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Identification of a TLR-Independent Pathway for *Borrelia burgdorferi*-Induced Expression of Matrix Metalloproteinases and Inflammatory Mediators through Binding to Integrin $\alpha_3\beta_1$

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*B. burgdorferi* stimulates a robust inflammatory response at sites of localization. Binding of borrelial lipoproteins to TLR-2 is one pathway important in the host response to *B. burgdorferi*. However, while TLR-2 is clearly important in control of infection, inflammation is actually worsened in the absence of TLR-2 or the shared TLR adapter molecule, MyD88, suggesting that there are alternative pathways regulating inflammation. Integrins are cell surface receptors that play an important role in cell to cell communications and that can activate inflammatory signaling pathways. In this study, we report for the first time that *B. burgdorferi* binds to integrin $\alpha_3\beta_1$ and that binding of *B. burgdorferi* to this integrin results in induction of proinflammatory cytokines, chemokines, and end-effector molecules such as matrix metalloproteinases in primary human chondrocyte cells. Expression of these same molecules is not affected by the absence of MyD88 in murine articular cartilage, suggesting that the two pathways act independently in activating host inflammatory responses to *B. burgdorferi*. *B. burgdorferi*-induced $\alpha_3$ signaling is mediated by JNK, but not p38 MAPK. In summary, we have identified a new host receptor for *B. burgdorferi*, integrin $\alpha_3\beta_1$; binding of *B. burgdorferi* to integrin $\alpha_3\beta_1$ results in the release of inflammatory mediators and is proposed as a TLR-independent pathway for activation of the innate immune response by the organism. *The Journal of Immunology*, 2006, 177: 657–664.

**Borrelia burgdorferi** is the causative agent of Lyme disease (1). Infection with *B. burgdorferi* results in the activation of inflammatory pathways that lead to the release of cytokines and chemokines and an influx of inflammatory cells that result in many of the clinical manifestations of Lyme disease (2–5). These manifestations include the erythema migrans rash, polyneuritis, meningitis, carditis, and, in later stages, arthritis.

Much of the recent attention on activation of inflammatory pathways by *B. burgdorferi* has focused on recognition of spirochetal products by members of the TLR family of receptors (6, 7). TLR-2 has been shown to recognize borrelial lipoproteins; binding of borrelial lipoproteins to TLR-2 results in activation of NF-κB and release of IL-8 (6). Studies of *B. burgdorferi* infection using knockout mice deficient in TLR-2 or MyD88, an adapter protein used by the majority of members of the TLR family, confirmed the importance of the TLR signaling in control of *B. burgdorferi* infection as bacterial burdens were greatly increased in the absence of either TLR-2 or MyD88. However, surprisingly, TLR-2 or MyD88 deficiency did not reduce inflammation at sites of infection, suggesting that other receptors are also involved in signaling for inflammation (8, 9).

Our laboratory has been interested in investigating the role of host matrix metalloproteinases (MMPs)\textsuperscript{3} in the pathogenesis of Lyme arthritis. Prior studies have shown that MMP-1 and MMP-3 are elevated in the joints of patients with Lyme arthritis and that inhibition of MMPs in an in vitro model of Lyme arthritis prevents the destruction of cartilage tissue (10–12). MMP-3 and MMP-19 are elevated in the joints of mice that develop arthritis in response to infection with *B. burgdorferi*, and levels of these MMPs correlate closely with the severity of arthritis seen in different strains of mice in response to infection (12). Induction of these MMPs appears to be mediated by multiple signaling pathways, including p38 MAPK, JNK, and JAK/STAT pathways (13). However, the receptors that are responsible for activation of these signaling pathways in joint tissue remain unknown. Gebbia et al. (14) have shown previously that TLR-2 is involved in the induction of MMP-9 from monocytes, but does not appear to be involved in the induction of MMP-1. MMP-9 has not been found to be elevated in the joints of humans or mice with Lyme arthritis (10).

