INFECTION AND IMMUNITY, June 2007, p. 3062–3069 Vol. 75, No. 6
0019-9567/07/$08.00+0 doi:10.1128/IAI.00202-07

CD14 Mediates Cross Talk between Mononuclear Cells and Fibroblasts for Upregulation of Matrix Metalloproteinase 9 by *Borrelia burgdorferi*[▽]

Zhihui Zhao,* Rhonda Fleming, Bilaal McCloud, and Mark S. Klempner

Section of Infectious Diseases, Department of Medicine, Boston University Medical Center, Boston, Massachusetts 02118

Received 6 February 2007/Returned for modification 13 March 2007/Accepted 22 March 2007

Lyme disease is an infection caused by a tick-borne spirochete, *Borrelia burgdorferi*. Matrix metalloproteinase 9 (MMP-9) was selectively upregulated in the erythema migrans skin lesions of patients with acute Lyme disease. In this study, the mechanism of upregulation of MMP-9 was investigated in vitro and in vivo. The concentrations of MMP-9 and soluble CD14 were markedly elevated in serum from patients with acute Lyme disease and were also upregulated in U937 cells by *B. burgdorferi* in a time- and concentration-dependent manner. MMP-9 mRNA was expressed at baseline in fibroblasts in the presence or absence of *B. burgdorferi*. However, when fibroblasts were incubated with supernatants from U937 cells with *B. burgdorferi* or recombinant CD14, the expression of MMP-9 was significantly increased. This effect was completely abolished by the anti-CD14 antibody. These data suggest that the upregulation of MMP-9 by *B. burgdorferi* involves the CD14 pathway in infiltrating inflammatory cells. Fibroblasts could be recruited to amplify local production of MMP-9 by acquiring CD14 from macrophages.

Lyme disease is an infection caused by a tick-borne spirochete, *Borrelia burgdorferi*. It is the most common vector-borne disease in the United States (18, 37). The hallmark of acute Lyme disease is the characteristic skin lesions, known as erythema migrans (EM) skin lesions. Other acute clinical manifestations result from dissemination of the spirochete to the central and peripheral nervous systems, the heart, and the musculoskeletal system (7, 21, 36).

Some bacteria that disseminate from a skin inoculation site secrete enzymes (e.g., collagenases and elastases) that are thought to participate in the spreading process by disrupting the extracellular matrix barrier (10, 22, 38). *B. burgdorferi* does not secrete any enzymes capable of digesting collagen, laminin, or gelatin (22). We have demonstrated that expression of matrix metalloproteinase 9 (MMP-9) was selectively upregulated in human EM skin lesions of acute Lyme disease. The activated fibroblasts and infiltrating mononuclear cells were the major sources of local MMP-9 production (43). Infiltration of the skin with mononuclear cells is frequently observed in EM lesions of acute Lyme disease (3). We hypothesize that bacterial induction of host proteases may play a major role in the dissemination of *B. burgdorferi* (43).

CD14 on blood monocytes mediate monocyte/macrophage activation by lipopolysaccharide (LPS). LPS bound to CD14 may contribute to atherogenesis by stimulating macrophages to produce tumor necrosis factor alpha, interleukin 1 (IL-1), IL-6, IL-8, IL-12, alpha interferon, migration inhibitory factors, chemokines, eicosanoids, and reactive oxygen species, which in turn stimulate the production of a second wave of chemokines, cytokines, and adhesion and signaling molecules. The mature CD14 protein is present in two isoforms: membrane bound and soluble.

Membrane-associated CD14 (mCD14) is expressed on myeloid cells, including tissue macrophages, monocytes, promonocytes, and activated granulocytes. It is a glycoprotein with a molecular mass of 55 kDa, attached to the cell membrane by a glycosylphosphatidylinositol (GPI) anchor that lacks both transmembrane and cytoplasmic domains (4, 25). It has been implicated in mediating inflammatory responses to many pathogen-derived ligands, including gram-positive bacterial peptidoglycan (PGN) and lipoteichoic acid (9, 42), which is the major component of the cell wall of gram-positive bacteria and lipoproteins from spirochetes (35).

Soluble CD14 (sCD14) varies from about 43 to 53 kDa and lacks the GPI anchor but has the same amino acid sequence as mCD14. Both mCD14 and sCD14 can function as receptors for LPS of gram-negative bacteria and from various cell wall products of gram-positive bacteria. sCD14 enhances mCD14-mediated cell activation by LPS and PGN by binding LPS and PGN and then by transferring LPS and PGN to mCD14 (15). sCD14 is a proinflammatory molecule which is increased in inflammation (24). The effects of CD14 in the patients of acute Lyme disease, especially the relationship between CD14 and MMP-9, are not completely understood. In this study, we explored the effects of CD14 in pathogenesis of acute Lyme disease and the relationship between CD14 and MMP-9.

MATERIALS AND METHODS

Patients. Serum was obtained from patients who met the Centers for Disease Control and Prevention clinical criteria for the diagnosis of acute Lyme disease. Serum was also obtained from healthy donors. Written informed consent was obtained from each study subject. Human experimentation guidelines of the U.S. Department of Health and Human Services and of the Boston University and Boston Medical Center institutional review boards were followed in the conduct of this research.

* Corresponding author. Mailing address: Boston Medical Center, EBRC 6th Floor, 650 Albany St., Boston, MA 02118. Phone: (617) 414-7965. Fax: (617) 414-5280. E-mail: zhao@bu.edu.

▽ Published ahead of print on 2 April 2007.

Antibodies and recombinant proteins. Monoclonal anti-human MMP-9, monoclonal anti-human MMP-2, monoclonal anti-human CD14 antibodies, recombinant MMP-9, recombinant MMP-2, and recombinant CD14 (rCD14) (endotoxin level, <0.1 ng per 1 μg of the cytokines, determined by the *Limulus* amebocyte lysate method) were purchased from R&D Systems (Minneapolis, MN). Polyclonal antibody against Toll-like receptor 2 (TLR2), polyclonal antibody against TLR4, polyclonal antibody against CD14, normal human immunoglobulin G1 (IgG1), and mouse IgG1 were purchased from Santa Cruz Biotechnology (Santa Cruz).

Culture of spirochetes. Low passages (passages 2 to 5) of *B. burgdorferi* strain N40 or B31 were cultured in Barbour-Stoenner-Kelly medium (Sigma). All spirochetes were grown to logarithmic phase, harvested by centrifugation, washed three times, and resuspended in cell culture medium.

Cell culture. U937 cells (ATCC CRL-1593.2; ATCC, Manassas, VA) were cultured in RPMI 1640 medium supplemented with 10 mM HEPES, 1 mM sodium pyruvate, 4.5 g/liter glucose, 1.5 g/liter sodium bicarbonate, and 10% fetal bovine serum (FBS). The human fibroblasts (ATCC SCRC-1042.2) were cultured in Dulbecco's modified Eagle medium with 4.5 g/liter glucose, 1.5 g/liter sodium bicarbonate, and 10% FBS. The cells of three to four passages at 80% confluence were changed to fresh medium containing 10% FBS or 1% FBS and subjected to various treatments.

