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Izadi, Hooman; Motameni, Amirreza T.; Bates, Tonya C.; Olivera, Elias R.; Villar-Suarez, Vega; Joshi, Ila; Garg, Renu; Osborne, Barbara A.; Davis, Roger J.; Rincon, Mercedes; and Anguita, Juan, "c-Jun N-terminal kinase 1 is required for Toll-like receptor 1 gene expression in macrophages" (2007). Davis Lab Publications. 57.
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Keywords
innate immune responses, Toll-like receptors (TLRs), c-Jun N-terminal kinase 1 (JNK1)

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c-Jun N-Terminal Kinase 1 Is Required for Toll-Like Receptor 1 Gene Expression in Macrophages

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Received 5 April 2007/Returned for modification 14 May 2007/Accepted 6 July 2007

The regulation of innate immune responses to pathogens occurs through the interaction of Toll-like receptors (TLRs) with pathogen-associated molecular patterns and the activation of several signaling pathways whose contribution to the overall innate immune response to pathogens is poorly understood. We demonstrate a mechanism of control of murine macrophage responses mediated by c-Jun N-terminal kinase 1 (JNK1) activity. JNK controls tumor necrosis factor alpha production and TLR-mediated macrophage responses to Borrelia burgdorferi, the causative agent of Lyme disease, and the TLR1/TLR2-specific agonist Pam3CysK, JNK1, but not JNK2, activity regulates the expression of the tlr1 gene in the macrophage cell line RAW264.7, as well as in primary CD11b+ cells. We also show that the proximal promoter region of the human tlr1 gene contains an AP-1 binding site that is subjected to regulation by the kinase and binds two complexes that involve the JNK substrates c-Jun, JunD, and ATF-2. These results demonstrate that JNK1 regulates the response to TLR1/2 ligands and suggest a positive feedback loop that may serve to increase the innate immune response to the spirochete.

Toll-like receptors (TLRs) play critical roles during the initiation of innate immunity and the development of specific cell-mediated immune responses (1). The members of the TLR family recognize specific components conserved among microorganisms (1), such as triacylated antigens of Borrelia burgdorferi, the causative agent of Lyme disease (2). In humans, the levels of expression of TLR1 have been involved in their capacity to respond to lipopidated OspA (2), the antigen used for the FDA-approved vaccine against Lyme borreliosis that has been retired from the market. Similarly, the capacity of B. burgdorferi to induce proinflammatory cytokine production is associated with the interaction of lipoproteins of the spirochete with TLR1/TLR2 complexes (2, 16). The comparison of disease in patients with polymorphic forms of different TLRs has demonstrated their importance in the progression of asthma and atherosclerosis (13, 25). TLR expression is regulated by their interaction with specific ligands and by cytokines. Thus, during leprosy, TLR1 expression is upregulated by gamma interferon (IFN-γ) (20), while B. burgdorferi lipoproteins increase the expression of TLR1 and TLR2 and induce the downregulation of TLR5 (7). Lipopolysaccharide has also been shown to induce increased expression of TLRs and accessory molecules, including MyD88 and MD-2 (24, 29). The mechanism by which this regulation occurs is not completely understood. The transcription factors AP-1, Ets, and PU.1 have been involved in the regulation of murine TLR4 expression (28, 34).

The interaction of TLRs with specific ligands results in the activation of several signaling pathways. The activation of the transcription factor NF-κB in response to TLR stimulation results in the expression of chemotactic factors, proinflammatory cytokines, and adhesion molecules in several cell types, such as macrophages, fibroblasts, and synovial cells (19). TLR engagement also results in the activation of the mitogen-activated protein p38 and c-Jun N-terminal kinases (JNK) (19). These pathways are involved in various physiological processes. The JNK pathway regulates apoptosis, development, cell transformation, T-cell activation and differentiation, and cytokine production (10, 18, 31, 35, 38, 39). Three isoforms of JNK have been identified: two ubiquitously expressed isoforms, JNK1 and JNK2, and the tissue-specific isoform JNK3 (9). JNK phosphorylates the transcription factor c-Jun and increases AP-1 transcriptional activity. Other substrates include JunD, ATF-2, ATF-4, Elk-1, Sap-1, and NFATc3 (9).

In this study, we show that JNK1 controls macrophage responses to B. burgdorferi by regulating the expression of the tlr1 gene. Our results clarify the role of this kinase in the innate immune response to B. burgdorferi.