In this study, we report on our studies examining cellular receptors that are important in the induction of MMPs and related inflammatory mediators from joint tissue by *B. burgdorferi*. We will show that induction of MMPs and multiple cytokines and chemokines can occur through activation of receptors other than those of the TLR system. In our search for other potential receptors that mediate the inflammatory response to *B. burgdorferi*, we have focused on receptors in the integrin family. Coburn et al. (15–18) have found that *B. burgdorferi* binds to at least several different

\textsuperscript{3}Abbreviations used in this paper: MMP, matrix metalloproteinase; CMA, control mouse ascites fluid; HBS, HEPES-buffered saline; HBSBD, HBS supplemented with BSA to 1% (w/v) and dextrose to 0.1% (w/v); HC, human chondrocyte; OspA, outer surface protein A; rt, real-time; siRNA, small interfering RNA; qRT-PCR, quantitative RT-PCR.
Integrins, including, but not limited to $\alpha_{\mathrm{m}}\beta_{3}$, $\alpha_{\mathrm{v}}\beta_{3}$, and $\alpha_{\beta_{1}}$. Integrin receptors are important in cell to cell communications, and binding to integrin receptors has been shown to activate many of the same signaling pathways found to be activated by $B.~burgdorferi$ infection. The $\beta_{3}$ integrin CD11b/CD18 has been shown to act as an accessory molecule in the recognition of Escherichia coli LPS (19–22). In this study, we present the first report of $B.~burgdorferi$ binding to another integrin, integrin $\alpha_{v}$, and show that binding of $B.~burgdorferi$ to this integrin results in induction of MMPs and inflammatory mediators, suggesting that it may play a role in non-TLR-dependent inflammation in response to the organism.

Materials and Methods

**Primary cultures of human chondrocyte (HC) cells and infection with $B.~burgdorferi$**

Primary HC cells from a healthy donor were purchased from Cambrex and maintained in chondrocyte growth medium (Cambrex) containing 10% FCS at 37°C with 5% CO$_2$. One day before infection, HCs were washed and the culture medium was replaced with chondrocyte basal medium without FCS. A clonal isolate of low passage (passages 2–6) $B.~burgdorferi$ sensu stricto strain N40 was cultured in Barbour-Stoenner-Kelly medium (Sigma-Aldrich), as previously described (23, 24). Spirochetes were washed three times and resuspended in chondrocyte basal medium without FCS. Cell cultures at 70–85% confluence were infected with $B.~burgdorferi$ at multiplicity of infection of 10 for various time periods. For blocking experiments, individual Abs were added to the cells in fresh serum-free medium 2 h before infection with $B.~burgdorferi$ and cells were harvested at 24 h postinfection. For integrin inhibition studies, cells were treated with 50 ng/ml mouse mAbs to different integrin subunits (Chemicon International) for 2 h and then infected with $B.~burgdorferi$ for 24 h. Similarly, for TLR-2-blocking studies, cells were treated with mouse anti-TLR-2-blocking mAb (tl2.1) (500 ng/ml) (25) for 2 h before infection with $B.~burgdorferi$. The concentrations of Abs used had no visible cytotoxic effect on the HCs, as judged by trypan blue exclusion. The outer surface of $B.~burgdorferi$ was contaminated by genomic DNA were discarded, and the original RNA was used to control for contamination by genomic DNA. cDNA samples contaminated by genomic DNA were discarded, and the original RNA was reisolated and DNase before qRT-PCR. Quantity of cDNA from specific mRNA transcripts was accomplished by quantitative RT-PCR (Bio-Rad) by using SYBR Green technology (Quantitect SYBR Green PCR kit; Qiagen), as previously described (13, 29). $\beta$-Actin and nidogen genes were used as internal control for human and murine samples, respectively. The primers used are listed in Table I.