Zymography. Gelatin zymography was performed using 10% Tris-glycine gels with 0.1% gelatin incorporated as a substrate according to the manufacturer's instructions (Invitrogen). The supernatants from the cultured cells were diluted with deionized water and sodium dodecyl sulfate sample buffer (2×) without heating prior to loading into gels. For inhibition studies 1,10-phenanthroline (10 mM) was included in the developing buffer prior to overnight incubation at 37°C. Gels were stained with Coomassie blue (0.5% [wt/vol]) and destained using an acetic acid destaining solution. Gelatinase activities were visualized as clear bands indicating proteolysis of the substrate. Zymographic results were confirmed by three or more replicate experiments. The Kodak Scientific Image System (Eastman Kodak) was used to quantify the data.

Isolation of RNA. Total RNA from the cultured cells was isolated using TRIZOL (Life Technologies) according to the manufacturer's instructions. Briefly, the cultured cells were lysed with TRIZOL reagent and were then mixed with chloroform, incubated on ice for 5 min, and centrifuged at 12,000 × *g* for 15 min at 4°C. The aqueous phase containing the RNA was transferred to a fresh tube and the RNA precipitated with isopropyl alcohol and centrifuged. The pellet containing the RNA was washed with ethanol and dissolved in RNase-free water. The amount and purity of the RNA were determined by measurement of optical density 260/280 and RNA electrophoresis on denaturing agarose gels.

MMP mRNA determination. Human MMP Nonrad-GEArrays (SuperArray Inc.) was used to analyze the mRNA expression of MMPs. Total RNA from the cultured cells was converted to biotinylated cDNA probes by reverse transcription with a deoxyribonucleoside triphosphate mix containing biotin-16-2′-deoxyuridine-5′-triphosphate (Biotin-dUTP). Biotinylated cDNA probes were hybridized to gene-specific cDNA fragments spotted on the membranes. The GEArray membrane was then blocked with GEAblocking solution and incubated with alkaline phosphatase-conjugated streptavidin. The relative expression levels of multiple MMP genes were detected by chemiluminescence using the alkaline phosphatase substrate, CDP-Star. The membrane was exposed to X-ray film for various lengths of time to determine the abundances of different transcripts. Semiquantitative expressions of genes were done by using Quantity One Software (Bio-Rad) and normalized with the internal controls, glyceraldehyde-3-phosphate dehydrogenase or β-actin.

Real-time RT-PCR. Real-time reverse transcription-PCR (RT-PCR) was performed with a TaqMan ABI 7000 sequence detection system (Applied Biosystems) using the TaqMan One-Step RT-PCR Master Mix reagents kit according to the manufacturer's instructions. 18S rRNA proprietary fluorescent dye-minor groove binder (VIC-MGB; Applied Biosystems) served as an internal control to assess the overall cDNA content. The oligonucleotide primers (300 nM) and probes (250 nM) were used for this study. PCR primers and TaqMan probes specific for the target molecules were designed using Primer Express software (Applied Biosystems). MMP-9 primers were as follows: 391F, 5′-GATCCAAAA CTACTCGGAAGACTTG-3′; 454R, 5′-GAAGGCGCGGGCAAA-3′; MMP-9 probe, 417T, 6FAM-CGCGGGCGGTGATTGACGAC-TAMRA. CD14 primers were as follows: 879F, 5′-CGCTCCGAGATGCATGTG-3′; 938R, 5′-AGCCCAGCG AACGACAGAT-3′; CD14 probe, 6FAM-CCAGCGCCCTGAACTCCCTCT-TA MRA. TLR2 primers were as follows: 2085F, 5′-CTACTGGGTGGAGAACCTTAT GGT-3′; 2160R, 5′-CCGCTTATGAAGACACAACTTGA-3′; TLR2 probe, 6FAM-CAGGAGCTGGAGAACTTCAATCCCCC-TAMRA.

Thermal cycling parameters for use with the TaqMan One-Step RT-PCR Master Mix reagents kit were as follows: reverse transcription, 48°C for 30 min; AmpII *Taq* Gold activation, 95°C for 10 min; there were 40 PCR cycles. A PCR cycle consisted of heating to 95°C for 15 s to denature and 60°C for 1 min for annealing/extension. Relative gene expression was calculated manually from the exported results, following the instructions of the ABI PRISM 7000 sequence detection system's manufacturer.

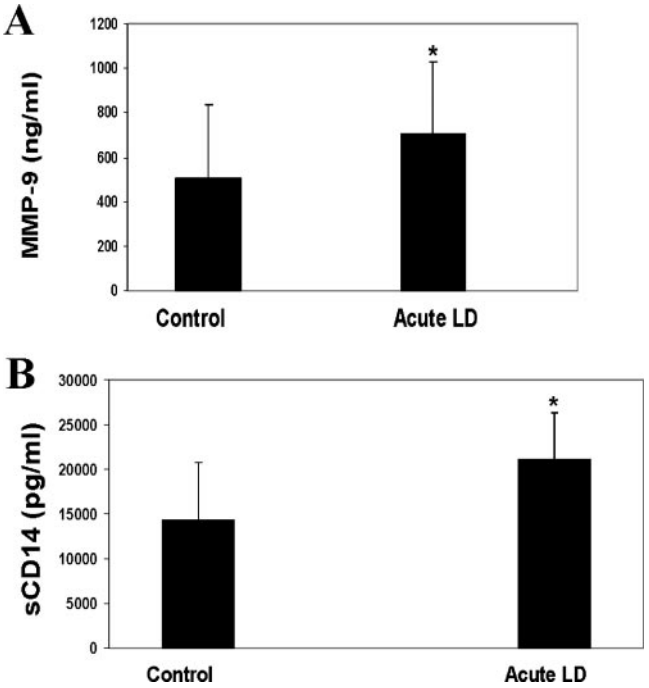

FIG. 1. Concentrations of MMP-9 and sCD14 in serum from patients with acute Lyme disease. (A) Concentration of MMP-9 in serum from patients with acute Lyme disease (LD) (n = 43) compared to that for healthy controls (n = 29), determined by using ELISA. *, $P < 0.05$. (B) Concentration of sCD14 from the patients with acute Lyme disease (n = 8) compared to that for healthy controls (n = 8), determined by using ELISA. *, $P < 0.05$. ELISAs were repeated twice in duplicate.

