN mice infected with *B. burgdorferi* develop severe arthritis of the ankle and knee joints. MMP-9$^{-/-}$ and WT mice on a C3H/HeN background were infected with *B. burgdorferi* by needle inoculation. Ankle thickness was measured weekly using calipers. As shown in Fig. 2A and B, ankle swelling for the MMP-9-deficient mice was statistically significantly reduced in the MMP-9 $^{-/-}$ mice compared to the WT controls. The levels of swelling begin to diverge at 2 weeks and are statistically significantly different through 3.5 weeks.

To determine whether ankle swelling correlated with his-

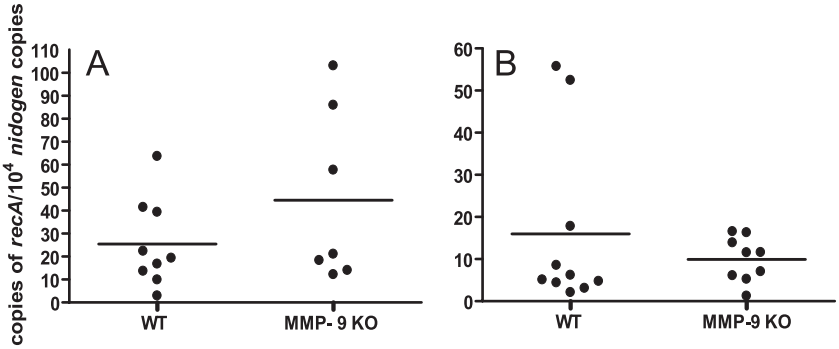
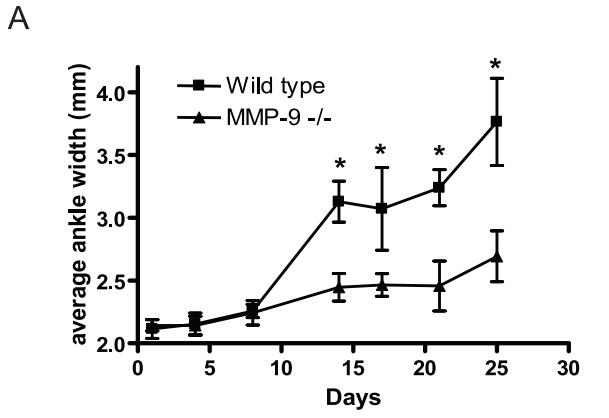
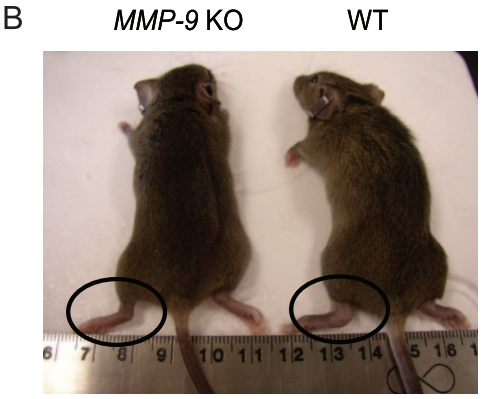
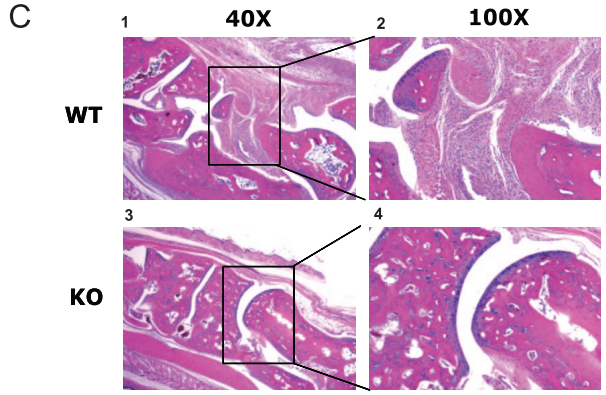


FIG. 1. MMP-9 is not required for the control of infection in the hearts or joints of mice. WT and MMP-9 KO mice were infected with *B. burgdorferi* by needle inoculation. Mice were sacrificed at 3.5 weeks postinfection. Total DNA from ankle joints and heart tissues was isolated and analyzed by quantitative PCR to quantify the number of *B. burgdorferi recA* copies using a known *recA* standard. The number of copies of *recA* was normalized to 10^4 copies of the mouse housekeeping gene *nidogen*. No significant difference was seen in the numbers of *B. burgdorferi* in the ankles ($P = 0.21$) (A) or hearts ($P = 0.40$) (B) of WT and MMP-9 KO mice. *P* values reflect the unpaired Student *t* test.