**MAPK assay**

After infection with $B.~burgdorferi$, total cellular protein extracts were prepared from chondrocyte cultures. Cell culture plates were transferred to ice and then washed twice with cold PBS. Cells were mechanically dislodged into cold PBS, to which 10 $\mu$g/ml leupeptin, 1 mM PMSF, and 0.5 mM DTT were added. Cells were pelleted by centrifugation at 4°C, the supernatant was removed, and pelleted cells were resuspended in cell lysis buffer (25 mM HEPES (pH 7.5), 300 mM NaCl, 1.5 mM MgCl$_2$, 2 mM EDTA (pH 8.0), 0.05% Triton X-100, 0.1 mM Na$_3$VO$_4$, 20 mM $\beta$-glycerol phosphate, leupeptin (10 $\mu$g/ml), 1 mM PMSF, 0.5 mM DTT). The resuspended cells were gently rocked at 4°C for 30 min; cellular debris was removed by centrifugation at 4°C. The cell extracts were collected and frozen at −80°C. The concentrations of the cell extracts were determined using the Bradford method (Bio-Rad). Two hundred micrograms of total protein was used for immunoprecipitation using phosphospecific Abs to p38 MAPK and JNK, and the kinase assays were performed using specific kits from Cell Signaling Technology, per the manufacturer’s instructions.

**Statistical analysis**

Experiments were repeated three to five times, as indicated, in each experiment. The statistical significance between groups was analyzed by using the nonparametric Mann-Whitney $U$ test. Differences were considered statistically significant when the $p$ value was ≤0.05.

**Results**

**Role of TLR-2 in $B.~burgdorferi$-induced expression of MMP-1 and MMP-3 from chondrocytes**

TLR-2 has been shown to be involved in $B.~burgdorferi$ induction of some, but not all, MMPs from human monocytes (14). We have shown previously that $B.~burgdorferi$ induces MMP-1 and MMP-3 from HC cells and that this induction involves MAPK (ERK1/2, p38 MAPK, and JNK) pathways that could potentially be activated...
through TLR-2 signaling (30–33). To determine whether induction of MMP-1 and MMP-3 from chondrocytes occurred through TLR-2 signaling, we first established that TLR-2 was expressed in our primary chondrocyte cells by q-RT-PCR and immunocytochemistry (data not shown). Having determined that TLR-2 was expressed in the chondrocyte cells, we next added a known TLR-2 ligand, lipoprotein OspA of *B. burgdorferi*, to in vitro cultures of chondrocytes. We have shown previously that MMP-1 and MMP-3 mRNA using q-RT-PCR (13). Addition of the lipoprotein OspA does induce expression of both MMP-1 and MMP-3 (3.2-fold) (13). We had access to *B. burgdorferi*–infected and sham-infected C57BL/6 mice (12). We used an animal model of Lyme arthritis. We have shown previously that TLR signaling was necessary for MMP induction, we next turned to an animal model of Lyme arthritis. We have shown previously that *B. burgdorferi* induction-induced MMP expression in the absence of MyD88 (8, 9). MyD88 is an adapter molecule used by TLR-2 and TLR-3 receptors recognizing other borrelial products may play a larger role in response to whole organisms.

### Table 1. Primer sequences for q-RT-PCR

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*B. burgdorferi*-infected mice induce MMP expression in the absence of MyD88

To confirm whether TLR signaling was necessary for MMP induction, we next turned to an animal model of Lyme arthritis. We have shown previously that *B. burgdorferi* induces MMP-3 and MMP-19 in C3H/HeN and C57BL/6 mice (12). We had access to *MyD88*-deficient mice, which have been shown to develop significant joint swelling and inflammation when infected with *B. burgdorferi* (8, 9). *MyD88*-deficient mice express inflammatory cytokines in the absence of MyD88

To further understand the role of TLR signaling on *B. burgdorferi* infection-induced inflammation, we examined the expression of key proinflammatory molecules in *MyD88*−/− mice. *B. burgdorferi* infection significantly induced mRNA expression of CXCL-2 (p = 0.011), MCP-1 (p = 0.011), and RANTES (p = 0.006) in...
the absence of MyD88 in murine joints (Fig. 3). Expression of TNF-α also was increased in infected group, but did not reach significance (p = 0.143) (Fig. 3). These results show that B. burgdorferi induction of proinflammatory cytokines and chemokines in an animal model of Lyme arthritis can also occur in a MyD88-independent manner.