Western blot analysis. The cell lysates or supernatants from the cultured cells were electrophoresed on a 4 to 20% sodium dodecyl sulfate-polyacrylamide gel and electrophoretically transferred to polyvinylidene fluoride membranes. Membranes were blocked overnight at 4°C with 5% nonfat milk in TBST (10 mM Tris-HCl, pH 7.5, 140 mM NaCl, 1.5 mM $MgCl_2$, 0.05% Tween 20) and incubated with the primary antibodies for 1 h. After three washes in TBST (10 min for each wash), the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies in TBST. Immunoreactivity was detected using an enhanced chemiluminescence system (Amersham).

Enzyme-linked immunosorbent assay (ELISA). Human MMP-9 (total) and sCD14 (R&D Systems, Minneapolis, MN) were measured by immunoassay according to the manufacturer's instructions. Briefly, the serum was diluted 200-fold for sCD14 and 100-fold for MMP-9 using a calibrator diluent. The optical density of each well was determined within 30 min, using a microplate reader set to 450 nm and 540 nm.

Statistics. The results were presented as means ± standard deviations and were analyzed using the Student t test. Statistical significance was determined to be a P value of <0.05.

RESULTS

Concentrations of MMP-9 and sCD14 in serum from the patients with acute Lyme disease. The concentration of MMP-9 and/or sCD14 in serum from patients with acute Lyme disease was determined by ELISA. As shown in Fig. 1, the concentrations of both of MMP-9 (Fig. 1A) and sCD14 (Fig.

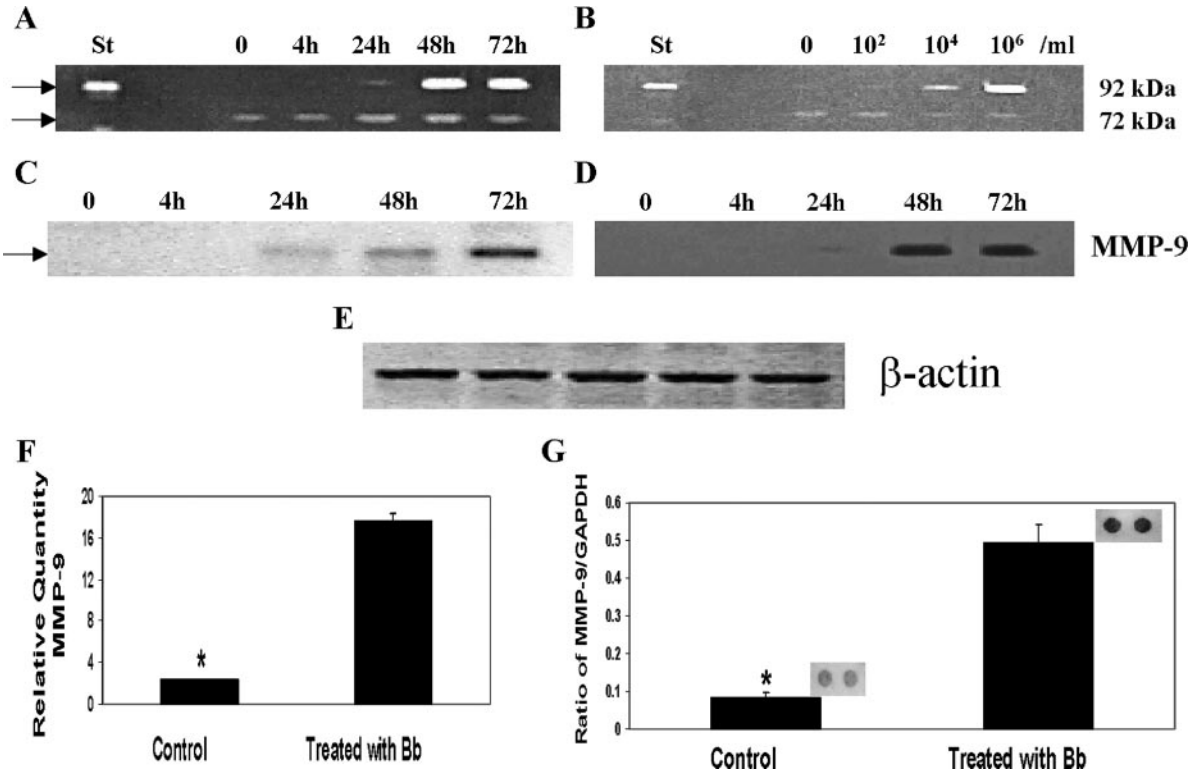

FIG. 2. Upregulation of MMP-9 by stimulation of *B. burgdorferi* in U937 cells. U937 cells were incubated in the presence or absence of *B. burgdorferi* (10^6 organisms/ml) for 0 to 72 h or of *B. burgdorferi* (0 to 10^6 organisms/ml) for 48 h. The activities of 92-kDa and 72-kDa proteins with stimulation by *B. burgdorferi* were analyzed by zymography in a time (A) or concentration (B) pattern (St, standard). Western blotting was performed with monoclonal antibody against human MMP-9 (92 kDa) or with monoclonal antibody against human MMP-2 (72 kDa; data not shown) in the supernatants (equal volume, 10 μl/well) (C) or in the cell lysates (D). β-Actin was used as a control (E). mRNA expression of MMP-9 in U937 cells with *B. burgdorferi* was assayed using real-time RT-PCR (F) or a targeted gene array of MMPs (G). *, $P < 0.01$; $n = 3$. Zymography and Western blot experiments were repeated three times, and representative experiments are shown. Real-time RT-PCR and targeted gene array experiments were repeated three times, and data presented are mean values for three separate experiments performed in duplicate.

1B) were significantly elevated for patients with acute Lyme disease compared to levels for healthy controls ($P < 0.05$).

Upregulation of MMP-9 by stimulation of *B. burgdorferi* in U937 cells and fibroblasts. As assessed by gelatin zymography, the upregulation of the 92-kDa protein by *B. burgdorferi* was time and concentration dependent in U937 cells. The activity of the 72-kDa protein was not significantly different in the presence or absence of *B. burgdorferi* (Fig. 2A and B). Western blot analysis demonstrated that not only was the expression of MMP-9 (92 kDa) in the supernatants collected from U937 cells elevated (Fig. 2C), the expression of MMP-9 (92 kDa) was also increased in U937 cell lysates, as determined using monoclonal antibody against MMP-9 (Fig. 2D) and monoclonal antibody against MMP-2 (for MMP-2, 72 kDa, data not shown). β-Actin was used as a control (Fig. 2E). All gelatinolytic activity was inhibited when zymography was performed in the presence of 10 mM 1,10-phenanthroline, a specific inhibitor of MMPs. Stimulation of MMP-9 production by *B. burgdorferi* in U937 cells was not affected by 50 μg/ml polymyxin B, an inhibitor of LPS activation (data not shown).

The mRNA expression of MMP-9 was also significantly increased in U937 cells in the presence of *B. burgdorferi*, as determined using real-time RT-PCR (Fig. 2F) and human MMP Nonrad-GEArrays (Fig. 2G).