topathological changes, mice were sacrificed at the peak of inflammation (3.5 weeks), and the ankle joints were sectioned, stained with H&E, and scored in a blinded fashion on a scale of 1 (no inflammation) to 4 (severe inflammation) by a pathologist (Fig. 2C and D). MMP-$9^{-/-}$ mice showed significantly less inflammation than WT controls (a knockout [KO] average score of 1.8 versus a WT average score 2.9, $P < 0.03$ [Fisher exact score]). The composition of the infiltrate (mononuclear







D

Pathology of MMP-$9^{-/-}$ mice infected with *B. burgdorferi*

genotype	Avg score ± SD (no. of mice)	
	Ankle	Heart
MMP-9 +/+	2.9 ± 1.18 (16)	2.1 ± 0.915 (16)
MMP-9 -/-	1.8 ± 1.08 (16)	2.0 ± 0.925 (16)
P	<0.03	0.69

FIG. 2. MMP-9-deficient mice develop less arthritis than WT counterparts. (A) WT and MMP-$9^{-/-}$ mice were infected with *B. burgdorferi* by needle inoculation. Ankle thicknesses were measured by using low-pressure calipers designed for measurements of mouse ankles. Measurements at each time point were taken three times, and the average of the three was recorded. Each point represents the average measurement from seven mice (two experiments). Error bars represent the standard deviation. *, $P < 0.001$ (unpaired Student *t* test). (B) Ankle joints from representative WT and KO mice. (C) Representative H&E-stained sections of ankle joints from WT and KO mice. The magnified box insets are shown in boxes 2 and 4. The ankle joints and hearts were processed and stained for H&E as described in Materials and Methods and then blindly scored for pathology on a scale of 1 (normal) to 4 (severe inflammation). Inflammation scores in WT and KO mice were compared by using the Fisher exact test.