Integrin α3 regulates B. burgdorferi-induced MMP expression

Given that MMPs and inflammatory cytokine and chemokine induction can occur in a TLR-2/MyD88-independent manner, we were interested in determining which other receptors might be involved in B. burgdorferi recognition. Among the other receptors that can activate the mitogen-activated protein, kinases (ERK1/2, p38 MAPK, and JNK) and JAK/STAT pathways that we have shown to be important in the induction of MMPs are the integrin family of receptors. Integrin signaling triggers inflammatory responses through activation of focal adhesion kinase, ERK1/2, JNKs, AP-1, and NF-κB (34–36). B. burgdorferi has also been shown to attach to different host cells through different integrin receptors (15, 16, 37). To determine whether attachment to integrins by B. burgdorferi could also result in activation of host signaling pathways leading to induction of inflammatory mediators, we attempted to block B. burgdorferi induction of MMPs from primary HC s using different anti-integrin Abs. All Abs used were mouse mAbs from ascites fluid. CMA containing Abs of the same isotype and concentration was used as a control in these experiments. Of the different Abs that we tested, only Ab to integrin α3, significantly inhibited B. burgdorferi-induced expression of MMP-1 (p = 0.014) and MMP-3 (p = 0.037) (~6 (83%)- and 3 (64%)-fold, respectively; Fig. 4, A and B). Abs directed against other integrin α subunits had no significant effect on MMP-1 and MMP-3 expression (Fig. 4, C and D). The effects of blocking Abs to some integrins (notably pan β3) could not be determined because of the effects of the Abs on cell attachment and growth. Ab to α3β1 did not appear to affect cell attachment or survival as measured by trypan blue exclusion.

Because blocking Abs can have unintended effects that may not be due to blocking of the receptor, we sought to confirm the role of α3β1 by using a different method to inhibit signaling through this receptor. We inhibited the expression of integrin α3 by siRNA. HCs were transfected with human integrin α3-specific siRNA (ITGA-3i) and control siRNA and subsequently infected with B. burgdorferi. Cells were harvested 24 h postinfection, and total RNA was analyzed by q-RT-PCR. Expression of α3 integrin was inhibited by 71% as determined by reduction of α3-specific RNA transcripts. Transcription of MMP-1 and MMP-3 in response to B. burgdorferi was inhibited by 56 and 55%, respectively, when compared with control siRNA-transfected chondrocytes (Fig. 5). Integrin α3-specific siRNA alone did not inhibit basal expression of MMP-1 and MMP-3 in the absence of B. burgdorferi infection. This result confirmed that integrin α3 mediates B. burgdorferi-induced MMP expression in HC cells.
B. burgdorferi binds to integrin α₃β₁

Although B. burgdorferi has been shown to bind to other integrin heterodimers, binding to α₃β₁ has not previously been shown. To ascertain whether the inhibitory effects of integrin α₃ on induction MMPs reflected an inhibition of ligand binding to integrin, we assessed the binding of B. burgdorferi to integrin α₃β₁. B. burgdorferi was added to plates coated with either purified integrin α₃β₁ or α₁β₃, and adherence was determined by ELISA. Integrin α₁β₃, which has previously been shown to bind to B. burgdorferi (16), was used as a positive control. B. burgdorferi strongly bound to α₃β₁ at a level equivalent to binding to integrin α₁β₃ (Fig. 6, first panel in the graph). Binding between B. burgdorferi and α₃β₁ was specific to integrin α₃ as the binding was significantly inhibited (p = 0.021) by anti-integrin α₃ Ab (Fig. 6, second panel in the graph). The binding between B. burgdorferi and integrin α₃β₁ was not affected by integrin α₃ Ab (Fig. 6, second panel in the graph), indicating specificity of the competitive inhibition between B. burgdorferi and integrin α₃ alone. Similarly, binding between B. burgdorferi and integrins α₁β₃ and α₃β₁ was not affected by the isotype-matched control Ab (CMA) used at the same concentration as the integrin Ab (Fig. 6, third panel in the graph), indicating the binding between B. burgdorferi and individual integrins is specific.