Functional activity of MMP-9 was not detected in fibroblasts in the presence or absence of *B. burgdorferi* by using gelatin zymography and a targeted gene array (data not shown). However, MMP-9 mRNA expression was detected when the more-sensitive technique, real-time RT-PCR, was used. The expression of MMP-9 was not changed in fibroblasts with *B. burgdorferi*, using real-time RT-PCR (Fig. 3). Nonetheless, when fibroblasts were incubated with the conditional medium (CM) (0.5 ml/ml) from the supernatants of U937 cells with *B. burgdorferi*, the expression of MMP-9 was significantly increased compared to results for fibroblasts with normal cultured medium (1 ml) (Fig. 3). Furthermore, MMP-9 expression in fibroblasts with conditional medium (0.5 ml/ml) was much higher when *B. burgdorferi* was added than for fibroblasts with normal cultured medium (1 ml) in the presence of *B. burgdorferi* (Fig. 3). However, there was no effect in the activity of MMP-9 in fibroblasts when the supernatants from U937 cells without *B. burgdorferi* were used.

Regulation of CD14 expression by stimulation of *B. burgdorferi* in U937 cells and fibroblasts. We assessed whether the expression of MMP-9 in U937 cells and fibroblasts with *B. burgdorferi* involved a CD14 signaling pathway. The U937 cells and fibroblasts were incubated in the presence or absence of *B. burgdorferi*. In Western blot analysis, not only the expression of

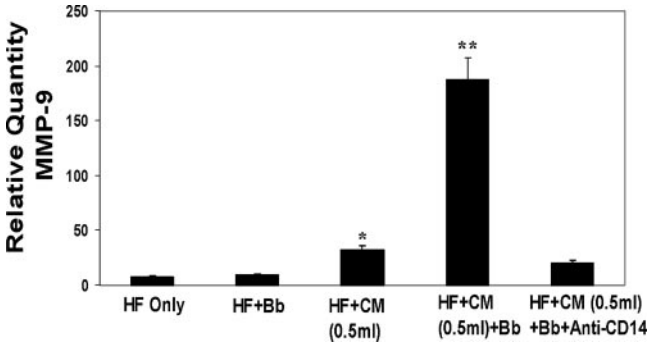

FIG. 3. mRNA expression of MMP-9 in fibroblasts. Fibroblasts were incubated in the presence or absence of supernatants of cultured U937 cells with *B. burgdorferi* (CM, 0.5 ml/ml) in the presence or absence of *B. burgdorferi*. mRNA expression of MMP-9 was analyzed by real-time RT-PCR. HF, human fibroblasts; Bb, *Borrelia burgdorferi*. For HF plus CM versus HF only, *, $P < 0.05$; for HF plus CM plus Bb versus HF plus CM or HF plus Bb, **, $P < 0.01$; $n = 5$. The real-time RT-PCR experiments were repeated five times, and data presented are mean values for five separate experiments performed in duplicate.

mCD14 (55 kDa) (Fig. 4A) but also the expression of sCD14 (48 kDa) (Fig. 4C and D, equal volume of 10 μl/well of supernatants) in U937 cells was elevated in the presence of *B. burgdorferi* in a time- and dose-dependent manner. β-Actin was used as a control (Fig. 4B). The expression pattern of CD14 showed the same time-dependent trend as that of MMP-9 by stimulation of *B. burgdorferi*.

The mRNA expression of CD14 was also significantly increased from U937 cells in the presence of *B. burgdorferi*, using real-time RT-PCR (Fig. 4E). Moreover, the expression of TLR2 was not significantly changed from U937 cells in the presence of *B. burgdorferi* (Fig. 4F). However, the expression of CD14 (mCD14 or sCD14) in fibroblasts was not found in the presence or absence of *B. burgdorferi* (data not shown).

Effect of anti-CD14 antibody on upregulation of MMP-9 by *B. burgdorferi* in U937 cells and fibroblasts. To determine whether the upregulation of MMP-9 by *B. burgdorferi* was associated with the CD14 signaling pathway specifically, the U937 cells and/or fibroblasts were cultured with or without anti-CD14 antibody (monoclonal or polyclonal antibodies). When anti-CD14 antibody was added to U937 cells, the upregulation of MMP-9 by *B. burgdorferi* was partially blocked in a concentration-dependent manner (Fig. 5A and B).

As mentioned above, not only the expression of mCD14 (Fig. 4A) but also the expression of sCD14 (Fig. 4C and D) in U937 cells was elevated in the presence of *B. burgdorferi* in a time- and dose-dependent manner as determined by Western blotting. When fibroblasts were incubated with CM (containing CD14), the expression of MMP-9 was significantly increased. Furthermore, the expression of MMP-9 was much higher when *B. burgdorferi* was added than for fibroblasts with normal cultured medium. This effect was completely blocked by anti-CD14 antibody (Fig. 3).

Recombinant CD14 directly activates MMP-9 enzymatic activity in fibroblasts. To determine whether the upregulation of MMP-9 in fibroblasts was associated with the expression of CD14, we incubated the fibroblasts with or without rCD14 (endotoxin level, <0.1 ng per 1 μg of the cytokines, determined by the LAL method). Indeed, the activity of MMP-9 was significantly elevated when fibroblasts were preincubated with rCD14 itself, and the activity of MMP-9 was much increased, especially in the presence of rCD14 and with *B. burgdorferi*, as

