The Antibiotics Doxycycline and Minocycline Inhibit the Inflammatory Responses to the Lyme Disease Spirochete *Borrelia burgdorferi*

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Tetracyclines moderate inflammatory responses of various etiologies. We hypothesized that tetracyclines, in addition to their antimicrobial function, could exert control over the inflammation elicited by *Borrelia burgdorferi*. To model systemic effects, we used the human monocytic cell line THP-1; to model effects in the central nervous system, we used rhesus monkey brain astrocytes and microglia. Cells were stimulated with live or sonicated *B. burgdorferi* or with the lipoprotein outer surface protein A in the presence of increasing concentrations of doxycycline or minocycline. Both antibiotics significantly reduced the production of tumor necrosis factor–α, interleukin (IL)–6, and IL-8 in a dose-dependent manner in all cell types. Microarray analyses of the effect of doxycycline on gene transcription in spirochete-stimulated monocytes revealed that the *NFKB* and *CHUK* (alias, *IKKA*) genes were down-regulated. Functionally, phosphorylation of IkBα and binding of NF-κB to target DNA were both reduced in these cells. Our results suggest that tetracyclines may have a dual therapeutic effect in Lyme disease.

Lyme borreliosis is a tick-transmitted disease caused by the spirochete *Borrelia burgdorferi*. The spirochete can invade and persist in a variety of tissues. This persistence has been correlated with severe disease and may be responsible for localized inflammation [1, 2]. The association between tissue invasion and localized inflammation may be explained by the fact that the spirochete possesses potent cytokine-stimulatory properties. It has been reported that, in vitro, *B. burgdorferi* can induce the production of proinflammatory cytokines [3–7] as well as other inflammatory mediators, such as chemokines [8, 9] and nitric oxide [10]. Thus, inflammation is thought to play an important role in the pathogenesis of Lyme disease.

Antibiotics—doxycycline, in particular—are used for the treatment of early and early disseminated Lyme borreliosis [11]. Doxycycline also may be used to treat late-stage Lyme arthritis, although for late neurologic manifestations of Lyme borreliosis ceftriaxone is recommended [11]. Doxycycline is a semisynthetic antibiotic with a broad spectrum of antimicrobial activity; it belongs to the tetracycline family [12]. All tetracyclines are bacteriostatic and exert this effect by inhibiting the synthesis of bacterial proteins. Protein synthesis inhibition takes place at the ribosome, by preventing the amino-acyl tRNA from binding to the acceptor site on the mRNA-ribosome complex [13].

In recent years, tetracyclines have been shown to moderate host-cell inflammatory responses in a variety of scenarios [14]. Doxycycline and another tetracycline, minocycline, were reported to induce neuroprotective effects in animal models of cerebral ischemia [15–18]. In addition, minocycline was shown to have beneficial effects in animal models of Huntington disease [19, 20], Parkinson disease [21], Alzheimer disease [22], amyotrophic lateral sclerosis [23], multiple sclerosis [24], and spinal cord injury [25]. The beneficial effects of the tetracyclines were shown to be related to a reduction in microglial activation and proliferation [16, 17], along with the inhibition of inducible nitric oxide synthase and interleukin (IL)–1β expression [26, 27]. Tetracy-
clines were shown to inhibit the p38 mitogen-activated protein kinase (MAPK) and NF-κB pathways [22, 26, 28], which play an important role in controlling the expression of proinflammatory mediators. Moreover, these antibiotics may promote central nervous system (CNS) cell survival through the inhibition of caspase-1 and -3 activity [29, 23, 25]. Thus, tetracyclines may attenuate multiple processes involved in mediating inflammation and cell death.

In the present study, we hypothesized that tetracyclines, in addition to their antimicrobial function, could exert control over the inflammatory effects elicited by *B. burgdorferi* spirochetes, their lipoproteins, and/or the bacterial debris left in the tissues after bacterial death. We evaluated this hypothesis by means of experiments performed in vitro, using cells involved in the innate immune response both in the periphery and in the CNS. To model systemic effects, we used the human monocytic cell line THP-1; to model effects in the CNS, we used primary cultures of rhesus monkey brain astrocytes and microglia. We present the results of this study here.