MATERIALS AND METHODS

Cells. JNK1-deficient (10) and JNK2-deficient (41) C57BL/6 (B6) mice were used to purify splenocyte macrophages by positive selection, using a biotinylated antibody (Ab) against CD11b (BD Pharmingen, La Jolla, CA). The purity of the cells, as determined by flow cytometry, was >85%. The macrophage cell line RAW264.7 (ATCC, Manassas, VA) was grown in RPMI medium (Sigma, St. Louis, MO) supplemented with 10% fetal calf serum.

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Published ahead of print on 30 July 2007.
TABLE 1. Primers used in the study

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<th>Gene/target</th>
<th>Sequence</th>
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<td>RT-PCR</td>
</tr>
<tr>
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<td>Real-time PCR</td>
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<td>AP-1 consensus</td>
<td>5′-CGC TTT AGG ACT TAG CCG GA-3′</td>
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* Underlining represents the consensus sequence.

All procedures that involved animals were in accordance with institutional guidelines for animal care at UNC Charlotte and the University of Massachusetts at Amherst.

Plasmids, small interfering RNA (siRNA), and transfections. Plasmids containing a mutant (dominant-negative) version of human JNK1 (dnJNK), constructed by replacement of Thr183 and Tyr185 by Ala and Phe, respectively (27), or the luciferase gene downstream of a 2×AP-1 (27) or 5×NF-kB (Stratagene, La Jolla, CA) response elements were used. The plasmids were transfected into 3 μl murine IFN-β264.7 cells using siPort Amine transfection agent (Ambion) following the manufacturer’s instructions. Control siRNA (Ambion) containing a random mixture of oligonucleotides was used in parallel. After 48 h, the cells were assessed for plk1 mRNA by reverse transcriptase (RT) PCR (Table 1) and stimulated as described below.

Stimulations. Low-passage B. burgdorferi N40 lysates were obtained from mid-log phase cultures by sonication. The protein concentration was determined by the Bradford method (Bio-Rad, Hercules, CA). The TRL1/TLR2 agonist PAM3-CSK4 was purchased from Invivogen (San Diego, CA). In vitro stimulations were performed using 10 μg/ml of a specific 32P end-labeled double-stranded oligonucleotide as described previously (26) in the absence or presence of unlabeled oligonucleotides. The reaction was performed at an annealing temperature of 55°C. Relative expression of the gene was determined by amplifying β-actin (Applied Biosystems, Foster City, CA) and was referred to control (SK)-transfected cells, according to the following formula:

\[
\text{Fold induction} = 2^{\frac{\text{Ct} _{\text{gene}} - \text{Ct} _{\beta\text{-actin}}}{\text{Ct} _{\beta\text{-actin}}}}
\]

where \( \text{Ct} \) represents the threshold cycle for each gene.

Construction of tlr1-luc and tlr1(luc)–luc plasmids. The proximal DNA fragment corresponding to 1 kb of the human tlr1 gene promoter region was subcloned upstream of the promoterless firefly luciferase (Luc) gene in the vector pGL3-basic (Promega). The 1-kb promoter fragment was generated by PCR (Table 1) using genomic DNA isolated from HeLa cells as a template. The fragment was cloned into the pBAD-TOPO (Invitrogen) vector and subcloned using the Smal and Sac restriction sites into the PGL3 vector (pG3-luc-luc). The construct was sequenced across both junctions to confirm the nucleotide sequence and the predicted orientation. Empty pG3luc was used as a control.

The PGL3-luc(luc)-luc plasmid, a derivative of pG3-luc(luc)-luc with the AP-1-binding site deleted (nucleotides –502 to –508 relative to the transcription start site), was constructed by using the QuikChange II XL site-directed mutagenesis kit (Strategene) according to the manufacturer’s instructions. The primers used for the deletion of the 7 bp are listed in Table 1. The mutations were confirmed by sequencing.

Nuclear extracts and electromobility shift assay (EMSA). Nuclear extracts were obtained as described previously (26) from 106 RAW264.7 cells transfected with 1 μg of anti-c-Jun (N), c-Fos (4), JunD (329), ATF-2 (C-19), and CREB (H-74) Abs (Santa Cruz Biotechnology).

Statistical analyses. Data are presented as the means and standard errors of at least three independent experiments or means and standard deviations for experiments performed in triplicate. The means of independent experiments were compared with two-way analysis of variance, followed by Bonferroni post-
tests, using the software Prism version 4.0. The means were considered statistically different when \( P < 0.05 \).