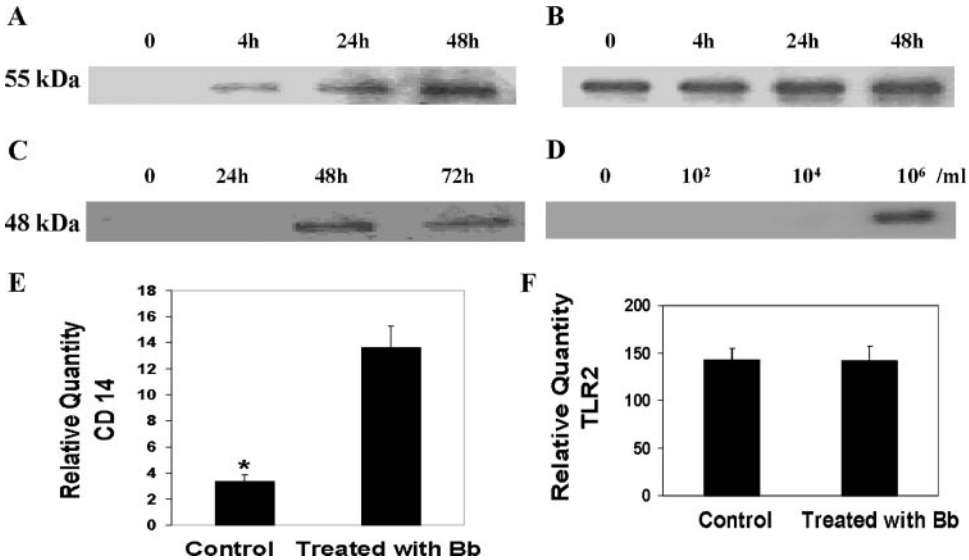

FIG. 4. Upregulation of CD14 by stimulation of *B. burgdorferi* in U937 cells. Western blotting was performed with cell lysates of U937 with or without *B. burgdorferi* (10^6 organisms/ml) using monoclonal antibody against human CD14 (55 kDa; membrane CD14) (A) or β-actin (B). Western blotting was also performed with supernatants of U937 cells with or without *B. burgdorferi* with a time-dependent (C) or concentration-dependent (D) pattern (48 kDa; sCD14) (equal volume of 10 μl/well of supernatants). RNA from treated U937 cells was isolated. mRNA expression of CD14 (E) or TLR2 (F) was determined in U937 cells with or without *B. burgdorferi*, using real-time RT-PCR. *, $P < 0.01$; $n = 3$. Western blot experiments were repeated three times, and representative experiments are shown. Real-time RT-PCR experiments were repeated three times, and data presented are mean values of three separate experiments performed in duplicate.

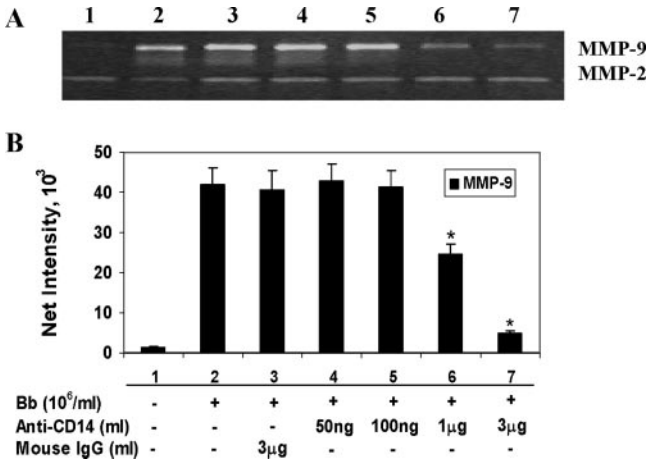

FIG. 5. Effect of anti-CD14 antibody on production of MMP-9. (A) Gelatinolytic activity of MMP-9 in U937 cells. Lane 1, medium control; lanes 2 to 7, *Borrelia burgdorferi* (Bb), 10^6 organisms/ml (lane 2, Bb only; lane 3, Bb plus mouse IgG [3 μg/ml]; lane 4, Bb plus monoclonal anti-CD14 antibody [50 ng/ml]; lane 5, Bb plus monoclonal anti-CD14 antibody [100 ng/ml]; lane 6, Bb plus monoclonal anti-CD14 antibody [1 μg/ml]; lane 7, Bb plus monoclonal anti-CD14 antibody [3 μg/ml]). Zymography experiments were repeated three times, and representative experiments are shown. (B) Quantitative analysis of activity of MMP-9 from values in all three separate experiments, using Kodak digital image analysis software. For 6 and 7 versus 2, *, $P < 0.01$; $n = 3$.

determined by zymography (Fig. 6A and B). Expression of MMP-9 was also significantly upregulated by rCD14 as determined using real-time RT-PCR (Fig. 6C). The effect was completely abolished by anti-CD14 antibody (Fig. 6A, B, and C). MMP-9 production by rCD14 with or without *B. burgdorferi* in fibroblasts was not affected by 50-μg/ml polymyxin B, an inhibitor of LPS activation.

DISCUSSION

We have demonstrated that the expression of MMP-9 was selectively upregulated in human EM skin lesions with acute Lyme disease. The activated fibroblasts and infiltrating mononuclear cells were the major sources of local MMP-9 production (43). Infiltration of skin with mononuclear cells is frequently observed in EM lesions with acute Lyme disease (3). Monocyte/macrophage infiltration is prominently observed in tissue specimens infected with *B. burgdorferi* for both humans and experimentally infected animals (29, 31). In this study, we demonstrate (i) that concentrations of MMP-9 and sCD14 are significantly elevated in serum of the patients with acute Lyme disease and (ii) that upregulation of MMP-9 involves a CD14 signaling pathway in cultured U937 cells and fibroblasts.

The expression of MMP-9 in primary neural cultures was induced in a dose- and time-dependent manner by *B. burgdorferi*. Human and rat type I astrocytes expressed MMP-9 when incubated with *B. burgdorferi* in the same manner as primary neural cultures (30). *B. burgdorferi* has been demonstrated to induce the secretion and activation of MMP-9 from primary human cells, including monocytes, neutrophils, keratinocytes, and astrocytes (11). But what mechanism of upregulation of MMP-9 by *B. burgdorferi* will be involved? *B. burgdorferi* possesses inherent stimulatory properties, believed to be mediated by spirochetal lipoproteins, which may be responsible for eliciting much of the pathology of Lyme disease (34). CD14 is a 55-kDa glycoprotein expressed on the surfaces of various cells, including monocytes, macrophages, neutrophils, and chondrocytes. CD14 can be found in a membrane-bound state and a circulating soluble state. Both human endothelial cells (sCD14) and neutrophils (mCD14) appear to attain a greater sensitivity to *B. burgdorferi* lipoproteins by utilizing CD14 for signaling. Enhanced expression of CD14 and HLA-DR by EM lesional neutrophils and macrophages indicated that these innate effector cells were highly activated (32). The pathway for *B. burgdorferi* to activate or elicit an increase in the release of MMP-9 by host cells could be either direct or indirect (i.e., either by acting on cells via receptors to increase their production of MMPs or by increasing the release of other mediators, such as cytokines, which could stimulate the release of MMPs). Our study indicated that the expression of both mCD14 and sCD14 was significantly increased from U937 cells in the presence of *B. burgdorferi*, which was associated with the upregulation of MMP-9 by *B. burgdorferi*. Indeed, the anti-CD14 antibody could partially block the expression of MMP-9 from U937 cells with *B. burgdorferi*. This suggested that the upregulation of MMP-9 by *B. burgdorferi* in U937 cells could involve a CD14 signaling pathway. MMP-9 but not MMP-1 was specifically induced in monocytic cells through TLR2 by *B. burgdorferi* (12). Due to the partially blocked expression of MMP-9 by anti-CD14 antibody, other pathways may also be involved, such as TLR2, TLR4, or other TLRs (4, 20).

In this study, functional activity of MMP-9 was not detected in human fibroblasts using zymography and a targeted gene array. But MMP-9 mRNA was expressed in fibroblasts at baseline when the more-sensitive technique, real-time RT-PCR, was used. However, the expression of MMP-9 was not impacted in the presence of *B. burgdorferi* because CD14 (mCD14 or sCD14) was not expressed in fibroblasts.