Integrin α₃ mediates B. burgdorferi-induced expression of proinflammatory cytokines and chemokines

MMP induction is typically downstream of activation of other inflammatory mediators, including numerous cytokines and chemokines. To determine whether integrin α₃ directly activates MMP expression or whether α₃ signaling induces proinflammatory cytokines and chemokines, which may in turn regulate the expression of MMPs, we sought to determine the effect of B. burgdorferi-induced α₃ signaling on a broader array of inflammatory mediators. HCs transfected with integrin α₃ siRNA were infected with B. burgdorferi for 24 h. Total cellular RNA was processed for q-RT-PCR, and transcription of a number of key proinflammatory cytokines and chemokines was examined. Expression of TNF-α (p = 0.019), IL-1β (p = 0.019), IL-6 (p = 0.019), IL-8 (p = 0.019), MCP-1 (p = 0.014), RANTES (p = 0.014), and IFN-inducible...
Materials and Methods
described in B. burgdorferi binding was determined by competition assay using anti-integrin any of these inflammatory molecules in the absence of B. burgdorferi infection. These data suggest that integrin FIGURE 7. Integrin α3 regulates B. burgdorferi-induced expression of proinflammatory cytokines and chemokines in HC cells. Primary HC cells were transfected with either human integrin α3 siRNA or control siRNA and at 24 h posttransfection infected with B. burgdorferi for 24 h. Total cellular RNA was isolated, and q-RT-PCR was done for TNF-α, IL-1β, IL-6, MCP-1, RANTES, and IFN-inducible protein 10. The relative expression of each treatment group is compared with siControl + Bb group, which is normalized to one. The experiment was repeated four times, and average of all experiments is shown. Bars represent mean ± SD. Average values are written within each bar. *, p < 0.05.

protein-10 (p = 0.019) was significantly inhibited by 86, 41, 53, 58, 75, 69, and 84%, respectively, in cells transfected with α3 siRNA compared with cells transfected with control siRNA (Fig. 7). Integrin α3-specific siRNA alone did not inhibit expression of any of these inflammatory molecules in the absence of B. burgdorferi infection. These data suggest that integrin α3 recognition of B. burgdorferi is important in the inflammatory response to infection and that MMP induction seen in chondrocytes occurs as part of a broader inflammatory response mediated by integrin binding.

Mechanism of B. burgdorferi-induced integrin α3-mediated effects
We have shown previously that B. burgdorferi-induced MMP-1 and MMP-3 expression is regulated through activation of p38 MAPK and JNK in primary HC cells (13). Both p38 and JNK may be activated through either TLR or integrin signaling. To determine whether binding of B. burgdorferi to integrin α3β1 resulted in activation of either p38 and/or JNK, we determined the effect of transfaction of HCs with integrin α3 siRNA on P38 and JNK activation by B. burgdorferi. Cells were transfected with integrin α3 siRNA or control; after 24 h, transfected cells were incubated with B. burgdorferi for 1 h and harvested. Phosphorylated p38 or JNK was immunoprecipitated from cell lysates using specific Abs and activity was measured using the appropriate substrate (activating transcription factor-2 for p38 and cJun for JNK). Consistent with prior reports, both JNK and p38 were activated following 1 h post-B. burgdorferi infection (Fig. 8). JNK activity returned almost to baseline in cells transfected with integrin α3 siRNA when compared with control siRNA (Fig. 8A). However, silencing of integrin α3 did not reduce activity of p38 MAPK; rather, there was a marginal increase in its activity, when compared with cells transfected with control siRNA (Fig. 8B). These data indicate that B. burgdorferi-induced integrin α3 signaling is mediated by activation of JNK, but not p38 MAPK.