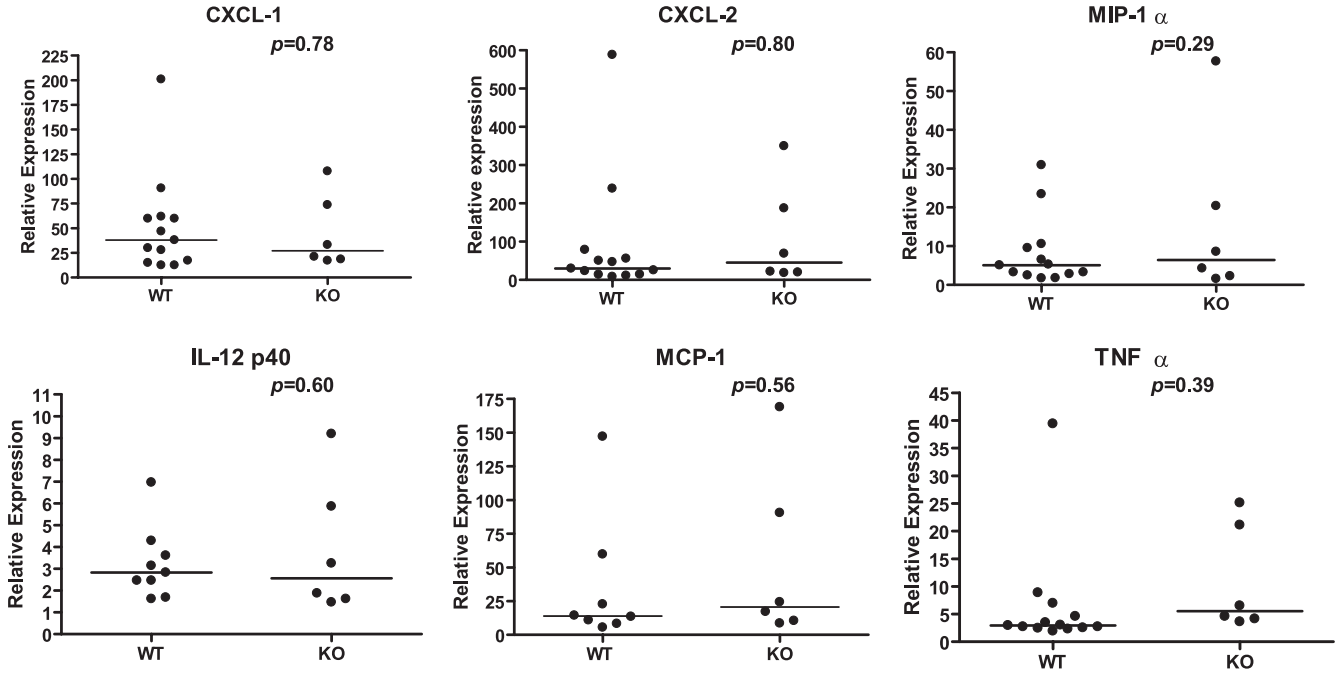


FIG. 3. Comparison of cytokine induction by *B. burgdorferi* infection in the ankles of MMP-9$^{-/-}$ and WT mice. Mice were infected with *B. burgdorferi* and sacrificed after 3.5 weeks of infection. Total RNA was isolated from homogenized ankle joints and reverse transcribed into cDNA as described in Materials and Methods. The amount of transcript for each chemokine or cytokine was normalized to the amount of mouse nidogen in the sample, and the relative expression was calculated by arbitrarily setting the level of transcript in uninfected mice to a value of 1. The *P* values comparing the results for MMP-9$^{-/-}$ and MMP-9$^{+/+}$ mice were calculated by using the unpaired Student *t* test. No significant differences were measured in the induction of CXCL-1, CXCL-2, MCP-1, IL-12 p40, MIP-1α, or TNF-α in the ankle joints of *B. burgdorferi*-infected MMP-9$^{-/-}$ or MMP-9$^{+/+}$ mice.

and polymorphonuclear cells) was similar between MMP-9$^{-/-}$ and WT joints (data not shown).

MMP-9 deficiency does not affect carditis. A second major component of Lyme disease in the murine mouse model is carditis. Hearts from MMP-9$^{-/-}$ and WT mice infected with *B. burgdorferi* for 3.5 weeks were sectioned and stained with H&E. The heart sections were then blindly scored on the same 1 to 4 scale for degrees of inflammation and pathology. Unlike the decreased inflammation seen in the joints (Fig. 2D), there was no statistical difference in the pathology of the hearts from the MMP-9 $^{-/-}$ mice compared to the WT controls (Fig. 2D).

Chemokine and cytokine transcriptional expression is similar in ankles from MMP-9$^{-/-}$ and MMP$^{+/+}$ mice. We next wanted to determine whether decreases in inflammation in the joints and hearts were due to decreases in the induction of chemokines and cytokines. It has previously been reported that CXC chemokine ligand 1 (CXCL-1), CXCL-2, the macrophage inflammatory protein 1α (MIP-1α), the monocyte chemoattractant protein 1 (MCP-1), the proinflammatory cytokines tumor necrosis factor alpha (TNF-α), and interleukin-12 (IL-12) p40 are increased in joints and heart tissues from mice infected with *B. burgdorferi* (6, 10). Induction of these cytokines was closely related to the spirochete tissue burden and to histological inflammation (6).

We examined the transcriptional expression levels of these chemokines and cytokines that have been related to *B. burgdorferi* infection. As shown in Fig. 3, there were no statistical differences in any of the transcript levels for CXCL-1, CXCL-2, MIP-1α, MCP-1, TNF-α, or IL-12 p40 between *B. burgdorferi*-infected MMP-9$^{-/-}$ mice versus WT mice. Thus, the transcriptional levels of these chemokines and cytokines cannot explain the dramatic differences in swelling and inflammation that were seen between the MMP-9$^{-/-}$ mice and the WT controls. This finding suggests that MMP-9 is playing some additional crucial role in coordinating the innate immune response to the site of *B. burgdorferi* infection that is independent of the spirochetal burden and/or the chemokine and cytokine response.

Type I collagen cleaved by MMP-9 results in the formation of a neochemotactic peptide. MMPs, but not MMP-9, have been shown to cleave collagens to create neopeptides with chemoattractive attributes (20, 47). To determine whether MMP-9 is capable of cleaving ECM protein in the joint to create chemoattractive peptides, we cleaved collagen types I, II, IV, and V with MMP-9 and tested the cleaved products for their chemoattractive properties for PBMC using a modified Boyden chamber. Concentrations of collagen were selected based on prior studies of chemotaxis of PBMC toward collagen cleaved with other proteases. MMP-9 cleavage of collagen type I significantly increased chemoattraction of PBMC compared to uncleaved collagen (Fig. 4). Cleavage of the other collagens did not significantly increase chemoattraction (Fig. 4).