One of the most interesting findings in this study was that the expression of MMP-9 was significantly increased when fibroblasts were incubated with the conditional medium that contained sCD14 receptor. This finding indicated that upregulation of MMP-9 was associated with increased sCD14 production. Upregulation of MMP-9 could be completely abolished by an anti-CD14 antibody. Although the origin of sCD14 is unclear, sCD14 is shed from the surfaces of monocytes upon activation by gamma interferon, LPS, and other stimuli, suggesting that sCD14 is derived from GPI-anchored mCD14 (6, 17, 27, 28, 39).

Our results also indicated that recombinant CD14 itself could induce the expression of MMP-9 in fibroblasts. Likewise, the concentration of sCD14 was elevated in the serum of patients with bacterial infections (14, 26, 27) as well as in that from those with autoimmune disorders and rheumatic diseases (1, 5, 8, 33). sCD14 is a proinflammatory molecule which is increased in inflammation (24). Nevertheless, the function of sCD14 in vivo is not yet known, and functional studies should complement the quantitative analyses. Moreover, the expression level of MMP-9 was much higher when fibroblasts were cultured in the presence of both recombinant sCD14 and *B. burgdorferi*. This effect was completely blocked by anti-CD14 antibody. The mechanism of the upregulation of MMP-9 by *B.*

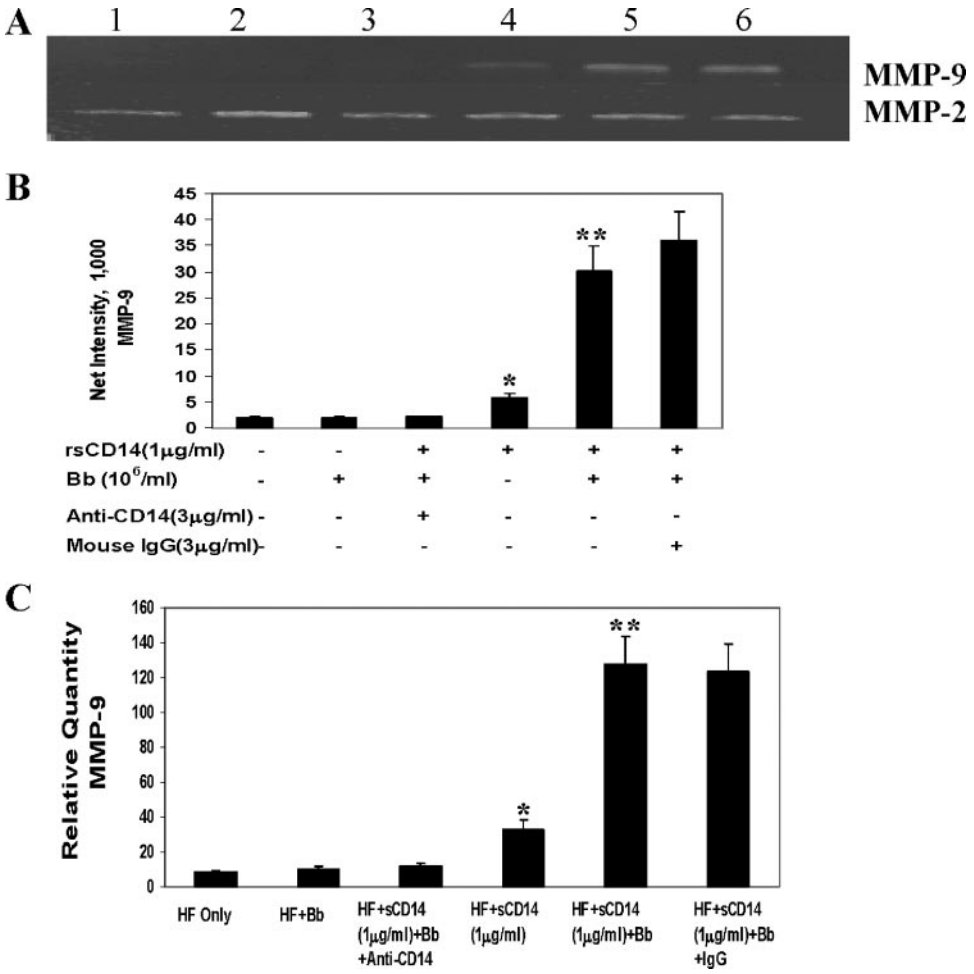

FIG. 6. Recombinant sCD14 directly activated MMP-9 enzymatic activity in human fibroblasts (HF). (A) Gelatinolytic activity of MMP-9 in HF in the presence or absence of recombinant CD14 (rCD14) and/or anti-CD14 antibody. Lane 1, HF medium control; lane 2, HF plus *Borrelia burgdorferi* (Bb); lane 3, HF plus Bb plus rsCD14 (1 μg/ml) plus anti-CD14 (3 μg/ml); lane 4, HF plus rCD14 (1 μg/ml); lane 5, HF plus rCD14 (1 μg/ml) plus Bb; lane 6, HF plus rCD14 (1 μg/ml) plus Bb plus mouse IgG (3 μg/ml). Zymography experiments were repeated four times, and representative experiments are shown. (B) Quantitative analysis of activity of MMP-9 from values in all four separate experiments, using Kodak digital image analysis software. 5 versus 4 or 1, **, $P < 0.01$; 4 versus 1, *, $P < 0.05$; $n = 4$. (C) mRNA expression of MMP-9 in fibroblasts with or without recombinant CD14 and/or anti-CD14 antibody. HF plus rsCD14 plus Bb versus HF plus rsCD14 or HF only, **, $P < 0.01$; HF plus rCD14 versus HF only, *, $P < 0.05$; $n = 5$. Real-time RT-PCR experiments were repeated five times, and data presented are mean values for five separate experiments performed in duplicate.

burgdorferi via a CD14 signaling pathway remained to be determined. It is possible that there is no direct binding of the bacterial cell wall to monocytes but that it occurs via the intermediate of CD14, which binds spirochetal lipoproteins and transfers these molecules to the cell membranes of sensitive cells, where they are recognized by specific receptors capable of directly mediating lipoprotein signaling (24). sCD14 can bind to whole bacteria and bacterial cell wall components and mediates bacterium-induced activation of cells that do not express membrane-bound CD14 as well as CD14-bearing cells (19, 23). Some studies demonstrated that OspA directly interacted with CD14 in vitro, forming a unique complex that migrated independently of free OspA and CD14 on native gels. Complex formation with CD14 exhibited some degree of specificity and appeared to be lipid mediated (41). *B. burgdorferi* lipoproteins induced cellular activation by a mechanism that did not involve the LPS receptor, and CD14 was involved in cellular activation by spirochetal lipoproteins, although likely by using a set of transmembrane proteins different from the one utilized by LPS to transduce the signals (13). *B. burgdorferi* lipoproteins also stimulated cytokine production via a CD14-mediated mechanism, thus supporting the contention that CD14 was a pattern recognition receptor as shown by its unique ability to recognize several structurally related microbial antigens (40). But contrary to an anticipated diminution in pathology, $CD14^{-/-}$ mice exhibited more severe and persistent inflammation than did $CD14^{+/+}$ mice (2).