**RESULTS**

**JNK activity regulates TNF-\( \alpha \) production in response to **B. burgdorferi**.** The role played by the JNK pathway in macrophage responses to pathogens mediated by TLRs is poorly understood. To address whether the interaction between B. burgdorferi antigens and macrophages results in the activation of JNK, we first stimulated RAW264.7 cells with a B. burgdorferi lysate. JNK activity was evident after 40 min of stimulation and decayed after 80 min (Fig. 1A). The activation of JNK was also detected in primary splenic macrophages (CD11b+ cells) from B6 mice stimulated with a B. burgdorferi lysate (Fig. 1A).

The involvement of JNK activity in macrophage responses to TLR ligands was then assessed. RAW264.7 cells were transfected with a dominant-negative form of the kinase (dnJNK) and stimulated with a B. burgdorferi lysate or the TLR1/TLR2 agonist PAM 3-CSK4, and the supernatants were evaluated for TNF-\( \alpha \) production. The repression of JNK activity resulted in a significant reduction of TNF-\( \alpha \) production compared to control transfected cells (\( P < 0.001 \) for B. burgdorferi lysate, \( P < 0.01 \) for PAM3-CSK4 stimulation) (Fig. 2A).

**FIG. 1.** JNK activity regulates TNF-\( \alpha \) production in response to B. burgdorferi. (A) RAW264.7 (top) and primary CD11b+ (bottom) cells were stimulated with a B. burgdorferi lysate for the indicated times (top) or 40 min (bottom) prior to assaying JNK activity in vitro with c-Jun as a substrate. Phospho-c-Jun was then detected by immunoblotting. The results are representative of three experiments performed. (B) RAW264.7 cells were transfected with a plasmid containing dnJNK1 or a plasmid control (SK) and stimulated with a B. burgdorferi lysate (Bb) or PAM 3-CSK4, and the TNF-\( \alpha \) levels in the supernatant supernatants were then quantified by ELISA. The results shown are the average standard error (SE) of three independent experiments, respectively. *, \( P < 0.001 \) and \( P < 0.01 \) for B. burgdorferi and PAM 3-CSK4 stimulation, respectively. (C) RAW264 cells were stimulated with 10 \( \mu \)g/ml of a B. burgdorferi lysate (Bb) or 1 \( \mu \)g/ml of PAM 3-CSK4 in the presence of increasing concentrations of the JNK inhibitor SP100625. TNF-\( \alpha \) was quantified in the stimulation supernatants after 16 h. The results are the average plus SE of three independent experiments.

**FIG. 2.** TLR1/2-mediated responses are dependent on JNK activity. (A) RAW264.7 cells were cotransfected with a plasmid containing the luciferase gene under the influence of AP-1 (A) or NF-\( \kappa B \) (B) response elements plus a plasmid containing dnJNK1 or a plasmid control (SK). The cells were then stimulated with a B. burgdorferi lysate (Bb) for 16 h, and luciferase activity was assayed. The results shown are the average standard error of four and three independent experiments, respectively. *, \( P < 0.001 \) (AP-1-luc) and \( P < 0.05 \) (NF-\( \kappa B \)-luc). (C) RAW264.7 cells were transfected with a plasmid containing the dnJNK form or a plasmid control (SK) and stimulated with a B. burgdorferi lysate for the indicated times. The cells were then lysed and assessed for I\( \kappa B \) content by immunoblotting (left). Band quantitation was performed with the Java-based software ImageJ (right). The experiment shown is representative of three performed. (D) RAW264.7 cells were transfected with a plasmid containing the dnJNK form or a plasmid control (SK) and stimulated with recombinant murine IFN-\( \gamma \) for 30 min. Phospho-STAT1 and total STAT1 levels were determined by immunoblotting. The results shown are representative of two experiments performed with similar results.
and $P < 0.01$ for PAM$_2$CSK$_4$ stimulation) (Fig. 1B). Similar results were obtained in macrophages isolated from the spleens of B6 mice (data not shown) and RAW264.7 cells stimulated with a B. burgdorferi extract or PAM$_2$CSK$_4$ in the presence of increasing concentrations of the JNK inhibitor SP600125 (5) (Fig. 1C). The reduced production of TNF-$\alpha$ was not the result of SP600125-induced cell death, as determined by the analysis of the cells by trypan blue exclusion (data not shown). These results indicated that TLR1/TLR2-mediated TNF-$\alpha$ production in macrophages involves JNK activity.

