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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 $\alpha_3\beta_1^1$

Aruna K. Behera,* Ethan Hildebrand,* Satoshi Uematsu,† Shizuo Akira,‡ Jenifer Coburn,* and Linden T. Hu²*  

*Borrelia 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.

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The Journal of Immunology
integrins, including, but not limited to $\alpha_m\beta_3$, $\alpha_v\beta_3$, and $\alpha_v\beta_1$. 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 $\beta_2$ 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 $\alpha_s$, 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% CO2. 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 µg/ml mouse mAbs to different integrin $\alpha$ 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 protein A (OspA) used in this study is a recombinant protein purified from *B. burgdorferi*. The concentrations of the cell extracts were determined by protein A (OspA) used in this study is a recombinant protein purified from *B. burgdorferi* for 24 h.

**Mice and *B. burgdorferi* infection**

MyD88-deficient mice were maintained as heterozygous breeding pairs at the fifth generation backcross on the C57BL6 background. MyD88$^{−/−}$, MyD88$^{+/−}$, and MyD88$^{+/+}$ littermates were genotyped, as described (28). The procedures used were reviewed and approved by the Tufts University Institutional Animal Care and Use Committee. Four- to 6-wk-old mice were infected intradermally by needle inoculation with *B. burgdorferi* (strain N40, 1 × 10$^6$) and were sacrificed 2 wk postinfection. Cartilage was microdissected from the ankle joints by using a stereomicroscope, and total RNA was isolated by using TRIzol (Invitrogen Life Technologies), according to the manufacturer’s protocol. Successful inoculation of individual mice was confirmed by culturing ear samples in Barbour-Stoenner-Kelly medium and monitoring the growth of *B. burgdorferi* by dark-field microscopy.

**Small interfering RNA (siRNA) transfection**

Primary HC cells were transfected with 100 nM integrin $\alpha_v$ siRNA (ITGA3; Dharmacon) or with 100 nM control siRNA (Dharmacon) using Lipofectamine 2000 (Invitrogen Life Technologies), following the manufacturer’s protocol. Cells were infected with *B. burgdorferi* 24 h after siRNA transfection and were harvested at 24 h postinfection. Transfection of siRNA had no cytotoxic effect on primary chondrocytes, as determined by trypan blue exclusion assay.

**Quantitation of *B. burgdorferi* binding to purified integrin**

Purified integrin $\alpha_v\beta_1$ was purchased from Chemicon International, and $\alpha_v$ was purified from human placenta by Arg-Gly-Asp (RGD)-Sepharose chromatography (16). Binding of purified integrins to *B. burgdorferi* was performed, as described previously (16). Briefly, purified integrins were diluted to 1 µg/ml in HEPES-buffered saline (HBS) and dispensed at 50 µl/well into prechilled Limbro 96-well plates (Valeant Pharmaceuticals). After incubation overnight at 4°C, the plates were washed once with HBS and then blocked by incubation for 1 h at ambient temperature with HBS supplemented with BSA to 1% (w/v) and dextrose to 0.1% (w/v) (HBSBD) at 200 µl per well. The blocking buffer was then replaced with 35 µl per well of HBSBD or with the same buffer containing 50 µg/ml either anti-integrin $\alpha_v$ mAb or control mouse ascites fluid (CMA). The concentration of the anti-integrin $\alpha_v$ mAb used was selected to match the concentration at which inhibition of MMP and inflammatory mediators was achieved. *B. burgdorferi* were washed in PBS supplemented with BSA to 0.2% (w/v) (15) and resuspended in HBSBD. After incubation of the plates with blocking Ab for 30 min at room temperature, *B. burgdorferi* suspensions of 1 × 10$^6$/ml in HBSBD were added at 35 µl per well. The plates were then centrifuged at 1,200 × g for 10 min and incubated for 1 h at room temperature. Inbound bacteria, which were removed by washing with HBS at 200 µl per well. None of the reagents tested affected either the motility of the bacteria or binding to uncoated wells. Plates were fixed by the addition of 3% (w/v) paraformaldehyde in PBS to 50 µl per well (17). The plates were rinsed with PBS and then blocked with 200 µl per well of HBSBD. Bound borreliae were identified by incubation with 50 µl per well of rabbit anti-Lyme spirochete serum (a gift from A. Steere, Massachusetts General Hospital, Boston, MA) diluted 1/10,000 in HBSBD, then with anti-rabbit Ig G-alkaline phosphatase conjugate diluted 1/10,000 in HBSBD, and finally with 1 mg/ml paranitrophenylphosphate in 100 mM Tris-HCl (pH 9.5), 100 mM NaCl, 5 mM MgCl2 at 50 µl per well. OD was read at 405 nm.