Our findings demonstrated that *B. burgdorferi* could induce the expression of MMP-9 via a CD14 signaling pathway. We also proved that sCD14 may play a role in the expression of MMP-9 from fibroblasts which showed a lack of CD14 (16). We postulated the possibility that the U937 cells stimulated by *B. burgdorferi* produced the proinflammatory cytokines and MMP-9 via an increased CD14 signaling pathway, and then the

released sCD14 may in turn function as receptors for fibroblasts to produce MMP-9 and/or proinflammatory cytokines. The upregulation of MMP-9 by *B. burgdorferi* involved a CD14 pathway in infiltrating mononuclear cells in acute Lyme disease. The activated fibroblasts can be recruited to amplify local production of MMP-9 by acquiring sCD14 from infiltrating mononuclear cells. This cascade may play an important role in the pathogenesis of acute Lyme disease.

ACKNOWLEDGMENT

This work was supported by the National Institutes of Health (grant R01 AI-37241).

REFERENCES

1. **Bazil, V., and J. L. Strominger.** 1991. Shedding as a mechanism of down-modulation of CD14 on stimulated human monocytes. J. Immunol. **147:** 1567–1574.
2. **Benhnia, M. R., D. Wroblewski, M. N. Akhtar, R. A. Patel, W. Lavezzi, S. C. Gangloff, S. M. Goyert, M. J. Caimano, J. D. Radolf, and T. J. Sellati.** 2005. Signaling through CD14 attenuates the inflammatory response to Borrelia burgdorferi, the agent of Lyme disease. J. Immunol. **174:**1539–1548.
3. **Berger, B. W.** 1989. Dermatologic manifestations of Lyme disease. Rev. Infect. Dis. **11:**S1475–S1481.
4. **Brettschneider, J., D. Ecker, A. Bitsch, D. Bahner, T. Bogumil, A. Dressel, E. Elitok, B. Kitze, S. Poser, F. Weber, and H. Tumani.** 2002. The macrophage activity marker sCD14 is increased in patients with multiple sclerosis and upregulated by interferon beta-1b. J. Neuroimmunol. **133:**193–197.
5. **Bufler, P., G. Stiegler, M. Schuchmann, S. Hess, C. Kruger, F. Stelter, C. Eckerskorn, C. Schutt, and H. Engelmann.** 1995. Soluble lipopolysaccharide receptor (CD14) is released via two different mechanisms from human monocytes and CD14 transfectants. Eur. J. Immunol. **25:**604–610.
6. **Bulut, Y., E. Faure, L. Thomas, O. Equils, and M. Arditi.** 2001. Cooperation of Toll-like receptor 2 and 6 for cellular activation by soluble tuberculosis factor and Borrelia burgdorferi outer surface protein A lipoprotein: role of Toll-interacting protein and IL-1 receptor signaling molecules in Toll-like receptor 2 signaling. J. Immunol. **167:**987–994.
7. **Chary-Valckenaere, I., B. Jaulhac, J. Champigneulle, Y. Piemont, D. Mainard, and J. Pourel.** 1998. Ultrastructural demonstration of intracellular localization of Borrelia burgdorferi in Lyme arthritis. Br. J. Rheumatol. **37:**468–470.
8. **Durieux, J. J., N. Vita, O. Popescu, F. Guette, J. Calzada-Wack, R. Munker, R. E. Schmidt, J. Lupker, P. Ferrara, H. W. Ziegler-Heitbrock, et al.** 1994. The two soluble forms of the lipopolysaccharide receptor, CD14: characterization and release by normal human monocytes. Eur. J. Immunol. **24:**2006–2012.
9. **Dziarski, R., S. Viriyakosol, T. N. Kirkland, and D. Gupta.** 2000. Soluble CD14 enhances membrane CD14-mediated responses to peptidoglycan: structural requirements differ from those for responses to lipopolysaccharide. Infect. Immun. **68:**5254–5260.
10. **Fuchs, H., R. Wallich, M. M. Simon, and M. D. Kramer.** 1994. The outer surface protein A of the spirochete Borrelia burgdorferi is a plasmin(ogen) receptor. Proc. Natl. Acad. Sci. USA **91:**12594–12598.
11. **Gebbia, J. A., J. L. Coleman, and J. L. Benach.** 2001. Borrelia spirochetes upregulate release and activation of matrix metalloproteinase gelatinase B (MMP-9) and collagenase 1 (MMP-1) in human cells. Infect. Immun. **69:** 456–462.
12. **Gebbia, J. A., J. L. Coleman, and J. L. Benach.** 2004. Selective induction of matrix metalloproteinases by Borrelia burgdorferi via toll-like receptor 2 in monocytes. J. Infect. Dis. **189:**113–119.
13. **Giambartolomei, G. H., V. A. Dennis, B. L. Lasater, and M. T. Philipp.** 1999. Induction of pro- and anti-inflammatory cytokines by *Borrelia burgdorferi* lipoproteins in monocytes is mediated by CD14. Infect. Immun. **67:**140–147.
14. **Gluck, T., J. Silver, M. Epstein, P. Cao, B. Farber, and S. M. Goyert.** 2001. Parameters influencing membrane CD14 expression and soluble CD14 levels in sepsis. Eur. J. Med. Res. **6:**351–358.
15. **Hailman, E., T. Vasselon, M. Kelley, L. A. Busse, M. C. Hu, H. S. Lichenstein, P. A. Detmers, and S. D. Wright.** 1996. Stimulation of macrophages and neutrophils by complexes of lipopolysaccharide and soluble CD14. J. Immunol. **156:**4384–4390.
16. **Hayashi, J., T. Masaka, I. Saito, and I. Ishikawa.** 1996. Soluble CD14 mediates lipopolysaccharide-induced intercellular adhesion molecule 1 expression in cultured human gingival fibroblasts. Infect. Immun. **64:**4946–4951.
17. **Hirschfeld, M., C. J. Kirschning, R. Schwandner, H. Wesche, J. H. Weis, R. M. Wooten, and J. J. Weis.** 1999. Cutting edge: inflammatory signaling by Borrelia burgdorferi lipoproteins is mediated by toll-like receptor 2. J. Immunol. **163:**2382–2386.
18. **Hu, L. T., and M. S. Klempner.** 1997. Host-pathogen interactions in the immunopathogenesis of Lyme disease. J. Clin. Immunol. **17:**354–365.
19. **Jack, R. S., U. Grunwald, F. Stelter, G. Workalemahu, and C. Schutt.** 1995. Both membrane-bound and soluble forms of CD14 bind to gram-negative bacteria. Eur. J. Immunol. **25:**1436–1441.