**METHODS**

**THP-1 cell culture.** The human monocytic cell line THP-1 was obtained from the American Type Culture Collection. Cells were cultured in RPMI 1640 that was modified so as to contain 2 mmol/L L-glutamine, 10 mmol/L HEPES, 1 mmol/L sodium pyruvate, 4.5 g/L glucose, and 1.5 g/L sodium bicarbonate (American Type Culture Collection) (hereafter, RPMI-M) and that was supplemented with 0.05 mmol/L 2-mercaptoethanol (Sigma-Aldrich), 10% fetal bovine serum (Hyclone), and penicillin/streptomycin (100 U/mL and 100 μg/mL, respectively; Gibco) at 37°C in a humidified atmosphere of 5% CO₂.

**Primary cultures of glial cells.** Brain tissues used in this study were collected from adult rhesus macaques (*Macaca mulatta*) of either Chinese or Indian origin. These animals were not infected with *B. burgdorferi* and were euthanized for purposes unrelated to this project. The procedure used for euthanasia was consistent with the recommendations of the American Veterinary Medical Association’s Panel on Euthanasia. Tissue was removed from the cortical region of the brain and immediately processed. Glial cells were isolated using a protocol that has been described elsewhere [29] and were maintained in Dulbecco’s modified Eagle medium (nutrient mixture F12) with L-glutamine and HEPES buffer, 10% fetal bovine serum (HyClone), 0.5 ng/mL granulocyte-macrophage colony-stimulating factor (Sigma-Aldrich), and penicillin/streptomycin (100 U/mL and 100 μg/mL, respectively) (hereafter, DMEM-G) at 37°C in a humidified atmosphere of 5% CO₂. After 14–21 days in culture, microglia were isolated by vigorously tapping the culture flasks. To obtain purified astrocytes, glial cells were incubated for 90 min in 10 mmol/L L-leucine methyl ester (Sigma-Aldrich). The purity of astrocytes and microglial cultures was assessed by staining with a specific microglia marker (anti-ionized calcium-binding adapter molecule-1) and was routinely 99%.

**Bacterial culture.** *B. burgdorferi* strain B31 (clone 5A19, possessing all plasmids) was cultured in BSK-H (Sigma-Aldrich) supplemented with 10% heat-inactivated (56°C for 30 min) rabbit serum (Sigma-Aldrich) and antimicrobial agents (rifampicin at 50 mg/mL, phosphomycin at 200 mg/mL, and amphotericin B at 8 mg/mL) at 34°C in a humidified atmosphere of 5% CO₂ and 95% N₂. For subsequent experiments, *B. burgdorferi* were washed with PBS and resuspended in DMEM-G or RPMI-M. No antibiotics were added to the RPMI-M or DMEM-G. Spirochetal viability was confirmed after a 24-h culture in these media by means of the LIVE/DEAD BacLight Bacterial Viability Kit (Molecular Probes), in accordance with the manufacturer’s instructions. We observed a spirochetal viability of 80%–85%.

**Measurement of cytokine and chemokine concentrations.** THP-1 cells (5 × 10⁵ cells/mL), microglia (2 × 10⁴ cells/mL), or astrocytes (5 × 10⁴ cells/mL) were preincubated for 24 h in RPMI-M or DMEM-G (as appropriate, with no added penicillin/streptomycin) either alone or with added doxycycline hyclate or minocycline hydrochloride (Sigma-Aldrich) at final concentrations of 0.0025, 0.005, or 0.01 mmol/L. The cells were washed in culture medium and incubated for 24 h with fresh doxycycline hyclate or minocycline hydrochloride in the appropriate culture medium either alone or with added live *B. burgdorferi* (MOI of 10), sonicated *B. burgdorferi* (quantity equivalent to an MOI of 10), or 0.25 μg/mL recombinant lipidated outer surface protein A (L-OspA; GlaxoSmithKline) for 24 h. Cell-free culture supernatants were collected and assayed for the presence of tumor necrosis factor (TNF)–α, IL-6, and IL-8 by sandwich ELISA (BD Biosciences), in accordance with the manufacturer’s instructions.