**Quantitative (q)-RT-PCR**

Total RNA was purified by using TRIzol (Invitrogen Life Technologies), according to the manufacturer’s instructions. Total RNA was DNase (Ambion) treated, following manufacturer’s protocol. First-strand synthesis of cDNA from DNase-treated total RNA was performed by using Improv II reverse transcriptase (Promega), according to the manufacturer’s instructions. Control reactions performed in the absence of reverse transcriptase were used to control for contamination by genomic DNA. cDNA samples contaminated by genomic DNA were discarded, and the original RNA was re-processed with DNase before reverse transcription. Quantitative cDNA from specific mRNA transcripts was accomplished by quantitative rt-RT-PCR (Bio-Rad) by using SYBR Green technology (Quantitect SYBR Green PCR kit; Qiagen), as previously described (13, 29). β-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 incubation 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 MgCl2, 2 mM EDTA (pH 8.0), 0.05% Triton X-100, 0.1 mM Na3VO4, 20 mM β-glycerol phosphate, leupeptin (10 µg/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 micromolars of test 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, lipidated OspA of *B. burgdorferi*, to in vitro cultures of chondrocytes. We have shown previously that MMP-1 and MMP-3 production by HC cells infected with *B. burgdorferi* is transcriptionally regulated, so we examined induction of 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 mRNA using q-RT-PCR (13). Addition of the lipoprotein OspA does induce expression of both 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 mRNA using q-RT-PCR (13). Addition of the lipoprotein OspA does induce expression of both 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 mRNA using q-RT-PCR (13). Addition of the lipoprotein OspA does induce expression of both 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 mRNA using q-RT-PCR (13). Addition of the lipoprotein OspA does induce expression of both MMP-1 and MMP-3 mRNA using q-RT-PCR (13).

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

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Reference</th>
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<tr>
<td>Human</td>
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<td>5′-CTGAAAGTGATGAAACAGGC3′</td>
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<tr>
<td></td>
<td>MMP-3</td>
<td>5′-CTGAAAGTGATGAAACAGGC3′</td>
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<tr>
<td></td>
<td>IL-1β</td>
<td>5′-CCATGGGCACACAAACTGAC3′</td>
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<tr>
<td></td>
<td>IL-6</td>
<td>5′-ATGAACTCTTCCTCCACAAAGG3′</td>
</tr>
<tr>
<td></td>
<td>RANTES</td>
<td>5′-GTGTTGCGTCTGGTCTGAC3′</td>
</tr>
<tr>
<td></td>
<td>IP-10</td>
<td>5′-TCCTAGCTCTTCTCCAACAC3′</td>
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<td></td>
<td>MCP-1</td>
<td>5′-TCTGCTGCTCGTCTGAC3′</td>
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<tr>
<td>Mouse</td>
<td>MMP-3</td>
<td>5′-AGCAGAGTATGAGTATG3′</td>
</tr>
<tr>
<td></td>
<td>MMP-19</td>
<td>5′-AGCAGAGTATGAGTATG3′</td>
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<tr>
<td></td>
<td>MCP-1</td>
<td>5′-ACTGAGTGAGTATG3′</td>
</tr>
<tr>
<td></td>
<td>RANTES</td>
<td>5′-ACTGAGTGAGTATG3′</td>
</tr>
<tr>
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<td></td>
<td>CXCL-2</td>
<td>5′-ACTGAGTGAGTATG3′</td>
</tr>
<tr>
<td></td>
<td>Nidogen</td>
<td>5′-ACTGAGTGAGTATG3′</td>
</tr>
</tbody>
</table>

<sup>a</sup> F, Forward; R, reverse.

* 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 is an adapter molecule used by TLR-2 and many other members of the TLR family in the signaling leading to activation of NF-κB. *B. burgdorferi* significantly induced expression of MMP-3 (3.2-fold) (*p* = 0.01) and MMP-19 (6.6-fold) (*p* = 0.006) in the joints of MyD88-deficient mice when compared with sham-infected control mice (Fig. 2). Similar fold induction of MMP-3 (5.6-fold) and MMP-19 (4.7-fold) was observed between sham-infected control mice (Fig. 2). This result confirms that *B. burgdorferi*-induced MMP expression in vivo occurs in a MyD88-independent manner.