20. **Kawanaka, N., M. Yamamura, T. Aita, Y. Morita, A. Okamoto, M. Kawashima, M. Iwahashi, A. Ueno, Y. Ohmoto, and H. Makino.** 2002. CD14+, CD16+ blood monocytes and joint inflammation in rheumatoid arthritis. Arthritis Rheum. **46:**2578–2586.
21. **Klempner, M. S., and B. T. Huber.** 1999. Is it thee or me?—autoimmunity in Lyme disease. Nat. Med. **5:**1346–1347.
22. **Klempner, M. S., R. Noring, M. P. Epstein, B. McCloud, R. Hu, S. A. Limentani, and R. A. Rogers.** 1995. Binding of human plasminogen and urokinase-type plasminogen activator to the Lyme disease spirochete, Borrelia burgdorferi. J. Infect. Dis. **171:**1258–1265.
23. **Labeta, M. O., K. Vidal, J. E. Nores, M. Arias, N. Vita, B. P. Morgan, J. C. Guillemot, D. Loyaux, P. Ferrara, D. Schmid, M. Affolter, L. K. Borysiewicz, A. Donnet-Hughes, and E. J. Schiffrin.** 2000. Innate recognition of bacteria in human milk is mediated by a milk-derived highly expressed pattern recognition receptor, soluble CD14. J. Exp. Med. **191:**1807–1812.
24. **Landmann, R., S. Link, S. Sansano, Z. Rajacic, and W. Zimmerli.** 1998. Soluble CD14 activates monocytic cells independently of lipopolysaccharide. Infect. Immun. **66:**2264–2271.
25. **Lapa e Silva, J. R., M. D. Possebon da Silva, J. Lefort, and B. B. Vargaftig.** 2000. Endotoxins, asthma, and allergic immune responses. Toxicology **152:** 31–35.
26. **Lin, B., R. Noring, A. C. Steere, M. S. Klempner, and L. T. Hu.** 2000. Soluble CD14 levels in the serum, synovial fluid, and cerebrospinal fluid of patients with various stages of Lyme disease. J. Infect. Dis. **181:**1185–1188.
27. **Nockher, W. A., M. Wick, and H. W. Pfister.** 1999. Cerebrospinal fluid levels of soluble CD14 in inflammatory and non-inflammatory diseases of the CNS: upregulation during bacterial infections and viral meningitis. J. Neuroimmunol. **101:**161–169.
28. **Nockher, W. A., R. Wigand, W. Schoeppe, and J. E. Scherberich.** 1994. Elevated levels of soluble CD14 in serum of patients with systemic lupus erythematosus. Clin. Exp. Immunol. **96:**15–19.
29. **Norgard, M. V., L. L. Arndt, D. R. Akins, L. L. Curetty, D. A. Harrich, and J. D. Radolf.** 1996. Activation of human monocytic cells by *Treponema pallidum* and *Borrelia burgdorferi* lipoproteins and synthetic lipopeptides proceeds via a pathway distinct from that of lipopolysaccharide but involves the transcriptional activator NF-kappa B. Infect. Immun. **64:**3845–3852.
30. **Perides, G., L. M. Tanner-Brown, M. A. Eskildsen, and M. S. Klempner.** 1999. Borrelia burgdorferi induces matrix metalloproteinases by neural cultures. J. Neurosci. Res. **58:**779–790.
31. **Philipp, M. T., M. K. Aydintug, R. P. Bohm, Jr., F. B. Cogswell, V. A. Dennis, H. N. Lanners, R. C. Lowrie, Jr., E. D. Roberts, M. D. Conway, M. Karacorlu, et al.** 1993. Early and early disseminated phases of Lyme disease in the rhesus monkey: a model for infection in humans. Infect. Immun. **61:**3047–3059.
32. **Salazar, J. C., C. D. Pope, T. J. Sellati, H. M. Feder, Jr., T. G. Kiely, K. R. Dardick, R. L. Buckman, M. W. Moore, M. J. Caimano, J. G. Pope, P. J. Krause, and J. D. Radolf.** 2003. Coevolution of markers of innate and adaptive immunity in skin and peripheral blood of patients with erythema migrans. J. Immunol. **171:**2660–2670.
33. **Scherberich, J. E., and W. A. Nockher.** 2000. Blood monocyte phenotypes and soluble endotoxin receptor CD14 in systemic inflammatory diseases and patients with chronic renal failure. Nephrol. Dial. Transplant. **15:**574–578.
34. **Sellati, T. J., D. A. Bouis, M. J. Caimano, J. A. Feulner, C. Ayers, E. Lien, and J. D. Radolf.** 1999. Activation of human monocytic cells by Borrelia burgdorferi and Treponema pallidum is facilitated by CD14 and correlates with surface exposure of spirochetal lipoproteins. J. Immunol. **163:**2049–2056.
35. **Sellati, T. J., D. A. Bouis, R. L. Kitchens, R. P. Darveau, J. Pugin, R. J. Ulevitch, S. C. Gangloff, S. M. Goyert, M. V. Norgard, and J. D. Radolf.** 1998. Treponema pallidum and Borrelia burgdorferi lipoproteins and synthetic lipopeptides activate monocytic cells via a CD14-dependent pathway distinct from that used by lipopolysaccharide. J. Immunol. **160:**5455–5464.
36. **Stanek, G., J. Klein, R. Bittner, and D. Glogar.** 1990. Isolation of Borrelia burgdorferi from the myocardium of a patient with longstanding cardiomyopathy. N. Engl. J. Med. **322:**249–252.
37. **Steere, A. C.** 1989. Lyme disease. N. Engl. J. Med. **321:**586–596.
38. **Steere, A. C.** 2001. Lyme disease. N. Engl. J. Med. **345:**115–125.
39. **Sugawara, S., A. Sugiyama, E. Nemoto, H. Rikiishi, and H. Takada.** 1998. Heterogeneous expression and release of CD14 by human gingival fibroblasts: characterization and CD14-mediated interleukin-8 secretion in response to lipopolysaccharide. Infect. Immun. **66:**3043–3049.

40. **Ulevitch, R. J., and P. S. Tobias.** 1995. Receptor-dependent mechanisms of cell stimulation by bacterial endotoxin. Annu. Rev. Immunol. **13:**437–457.
41. **Wooten, R. M., T. B. Morrison, J. H. Weis, S. D. Wright, R. Thieringer, and J. J. Weis.** 1998. The role of CD14 in signaling mediated by outer membrane lipoproteins of Borrelia burgdorferi. J. Immunol. **160:**5485–5492.
42. **Wright, S. D.** 1995. CD14 and innate recognition of bacteria. J. Immunol. **155:**6–8.
43. **Zhao, Z., H. Chang, R. P. Trevino, K. Whren, J. Bhawan, and M. S. Klempner.** 2003. Selective up-regulation of matrix metalloproteinase-9 expression in human erythema migrans skin lesions of acute lyme disease. J. Infect. Dis. **188:**1098–1104.

Editor: R. P. Morrison