**TLR-mediated responses are dependent on JNK activity.** We further substantiated the contribution of JNK activity to TLR-induced signaling events in macrophages by analyzing the activation of JNK-dependent and independent transcription factors. JNK activity results in the phosphorylation of c-Jun and the formation of AP-1 complexes (9). As expected, the repression of JNK activity during stimulation with B. burgdorferi resulted in decreased AP-1 transcriptional activity ($P < 0.001$) (Fig. 2A). As a control, we also analyzed the activation of JNK-independent signaling pathways in response to B. burgdorferi antigens. Surprisingly, the repression of JNK activity also resulted in reduced NF-$\kappa$B transcriptional activity ($P < 0.05$) (Fig. 2B). No reports have associated JNK activity and the activation of NF-$\kappa$B. Since the repression of JNK activity during stimulation with TLR ligands results in lower TNF-$\alpha$ production, and this cytokine also activates NF-$\kappa$B (36), we analyzed I$\kappa$B degradation in the cells that had been transfected with the dnJNK plasmid at times when the production of the cytokine was not evident (data not shown). The stimulation of RAW264.7 cells transfected with the dnJNK plasmid also resulted in decreased I$\kappa$B degradation in response to IFN-$\gamma$-induced signals. The phosphorylation of STAT1 induced by IFN-$\gamma$ was not affected by the presence of the dnJNK-containing plasmid in RAW264.7 cells (Fig. 2D), indicating that JNK affects TLR-mediated re-
responses, but not the TLR-independent, IFN-γ-mediated response, in macrophages.

**JNK1 regulates the expression of the tlr1 gene.** Our results suggested that TLR-mediated responses in macrophages are dependent on JNK activity. These data could be explained by a direct effect of JNK activity on the regulation of the expression of the components involved in the responses, including TLR1 and TLR2, or signaling intermediates that lead to the activation of downstream signaling pathways. To test whether JNK regulates the expression of the components involved in TLR-mediated responses, we analyzed tlr1 and tlr2 mRNA levels in macrophages with repressed or absent JNK activity. RAW264.7 cells transfected with dnJNK showed lower expression levels of tlr1, with no effect on tlr2 (Fig. 3A). These results correlated with lower TLR1 protein levels than in control transfected cells, while TLR2 protein levels were not affected by the dnJNK plasmid (Fig. 3B). The reduction of TLR1 surface protein expression induced by the repression of JNK activity was also observed by flow cytometry in THP-1 cells transfected with dnJNK (data not shown).

To further demonstrate the regulation of tlr1 gene expression by JNK, we analyzed the levels of expression of both tlr1 and tlr2 genes in primary macrophages from JNK1- and JNK2-deficient mice. While tlr2 mRNA was not affected by the lack of either JNK1 or JNK2, tlr1 mRNA was reduced in JNK1-deficient macrophages (Fig. 3C). Moreover, the silencing of JNK1-encoding mRNA in RAW264.7 cells resulted in lower tlr1 gene expression than in controls (Fig. 3D), suggesting that JNK1 activity specifically regulates the expression of TLR1.

To assess the contribution of the JNK isoforms to TNF-α production in response to *B. burgdorferi*, we stimulated JNK1- and JNK2-deficient CD11b⁺ cells with a *B. burgdorferi* lysate. Both JNK1- and JNK2-deficient macrophages produced lower levels of TNF-α than wild-type macrophages, although the differences were not statistically significant (Fig. 3E). Correspondingly, JNK1 siRNA-transfected RAW264.7 cells produced lower levels of TNF-α in response to a *B. burgdorferi* lysate and PAM3-CSK4 (*P* < 0.05 for both stimuli) (Fig. 3G), indicating that JNK1 and JNK2 regulate the production of TNF-α through different mechanisms and that it involves the regulation of tlr1 gene expression by JNK1.

To determine whether JNK1 regulates TLR1/2-mediated responses solely through the control of TLR1 expression, we transfected RAW264.7 cells with a plasmid that drove the constitutive expression of the receptor. The ectopic expression of TLR1 prevented the inhibition of TNF-α production in the presence of the dnJNK plasmid in response to *B. burgdorferi* antigens and PAM3-CSK4 (Fig. 3H), confirming that JNK1 controls TLR1/2-mediated signals in macrophages by regulating the expression of TLR1.