**Cell viability.** Cell suspensions were mixed with an equal volume of 0.4% isotonic trypan blue solution (Invitrogen). Total cells and dye-accumulating cells (nonviable) were counted using a hemocytometer under a light microscope.

**RNA isolation.** RNA was isolated from 2 × 10⁶ THP-1 cells. Cells either were incubated in medium alone or were pretreated for 24 h with doxycycline hyclate at 0.01 mmol/L, followed by incubation with fresh doxycycline hyclate at the stated concentration and sonicated *B. burgdorferi* (quantity equivalent to an MOI of 10) for 2, 8, or 12 h, after which RNA was collected using the RNeasy Mini Kit (Qiagen), in accordance with the manufacturer’s protocol. The DNA-free Kit (Ambion) was used to remove DNA contamination. The RNA concentration was determined spectrophotometrically (optical density read at 260 nm).

**Human DNA microarray.** Microarray experiments and analyses of data were performed according to protocols that have been described elsewhere [30]. We used the 44,544-element 70mer HEEBO (Human Exonic Evidence-Based Oligonucleotide) microarray, supplied by the Stanford Function Genomics
Facility (http://www.microarray.org/sfgf/heebod). Five micrograms of mRNA from THP-1 cells was used to incorporate Cy3 (samples with no doxycycline) or Cy5 (samples with added doxycycline). Labeling, hybridization, and scanning were performed per protocols that have been described elsewhere [30].

**Microarray analysis.** Human microarrays were scanned in a dual-confocal continuous microarray scanner (GenePix 4000B; Molecular Devices), using GenePix Pro (version 6.1) as the image-acquisition and extraction software. All of the microarray data were based on duplicate measurements (i.e., using RNA derived from 2 independent experiments with THP-1 cells for each of the experimental conditions described). The resulting text data were imported into DecisionSite for Functional Genomics software (version 3.0; Spotfire) and filtered to remove unreliable data [31]. Data were then normalized (locally weighted scatterplot smoothing) using R-Bioconductor software (version 2.1) and subjected to statistical analysis [32]. Genes whose expression changed by 2.0-fold with a corrected P < .05 by Student’s t test were considered to be differentially expressed in a statistically significant manner. Pathway analysis was performed by uploading significant data sets into Ingenuity Pathways Analysis (IPA) software (version 5.0; Ingenuity). Pathways that were perturbed in a statistically significant manner (P < .05) were included in the analysis.

**Extraction of nuclear proteins.** THP-1 cells (5 × 10^6 cells/mL) were either pretreated for 24 h with doxycycline hydrochlorate or minocycline hydrochloride (each at 0.005 or 0.01 mmol/L) or left in medium alone. Cells were then washed and incubated with fresh doxycycline hydrochlorate or minocycline hydrochloride at the same respective concentrations as above and stimulated with sonicated *B. burgdorferi* (MOI of 10) for 2 or 4 h. Nuclear extracts were isolated using the Nuclear Extract Kit (Active Motif), in accordance with the manufacturer’s instructions. Protein concentrations in the nuclear extracts were determined by the Bradford assay (Bio-Rad Protein Assay).

**NF-κB activation assay.** Nuclear extracts (2 μg) were assayed for NF-κB DNA–binding activity by ELISA, using the TransAM Kit (Active Motif) in accordance with the manufacturer’s protocol. The NF-κB TransAM Kit includes a 96-well plate with immobilized double-stranded oligonucleotides (5’-GGGACTT-TCC-3’) that specifically bind to the active form of NF-κB contained in the nuclear extract. The primary antibody directed against the NF-κB p65 subunit recognizes the transcriptionally active NF-κB complex bound to the oligonucleotide. A secondary antibody conjugated to horseradish peroxidase provides the colorimetric readout (after adding a proprietary color-development reagent and stop solution), which is quantified spectrophotometrically at 450 nm with a reference wavelength of 655 nm.