DISCUSSION

Previous studies have implicated MMP-9 as playing an important role in the dissemination of *B. burgdorferi* during the course of infection. In these experiments, we report that

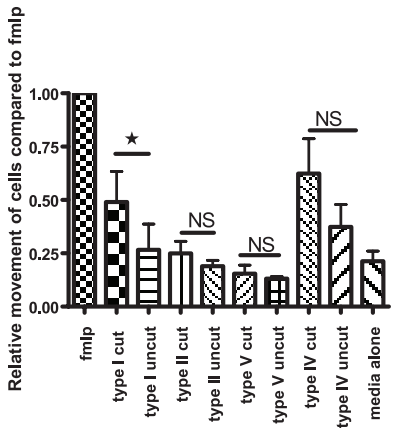


FIG. 4. Release of chemotactic molecules after cleavage of collagen with MMP-9. Type I, II, IV, and V collagens were digested in vitro by activated MMP-9. Digestion of collagen was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Coomassie blue staining (data not shown). Chemotaxis of PBMC toward the digested collagen was measured using a modified Boyden chamber. The polycarbonate membrane containing the migrated cells was fixed, stained, and photographed, and the number of cells that migrated into the membrane was measured by determining the optical density as described in Materials and Methods. The error bars represent standard error of the mean. NS, not significant; *, $P < 0.05$ (paired Student *t* test).

MMP-9 was not required for the dissemination of the spirochete since the time to dissemination to distant sites and the spirochetal burden in the hearts and ankle joints of MMP-$9^{-/-}$ mice were similar to those found in MMP-$9^{+/+}$ mice. There was a slight trend toward earlier dissemination in the WT mice versus the MMP-$9^{-/-}$ mice. Gebbia et al. (27) have previously shown that MMP-9 could enhance penetration through ECM components in vitro. Thus, while we cannot rule out that MMP-9 plays some role in dissemination, it is clearly not required. It is certainly possible that MMP-9 is one of a number of different but redundant proteases utilized by *B. burgdorferi* in order to digest ECM proteins to spread from the site of inoculation. *B. burgdorferi* binds plasmin, which is also capable of digesting ECM proteins directly, as well as activating MMP-9 and other MMPs. In vitro, plasmin increases penetration of *B. burgdorferi* through ECM proteins by direct digestion and by activation of other MMPs (16, 17, 29, 32, 33, 35, 36). Studies in plasmin(ogen)-deficient mice also did not show an effect on dissemination of the organism to distant organs, although there was a slight effect on bacteremia (15).

In spite of similar spirochetal tissue burdens, the MMP-$9^{-/-}$ mice showed significantly reduced swelling and pathology in the ankle joints compared to the WT controls. Because the mice were backcrossed to the arthritis-susceptible C3H background from a resistant C57BL/6 background, there is a small chance that a retained gene from C57BL/6 accounts for the lack of arthritis. However, this is very unlikely with 10 backcrosses. In addition, the proinflammatory chemokine and cytokine induction by *B. burgdorferi* were similar between the two groups of mice, suggesting that the signals to attract inflammatory cells to the areas of *B. burgdorferi* localization are similar in the WT and KO mice. There are several potential explanations for why inflammation may be decreased in MMP-$9^{-/-}$ mice despite similar levels of inflammatory cytokines.

One possibility is that MMP-9 is utilized by inflammatory cells to break down ECM barriers and follow chemotactic gradients to areas where the organism has localized. In this scenario, the lack of MMP-9 would result in inflammatory cells being unable to respond to chemoattractive signals due to their inability to degrade and maneuver through the ECM. There are some data to suggest that MMP-9-mediated degradation of the ECM is essential for the immune effector cells to extravasate to the site of infection (21). In the absence of this degradation, cellular honing to the site of infection does not occur, and inflammation is consequently dampened. However, this remains controversial since other studies have reported different results for the requirement of MMP-9 ECM degradation in this process. These studies showed similar movement of effector cells in response to either IL-8 or murine TNF-α in spite of the absence of MMP-9 (3, 22). With *B. burgdorferi* infection, we observed an effect of MMP-9 deficiency on inflammation in the joint but not in the heart. This suggests to us that the ability of inflammatory cells to migrate was not compromised in the absence of MMP-9. However, we cannot rule out the possibility that MMP-9 is required for migration into the joint space but not the heart due to differences in tissue composition. However, since neither the joint space or cardiac tissue present specific barriers to cell migration through the endothelium, it is less likely that any tissue differences account for a difference in migration.