**JNK1 regulates tlr1 promoter activity.** To further demonstrate that JNK activity regulates tlr1 gene transcription, we cloned the proximal 1-kb promoter region of the tlr1 gene (Fig. 4) upstream of a promoterless luciferase gene. The transfec-
Three putative AP-1 binding sites that potentially could serve as AP-1 binding sites in the tlr1 promoter were identified (Fig. 4). Double-stranded oligonucleotides that spanned these regions were used in EMSAs (Table 1). Only the oligonucleotide based on the sequence found at −502 to −508 showed binding of nuclear extract preparations of unstimulated (Fig. 5B) and B. burgdorferi-stimulated (not shown) RAW264.7 cells. We observed two binding complexes at this site. The lower complex increased in B. burgdorferi-stimulated RAW264.7 compared to unstimulated cells (Fig. 5C) and was competed when the binding reactions contained anti-c-Jun and anti-c-Fos Abs (Fig. 5C). This complex was competed by unlabeled double-stranded oligonucleotides corresponding to the AP-1 consensus binding site (Fig. 5C) and coincided with the complex obtained with a consensus AP-1 binding site oligonucleotide (Fig. 5D). The presence of ATF-2 and JunD Abs competed the formation of both complexes, while Abs to CREB did not result in competition (Fig. 5C). The upper band observed with anti-CREB was not consistently seen in other experiments performed. These results suggested that this region serves as a docking area for two complexes that contain ATF-2 and JunD, which coincided with the complex obtained with a consensus AP-1 binding site oligonucleotide (Fig. 5D).

FIG. 5. JNK1 regulates tlr1 promoter activity. (A) (Top) RAW264.7 cells were cotransfected with a plasmid containing the luciferase gene downstream of the 1-kb proximal tlr1 promoter and the dnJNK plasmid or a plasmid control (SK). After 30 min of incubation with PAM3-CSK4 or left unstimulated (Unst.) Luciferase activity (AU, arbitrary units) was measured 16 h after stimulation. The data presented correspond to the average plus standard deviation of triplicate determinations of one of four experiments performed. *, P < 0.05. (Bottom) Western blot of RAW264.7 cells stimulated with a B. burgdorferi lysate (Bb) or PAM3-CSK4 or left unstimulated (Unst.). Luciferase activity (AU, arbitrary units) was measured after 16 h of stimulation. The data presented correspond to the average plus standard deviation of triplicate determinations of one of four experiments performed. *, P < 0.05. (B) EMSA showing nuclear extract binding to oligonucleotides (oligo) containing putative AP-1 binding sites in the proximal 1-kb tlr1 promoter. The reactions were performed in the absence or presence of an excess (100×) of unlabeled (cold) oligonucleotides. (C) EMSA of nuclear extracts of RAW264.7 cells unstimulated and stimulated with a B. burgdorferi lysate for 30 min. The reactions were performed in the absence or presence of Abs against c-Jun, JunD, c-Fos, CREB, and ATF-2 and in the absence or presence of unlabeled (cold) oligonucleotides corresponding to the tlr1 promoter or the AP-1 consensus binding sequence. The arrows indicate the constitutive (top) and B. burgdorferi-enhanced (bottom) complexes formed. (D) EMSA of tlr1-derived and consensus AP-1 binding oligonucleotides with nuclear extracts from unstimulated or B. burgdorferi-stimulated RAW264.7 cells. (E) RAW264.7 cells were cotransfected with plasmids containing tlr1− or tlr1Δ− (−502, −508) [tlr1(smarr)]-luciferase and a plasmid containing dnJNK or a plasmid control (SK). After 48 h, the cells were stimulated with a B. burgdorferi lysate (Bb) or PAM3-CSK4 or left unstimulated (Unst.). Luciferase activity was measured 16 h poststimulation. The results shown are the average plus standard error of three independent experiments. *, P < 0.05.

DISCUSSION

The interaction of pathogen-associated molecular patterns with the germ line-encoded TLRs is a fundamental feature of the immune response associated with infection. It is becoming evident that these interactions in innate immune cells have profound consequences for the ability of the body to mount efficient or detrimental responses to pathogens. Thus, deficiencies in TLR1, TLR2, or the adaptor protein MyD88 lead to increased B. burgdorferi burdens in mice (2, 4, 6, 21, 40), underscoring the importance of these receptors for the efficient
control of infection. Moreover, the severity of pathology associated with infection with *Mycoplasma leprae* is associated with the host’s capacity to respond to mycobacterial antigens and is also related to TLR expression (20). In addition, TLR ligands are being considered as potential adjuvants that could help boost the response to vaccines compared to the currently limited choices in humans (17, 32). TLR-mediated signals are also required for the successful vaccination of individuals against infectious diseases. For example, the deficient expression of TLR1 in patients hyporesponsive to lipidated outer surface protein A of *B. burgdorferi* is associated with decreased responses to the vaccine against the spirochete (2). It is therefore imperative to understand the control of these responses, including how the expression of TLRs is regulated in innate immune cells.