**Western blot analysis.** THP-1 cells (1 × 10^6 cells/mL), pretreated for 24 h with either doxycycline hydrochlorate at 0.01 mmol/L or medium alone, were washed and incubated with fresh doxycycline hydrochlorate (0.01 mmol/L) and sonicated *B. burgdorferi* (MOI of 10) for 15, 30, or 60 min. Cells were lysed in RIPA buffer with protease inhibitors (Protease Inhibitor Cocktail Kit; Pierce Biotechnology). Protein concentration was determined by the Bradford assay (Bio-Rad). Equal amounts of sample (50 μg/lane) were separated in 12% acrylamide Tris-HCl precast gels (Bio-Rad), transferred to Protran nitrocellulose membranes (Schleicher and Schuell BioScience), and blocked in PBS with 0.05% Tween 20 and 3% bovine serum albumin fraction V (Sigma-Aldrich). Membranes were probed with primary antibody against phospho-IκBα (1:1000; Cell Signaling Technology) and antibody to β-tubulin (1:6000; Abcam), followed by incubation with the appropriate secondary antibody (Santa Cruz Biotechnology) conjugated with horseradish peroxidase. Immunoreactive proteins were visualized using 3,3’-diaminobenzidine as chromogen.

**Statistical analysis.** Results are presented as means ± SDs for the number of determinations specified in each case. Cytokine concentrations and optical density values from the NF-κB activation assay were examined by 1-way analysis of variance, using Prism software (version 3.0; GraphPad Software). Differences were considered statistically significant at P < .05.

**RESULTS**

**Effects of doxycycline and minocycline on the production of proinflammatory mediators by cells stimulated with L-OspA or with live or sonicated B. burgdorferi.** To explore the effects that doxycycline and minocycline have on inflammation elicited by *B. burgdorferi* or by spirochetal antigens, we quantified the differential production of 3 proinflammatory mediators, IL-6, IL-8, and TNF-α, in 3 different cell types, THP-1 monocytes, astrocytes, and microglia. After pretreatment with medium alone or with increasing concentrations of doxycycline or minocycline, cells were stimulated for 24 h with L-OspA or with live or sonicated *B. burgdorferi* in the presence of fresh antibiotics at the same concentrations described above. The production of TNF-α, IL-6, and IL-8 was significantly reduced by doxycycline and minocycline in a dose-dependent manner in both stimulated THP-1 cells (figure 1) and glial cells (figure 2). The most marked anti-inflammatory effect was the one that doxycycline had on microglia. At a doxycycline concentration of 0.005 mmol/L, the production of IL-6 by these cells after stimulation with L-OspA or with live or sonicated *B. burgdorferi* was diminished by 70.4%, 51.9%, and 59.2%, respectively, and that of IL-8 was diminished by 56.1%, 15.8%, and 39.9% (figure 2A and 2B). Concentrations of doxycycline or minocycline up to 0.005 mmol/L for glial cells and up to 0.01 mmol/L for THP-1 cells were found to be nontoxic. Thus, on the basis of viability experiments, >95% of glial cells and >92% of THP-1 cells were viable at 0.005 and 0.01 mmol/L doxycycline, respectively.

**Microarray analysis of gene expression in stimulated THP-1 cells treated with doxycycline.** To further evaluate the effects that doxycycline has on the control of inflammation, we
used microarray analysis to detect genes in THP-1 cells whose expression on stimulation with sonicated *B. burgdorferi* was affected by doxycycline. We observed that the expression of 326 genes (148 up-regulated and 178 down-regulated) was altered in THP-1 cells after treatment with 0.01 mmol/L doxycycline and stimulation for 2 h with sonicated *B. burgdorferi* (table 1). Using the IPA algorithm to examine pertinent regulatory pathways affected by doxycycline, we identified one such pathway in which the NFKB gene, which encodes a transcription factor that regulates the expression of a large number of genes involved in inflammation, was among those found to be down-regulated (figure 3A).

To expand our evaluation of the effect of doxycycline, we performed microarray analysis of doxycycline-treated THP-1 cells