Discussion
Innate immune recognition of pathogens or pathogen products forms the initial defense against infectious agents. This recognition occurs through a battery of receptors, including, among others, the TLRs, scavenger receptors, complement receptors, members of the C-type lectin receptor family, and integrins. TLR-2 has been shown to be a mediator of the inflammatory events associated with Lyme arthritis (6). Nevertheless, deletion of TLR-2 or the central adapter molecule of TLR signaling, MyD88, did not inhibit the development of swelling and migration of inflammatory cells in response to B. burgdorferi. In fact, absence of signaling through TLR-2 or MyD88 often resulted in site-specific increases in inflammation, indicating that receptors other than TLRs are also involved in B. burgdorferi-induced inflammation (7–9). Consistent with these results, in this study we have been able to show that inflammatory cytokine, chemokine, and/or MMP induction can occur in the absence of TLR-2 or MyD88 signaling both in an in vitro and knockout animal model. We have identified a separate receptor, integrin α3β1, as a receptor for B. burgdorferi that activates...
signaling cascades that lead to the induction of these inflammatory mediators.

Binding of \textit{B. burgdorferi} to integrin \(\alpha_3\beta_1\) has not previously been reported. \textit{B. burgdorferi} bound to integrin \(\alpha_3\beta_1\) with similar intensity to other integrins that have been shown to bind to \textit{B. burgdorferi}, including integrins \(\alpha_m\beta_3\), \(\alpha_3\beta_1\), and \(\alpha_5\beta_1\). These integrins have been suggested to play a primary role in adhesion of \textit{B. burgdorferi} to different host cells and may be important in the pathogenesis of Lyme disease by affecting the ability of spirochetes to localize to specific cell types (37). However, binding of \textit{B. burgdorferi} to these integrins has not previously been reported to activate cell signaling pathways. In our study, Ab blocking of borrelial binding to other integrins expressed by chondrocytes did not seem to affect induction of MMPs. Thus, while multiple integrins may play a role in bacterial adhesion (and potentially be responsible for the ability of the organism to colonize many different tissues and organs), it appears that of the integrin receptors, \(\alpha_3\beta_1\) may play the most significant role in initiating the host response to the organism.

\(\alpha_3\beta_1\) is widely expressed on nearly all tissue types, including chondrocytes (38). Substrates for binding to \(\alpha_3\beta_1\) integrins are diverse and \(\alpha_3\beta_1\) can bind ligands with and without classical RGD integrin-binding motifs (39, 40). \(\alpha_3\beta_1\) is a known receptor for host products, including collagen (type I and VI), laminin (\(\alpha_3\beta_1\gamma_1\)), laminin-5 (\(\alpha_3\beta_1\gamma_2\)), laminin-10 (\(\alpha_3\beta_1\gamma_1\)), fibrinectin, entactin, nidogen, and thrombospondin-1 (29, 40–46). Fibrinectin and thrombospondin also bind to the \(\beta_1\) chain integrins, which bind to \textit{B. burgdorferi} (47, 48). \(\alpha_3\beta_1\) also acts as an alternative receptor for adenosine receptor types 2 and 5 (49).