* B. burgdorferi-infected mice express proinflammatory cytokines and chemokines 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<sup>−/−</sup> 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...
shown to attach to different host cells through different integrin

A blocking Ab, tl2.1 (500 ng/ml), and subsequently either exposed to lipi-

MMP-1 and MMP-3. HC cells were treated with either CMA or TLR2-

was examined by q-RT-PCR, and the relative expression of each MMP

expression by rt-RT-PCR for MMP-3 and MMP-19. Each sym-

sacrificed 2 wk postinfection. Ankle joint cartilage was microdissected, and

induction can occur in a TLR-2/MyD88-independent manner, we

notably pan ITGA-3i) and control siRNA and subsequently infected with

B. burgdorferi strain N40 and sacrificed 2 wk postinfection. Ankle joint cartilage was microdissected, and
total RNA was harvested. cDNA generated from total RNA was analyzed

for MMP expression by rt-RT-PCR. Expression of each MMP normalized to that of β-actin was plotted. Experiments were repeated three
times, and the average of all experiments was plotted. Bars represent SD. * p < 0.05.

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. burg-
dorferi 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 in-

volved 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 re-
sponses 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 inte-
grins by B. burgdorferi could also result in activation of host sig-

naling pathways leading to induction of inflammatory mediators,

we attempted to block B. burgdorferi induction of MMPs from

primary HCcs 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 experi-

ments. 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 be-
cause of the effects of the Abs on cell attachment and growth. Ab
to αβ3 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

αβ3 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 com-
pared with control siRNA-transfected chondrocytes (Fig. 5). Inte-
grin 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 \( \alpha_3\beta_1 \)

Although *B. burgdorferi* has been shown to bind to other integrin heterodimers, binding to \( \alpha_3\beta_1 \) has not previously been shown. To ascertain whether the inhibitory effects of integrin \( \alpha_3\beta_1 \) on induction of MMPs reflected an inhibition of ligand binding to integrin, we assessed the binding of *B. burgdorferi* to integrin \( \alpha_3\beta_1 \). *B. burgdorferi* was added to plates coated with either purified integrin \( \alpha_3\beta_1 \) or \( \alpha_v\beta_3 \), and adherence was determined by ELISA. Integrin \( \alpha_v\beta_3 \), which has previously been shown to bind to *B. burgdorferi* (16), was used as a positive control. *B. burgdorferi* strongly bound to \( \alpha_3\beta_1 \) at a level equivalent to binding to integrin \( \alpha_v\beta_3 \) (Fig. 6, first panel in the graph). Binding between *B. burgdorferi* and \( \alpha_3\beta_1 \) was specific to integrin \( \alpha_3 \) as the binding was significantly inhibited \((p = 0.021)\) by anti-integrin \( \alpha_3 \) Ab (Fig. 6, second panel in the graph). The binding between *B. burgdorferi* and integrin \( \alpha_3\beta_1 \) was not affected by integrin \( \alpha_3 \) Ab (Fig. 6, second panel in the graph), indicating specificity of the competitive inhibition between *B. burgdorferi* and integrin \( \alpha_3 \) alone. Similarly, binding between *B. burgdorferi* and integrins \( \alpha_3\beta_1 \) and \( \alpha_v\beta_3 \) 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 \( \alpha_3 \) 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 \( \alpha_3 \) directly activates MMP expression or whether \( \alpha_3 \) 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 \( \alpha_3 \) signaling on a broader array of inflammatory mediators. HCs transfected with integrin \( \alpha_3 \) 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-\( \alpha \) \((p = 0.019)\), IL-1\( \beta \) \((p = 0.019)\), IL-6 \((p = 0.019)\), IL-8 \((p = 0.019)\), MCP-1 \((p = 0.014)\), RANTES \((p = 0.014)\), and IFN-inducible

---

**FIGURE 4.** Integrin \( \alpha_3 \) regulates *B. burgdorferi*-induced MMP expression. Primary HC cells were treated with monoclonal anti-integrin \( \alpha_3 \) (A and B) or mAbs directed against \( \alpha_2, \alpha_5, \alpha_6, \) and \( \alpha_6 \) (C and D) (50 \( \mu \)g/ml) for 2 h and then infected with *B. burgdorferi* for 24 h. CMA was used as control Ab. Total RNA was isolated, and rt-RT-PCR was performed for MMP-1 (A and C) and MMP-3 (B and D). The experiment was repeated four (A and B) and two (C and D) times in duplicate, and the average of all the experiments is shown. Bars represent SD. * \( p < 0.05 \).