Our results demonstrate that JNK activation upon engagement of TLR1/2 complexes with specific ligands initiates a positive feedback cascade that increases the expression of TLR1. They also show that in our model the ectopic expression of the receptor restores the capacity of macrophages to respond to *B. burgdorferi* and PAM~3~-CSK~4~, indicating that in our system, JNK1 activity contributes exclusively to TLR1 expression. Experiments with splenic macrophages derived from deficient mice demonstrated that JNK1, but not JNK2, activity is involved in the control of TLR1 expression, while neither JNK1 nor JNK2 regulates the expression of TLR2 in RAW264.7 cells or primary macrophages. These results correlate with previous observations in the macrophage cell line RAW264.7 that showed no implication of JNK in the expression of the tlr2 gene (22). Furthermore, our results show that JNK2 also contributes to the induction of TNF-α in response to *B. burgdorferi* antigens and the TLR1/2 agonist PAM~3~-CSK~4~, through a mechanism that does not involve the regulation of TLR1. In correlation, the induction of TNF-α by the TLR4, TLR9, and TLR5 agonists lipopolysaccharide, poly(dI-dC), and flagellin, respectively, was lower in cells transfected with the plasmid containing dnJNK (Fig. 3F). These data therefore imply nonoverlapping functions of the two kinases that have also been demonstrated in T cells and other cell types (8, 10, 30, 41) and that have been associated with differential binding of both isoforms with their substrates (11).

Several reports have shown the regulation of TLR expression by different stimuli, including *B. burgdorferi* stimulation and cytokines (7, 20, 24). Similarly, corticotropin-release factor and the urocortins regulate the expression of TLR4 through the activation of the transcription factors PU.1 and AP-1 (34), although the exact mechanisms of regulation have not been described. The regulation of TLR expression may be stimulus dependent, since *B. burgdorferi* does not affect the expression of the tlr4 gene (reference 7 and data not shown). Similarly, the regulation of tlr2 gene expression by NF-κB has been documented (22–24), and NF-κB binding sites have been described in the tlr2 promoter (23, 37). However, our results indicate that despite lower NF-κB activation induced by TLR ligands in the absence of JNK activity due to reduced expression levels of TLR1, the levels of TLR2 remained unchanged.

The interaction of *B. burgdorferi* antigens with macrophages potentially occurs through different TLRs: TLR1/TLR2 dimers respond to triacylated lipoproteins, such as lipidated OspA (2), while TLR5 and TLR9 probably contribute to the response of bacterial lysates through their interaction with flagellin and hypomethylated CpG motifs, respectively (14, 15). In turn, these interactions may be physiologically relevant, due to bacterial death in vivo. Our results strongly suggest, however, that the predominant response under our experimental conditions occurred through TLR1/TLR2, since (i) the response was mimicked by the use of the TLR1/TLR2 agonist PAM~3~-CSK~4~ and (ii) the ectopic expression of TLR1 in JNK-repressed RAW264.7 cells completely restored the responses to both *B. burgdorferi* extracts and PAM~3~-CSK~4~. In light of our results, we propose that the interactions of spirochetal antigens with TLRs result in the activation of JNK. JNK substrates contribute to the upregulation of the *tlr1* gene, which in turn increases the response of macrophages to TLR1/TLR2 ligands. Thus, we propose that JNK1 contributes to the response of macrophages to *B. burgdorferi* by regulating the expression of TLR1.

Several signaling pathways are activated as a result of the ligation of TLRs with specific ligands. The specific contribution of each pathway to phagocytic responses is still unclear. During infection with *B. burgdorferi*, p38 mitogen-activated protein kinase controls inflammation (3). Our results indicate that the different signal pathways activated as a result of the interactions between TLRs and their ligands are not redundant, although they may all be needed for a full response to infectious agents. Since macrophage responses to *B. burgdorferi* are largely dependent on TLR-mediated interaction with ligands present in the bacterium, our results can provide the basis for a full understanding of the immune response to this prevalent infectious agent.

ACKNOWLEDGMENT

This work was supported by NIH grant AR048265 to J.A.

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