The relative contributions of signaling mediated by integrin \(\alpha_3\) and TLRs to induction of inflammatory mediators remain unknown. Our in vitro data with TLR-2-blocking Ab showing that blocking of TLR-2 could block induction of MMPs when cells were stimulated with a purified borrelial lipoprotein (OspA), but not with whole borrelia, would seem to indicate a significant role for non-TLR-2 signaling pathways. Although animal studies with TLR-2 or MyD88 knockout mice showing high levels of inflammation also seem to suggest a lesser role for the TLR family in mediating the inflammatory response to \textit{B. burgdorferi}, interpretation of these studies is complicated by the higher spirochetal burdens seen in these knockout animals. We cannot rule out the possibility that the majority of inflammatory signaling in the presence of an intact immune system occurs through the TLR system, while other receptors such as \(\alpha_3\beta_1\) are activated only in the presence of high levels of ligand or in specific settings. In our in vitro findings would indicate that this is a less likely explanation.

The ideal way to test the contribution of signaling through integrin \(\alpha_3\) would be through the use of an animal model of disease. Unfortunately, integrin \(\alpha_3\) knockout mice show a very significant phenotype with severe defects in kidney, lung, and skin development resulting in perinatal lethality, making them inappropriate for infections with \textit{B. burgdorferi}, which take weeks to months to evaluate (50). We are also unaware of specific inhibitors of integrin \(\alpha_3\) that are appropriate for administration to animals. Recently, Glaser et al. (51) have shown that a small molecule, S18407, that inhibits integrin \(\alpha_3\beta_1\) can reduce the development of Lyme arthritis in a mouse model. S18407 was shown to have specificity for integrin \(\alpha_3\beta_1\), and no significant effect on \(\alpha_2\beta_1\), \(\alpha_3\beta_1\), \(\alpha_5\beta_1\), integrin interactions. The effects on \(\alpha_3\beta_1\) were not reported. In our studies, we have not seen \textit{B. burgdorferi} binding to \(\alpha_3\beta_1\) and there was no decrease in signaling with use of an \(\alpha_3\beta_1\) Ab.

The mechanism by which the \textit{B. burgdorferi}-integrin \(\alpha_3\beta_1\) interaction activates downstream signaling pathways appears to be through activation of JNK, but not p38 MAPK. It has been shown previously that \textit{B. burgdorferi} infection of chondrocytes results in activation of both JNK and p38 pathways (13); however, consistent with our findings, production of MMP-1 was only partially inhibited by p38 inhibitors and almost completely inhibited by JNK inhibitors. The effect of the inhibitors was more similar for MMP-3. For both MMP-1 and MMP-3, an additive effect of JNK and p38 inhibitors was reported. Together with the data presented in this study, this suggests that separate receptors are involved in activating these pathways in response to \textit{B. burgdorferi}. As p38 signaling is a well-described downstream effect of TLR-2 activation, it is possible that activation of p38 by binding of \textit{B. burgdorferi} products to TLR-2 is responsible for a minor contribution to the release of inflammatory mediators from chondrocytes; however, we cannot rule out that p38 activation is the result of activation of a different class of receptors.

Inflammatory signaling mediated by LPS binding to CD11b/CD18 does not appear to require the cytoplasmic tail of the integrin, and signaling appears to be mediated through interaction of this \(\beta_2\) integrin with CD14 and TLR-4 (19–22, 52). However, a similar indirect signaling pathway through interactions with TLRs in \(\alpha_3\beta_1\) binding to \textit{B. burgdorferi} seems unlikely, as blockage or deletion of TLR-2 or the common adapter protein MyD88 does not abrogate the inflammatory response to \textit{B. burgdorferi} as was seen with the deletion of TLR-4 in CD11b/CD18-mediated LPS signaling (8, 9, 19, 20, 53). However, this will require confirmation by future studies.

In summary, we have identified an alternative receptor pathway by which cells can recognize \textit{B. burgdorferi} and activate an inflammatory response. Identifying the \textit{B. burgdorferi} proteins that serve as the ligand(s) for \(\alpha_3\beta_1\) and determining the relative contribution of \(\alpha_3\beta_1\) recognition of \textit{B. burgdorferi} to overall inflammatory signaling is the subject of continuing research in our laboratory.

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Disclosures

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