**FIGURE 5.** Inhibition of integrin \( \alpha_3 \) by siRNA inhibits *B. burgdorferi*-induced MMP expression. Primary HC cells were transfected with siRNA to human integrin \( \alpha_3 \) (ITGA-3i) or control siRNA (siCONTROL) and 24 h later infected with *B. burgdorferi* for 24 h. Total cellular RNA was subjected to q-RT-PCR for integrin \( \alpha_3 \), MMP-1, and MMP-3. The experiment was repeated four times, and the average of all the experiments is shown. Bars represent SD. Values of \( p \) are written on top of each graph.
B. burgdorferi-induced inflammation mediated by \( \alpha_3\beta_1 \)

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 \( \alpha_3\beta_1 \), as a receptor for B. burgdorferi that activates...
signaling cascades that lead to the induction of these inflammatory mediators.

Binding of B. burgdorferi to integrin \(\alpha_\beta_1\) has not previously been reported. B. burgdorferi bound to integrin \(\alpha_\beta_1\) with similar intensity to other integrins that have been shown to bind to B. burgdorferi, including integrins \(\alpha_m\beta_3\), \(\alpha_\beta_1\), and \(\alpha_\beta_1\). These integrins have been suggested to play a primary role in adhesion of 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 B. burgdorferi to these integrins has not previously been reported to activate cell signaling pathways. In our study, Ab blocking of borreial 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 the integrin receptors, \(\alpha_\beta_1\), may play the most significant role in initiating the host response to the organism.

\(\alpha_\beta_1\) is widely expressed on nearly all tissue types, including chondrocytes (38). Substrates for binding to \(\alpha_\beta_1\) integrins are diverse and \(\alpha_\beta_1\) can bind ligands with and without classical RGD integrin-binding motifs (39, 40). \(\alpha_\beta_1\) is a known receptor for host products, including collagen (type I and VI), laminin (\(\alpha_\beta_1\), laminin-5 (\(\alpha_\beta_2\gamma_1\)), laminin-10 (\(\alpha_\beta_3\gamma_1\)), laminin-11 (\(\alpha_\beta_3\gamma_1\)), fibronectin, entactin, nidogen, and thrombospondin-1 (29, 40–46). Fibronectin and thrombospondin also bind to the \(\beta_1\) chain integrins, which bind to B. burgdorferi (47, 48). \(\alpha_\beta_1\) also acts as an alternative receptor for adenosine serotypes 2 and 5 (49).

The relative contributions of signaling mediated by integrin \(\alpha_\beta_1\) 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 borreial lipoprotein (OspA), but not with whole borreia, 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 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 \(\alpha_\beta_1\) are activated only in the presence of high levels of ligand or in specific settings. Our in vitro findings would indicate that this is a less likely explanation.

The ideal way to test the contribution of signaling through integrin \(\alpha_\beta_1\) would be through the use of an animal model of disease. Unfortunately, integrin \(\alpha_\beta_1\) 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 B. burgdorferi, which take weeks to months to evaluate (50). We are also unaware of specific inhibitors of integrin \(\alpha_\beta_1\) that are appropriate for administration to animals. Recently, Glaser et al. (51) have shown that a small molecule, \(S18407\), that inhibits integrin \(\alpha_\beta_1\) can reduce the development of Lyme arthritis in a mouse model. \(S18407\) was shown to have specificity for integrin \(\alpha_\beta_1\), and no significant effect on \(\alpha_\beta_2\), \(\alpha_\beta_3\), \(\alpha_\beta_4\), \(\alpha_\beta_6\) integrin interactions. The effects on \(\alpha_\beta_1\) were not reported. In our studies, we have not seen B. burgdorferi binding to \(\alpha_\beta_1\) and there was no decrease in signaling with use of an \(\alpha_\beta_1\) Ab.

The mechanism by which the B. burgdorferi-integrin \(\alpha_\beta_1\) interaction activates downstream signaling pathways appears to be through activation of JNK, but not p38 MAPK. It has been shown previously that 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 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 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 \(\alpha_\beta_2\) binding to B. burgdorferi seems unlikely, as blockage or deletion of TLR-2 or the common adapter protein MyD88 does not abrogate the inflammatory response to 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 B. burgdorferi and activate an inflammatory response. Identifying the B. burgdorferi proteins that serve as the ligand(s) for \(\alpha_\beta_1\) and determining the relative contribution of \(\alpha_\beta_1\) recognition of B. burgdorferi to overall inflammatory signaling is the subject of continuing research in our laboratory.

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Disclosures

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