An alternative possibility is that the proteolytic activity of MMP-9 is required to activate an important inflammatory factor. MMP-9 cleavage of certain chemokines has been shown to increase their potency as proinflammatory molecules. For example, truncation of the amino-terminal end of IL-8 by MMP-9 potentiates its chemotactic properties by tenfold and the mouse neutrophilic chemoattractant GCP-2/LIX by twofold (52). MMP-9 can also change the proinflammatory environment created by specific cytokines. Activation of IL-1β, a potent proinflammatory cytokine, from its dormant precursor by MMP-9 also potentiates the innate immune response through the removal of its activity-blocking prodomain (46). Similarly, TNF-α is proteolytically cleaved from its 233-amino-acid anchored form to its 157-amino-acid activated soluble form (25, 26). Increases in potency of chemokines through cleavage and activation by MMP-9 would not be detected by the transcriptional PCR analysis we performed. Thus, it is possible that, in MMP-$9^{-/-}$ mice, a similar profile of proinflammatory chemokines and cytokines is upregulated by the presence of the organism; however, without further processing by MMP-9, many of the inflammatory molecules may not be fully activated.

In addition to directly cleaving chemokines and cytokines, MMP-9 also can control inflammation by controlling the release of other soluble inflammatory mediators that are usually bound to the ECM. Once released from the ECM, these mediators then create a concentration gradient that can attract the innate immune effector cells to the site of infection. An example of this type of MMP-9 inflammatory regulation involves the release of proinflammatory proteoglycans from the ECM. Upon stimulation with CXCL-12, HeLa cells upregulate MMP-9, which in turn can cause the release of specific pro-

teoglycans called syndecans (11). These shed syndecans have been shown to mediate inflammation (23).

Recent research has revealed that the components of the ECM do not merely provide scaffolding for other cells but can in fact play a direct role in immune modulation. Fragments of degraded ECM components have been shown to mediate inflammation by serving as chemotactic peptides that attract innate immune effector cells to the site of infection (2). Collagen, elastins, laminins, and fibronectins which are all substrates of MMP-9, have been shown to become chemotactic after they are degraded by various proteases (including bacterial collegenase, human neutrophil elastase, MMP-2, and thermolysin), with the resulting peptide fragments having chemotactic properties for both neutrophils and macrophages (1, 14, 28, 37, 44, 49). This was suggested as a possible mechanism for the decreased inflammation seen in MMP-9$^{-/-}$ mice challenged with *F. tularensis*. MMP-9$^{-/-}$ mice showed lower levels of the neutrophil proinflammatory tripeptide Pro-Gly-Pro, which is a by-product of ECM degradation by MMP-9 (40).

To explore the hypothesis that MMP-9 may be degrading components of the ECM, and collagen more specifically, we digested various forms of collagen with MMP-9 and tested whether these fragments could cause the chemotaxis of human PBMC and potentially play a role in the progression of Lyme arthritis. We found that degraded type I collagen and potentially type IV collagen were chemotactic after digestion with MMP-9. To our knowledge, this is the first report of MMP-9 cleavage of collagen resulting in the development of a chemotactic molecule(s). However, although intriguing, the fact that MMP-9 cleavage of collagen in vitro results in increased chemotaxis, does not confirm that this is the mechanism for MMP-9-associated increases in inflammation. Of note, in our studies of murine Lyme arthritis, we found differences in inflammation between the WT and MMP-9 $^{-/-}$ mice in the ankle joints but not in the heart. Both type I and type IV collagen are located at these sites (9, 30); however, given the differences in tissue structure and the accessibility of the collagen to MMP-9, the resultant concentrations of the chemotactic molecule may be different. Alternatively, we may have missed a difference in inflammation in the hearts since we only looked for carditis 4 weeks postinfection. Murine Lyme-induced carditis usually peaks around 2 weeks postinfection, whereas arthritis peaks at 3 to 4 weeks (4). By sacrificing the mice at 3.5 weeks, we would have missed any differences that are detectable only at the peak of inflammation.

An interesting point that is raised by our data is whether the recruitment of large numbers of inflammatory cells is needed to control spirochete infection. In spite of the lower amounts of macrophages and neutrophils in the infiltrate, the spirochetal burdens in the two groups of mice were remarkably similar. Other studies have shown that the converse may also be true. Heavy inflammatory cell infiltration in MyD88$^{-/-}$ mice was not effective in clearing bacteria and these mice showed 1- to 2-log higher bacterial burdens despite an increase in inflammatory cells. In our study, loss of MMP-9 that resulted in decreased inflammation did not hinder the ability of the animal to control infection to WT levels. Given that damage to host tissues in *B. burgdorferi* infection occurs largely through the action of host proteases rather than proteases produced by the bacteria, these data suggest that it may be possible to limit inflammation and cellular damage without hindering immune mediated clearance of the organism. Further studies will be required to understand the contributions of MMPs and host immune cells to cellular damage and control of *B. burgdorferi* infection.

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