![Figure 1. Effect of doxycycline and minocycline on the secretion of proinflammatory mediators by stimulated THP-1 cells. Concentrations of interleukin (IL)-6 (A and D), tumor necrosis factor (TNF)-α (B and E), and IL-8 (C and F) secreted by THP-1 cells were determined after incubation in medium alone or with doxycycline (left) or minocycline (right) at 0.005 or 0.01 mmol/L for 24 h, followed by a 24-h incubation with fresh doxycycline or minocycline at the above-indicated concentrations or medium alone and with lipidated outer surface protein A (L-OspA) at 0.25 μg/mL, live or sonicated *Borrelia burgdorferi* (at an MOI of 10 or its equivalent), or no stimulant. Shown are means ± SDs for triplicate specimens. Similar results were obtained using supernatants from cells in 3 additional independent experiments. *P < .05 and **P < .005, for the comparison between untreated and doxycycline- or minocycline-treated cells (differences were significant in a dose-dependent manner).
incubated with sonicated *B. burgdorferi* for 12 h. On increasing the stimulation time, the number of genes affected by doxycycline increased to 3567, with 1890 up-regulated and 1677 down-regulated (table 2). The IPA algorithm revealed a significantly perturbed pathway that involved the CHUK (alias, IKKA) gene, which encodes a catalytic subunit of the IKK kinase complex, a critical mediator of NF-κB activation (figure 3B). We also examined by means of the IPA algorithm whether doxycycline had an effect on MAPK pathways that are known to be affected by tetracyclines in microglia stimulated with lipopolysaccharide (LPS), namely, p38, c-Jun-N-terminal activated protein kinase (JNK) 1/2, and extracellular signal regulated kinase (ERK) 1/2 [28]. Of these, only the JNK pathway was significantly perturbed (data not shown).

**Effect of doxycycline on the DNA binding activity of NF-κB in stimulated THP-1 cells.** To investigate whether the effect that doxycycline has on NF-κB expression observed at the mRNA level was functionally manifest, we quantified functional NF-κB in nuclear extracts of THP-1 cells that had been incubated with sonicated *B. burgdorferi* for 2 or 4 h in the presence of increasing concentrations of doxycycline or minocycline. The NF-κB binding activity was significantly reduced by incubation

### Table 1. Genes whose expression was altered in THP-1 cells after treatment with doxycycline at 0.01 mmol/L and stimulation with sonicated *Borrelia burgdorferi* for 2 h.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Expression Change</th>
</tr>
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<tbody>
<tr>
<td>IL-6</td>
<td>Increased</td>
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<tr>
<td>IL-8</td>
<td>Decreased</td>
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The table is available in its entirety in the online edition of the *Journal of Infectious Diseases.*

### Table 2. Genes whose expression was altered in THP-1 cells after treatment with doxycycline at 0.01 mmol/L and stimulation with sonicated *Borrelia burgdorferi* for 12 h.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Expression Change</th>
</tr>
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<tbody>
<tr>
<td>NF-κB</td>
<td>Decreased</td>
</tr>
</tbody>
</table>

The table is available in its entirety in the online edition of the *Journal of Infectious Diseases.*
Figure 3. Microarray analysis showing the NF-κB signaling pathway affected by doxycycline in stimulated THP-1 cells. Cells were incubated in medium alone or pretreated with doxycycline at 0.01 mmol/L for 24 h, followed by incubation with fresh doxycycline at 0.01 mmol/L and sonicated *Borrelia burgdorferi* (quantity equivalent to an MOI of 10) for 2 or 12 h. Pathway analysis was performed using Ingenuity Pathways Analysis software (version 5.0). Two pathways with a high significance for genes regulated by doxycycline in THP-1 cells at 2 (A) and 12 (B) h were identified and merged. Color indicates the degree of up-regulation (red) or down-regulation (green) of the genes identified by microarray analysis.
and IKKβ kinase complex mediates the phosphorylation of IkB proteins, freeing NF-κB for translocation into the nucleus. We demonstrated that the phosphorylation of IkBα is reduced in doxycycline-pretreated THP-1 cells after a 30-min stimulation with sonicated *B. burgdorferi* (figure 5). The concentration of doxycycline was 0.01 mmol/L.

**DISCUSSION**

Both doxycycline and minocycline play an immune modulatory role in several models of neurodegenerative disease [15–25, 27]. Minocycline has been shown to exert an anti-inflammatory effect in CNS bacterial infection independently of its antimicrobial properties. Thus, a previous study demonstrated that minocycline modulates the host response to acute infection of mouse CNS parenchyma with a minocycline-resistant strain of *Staphylococcus aureus*, reducing abscess-associated mortality and improving brain survival [33].

In Lyme borreliosis, the pathological processes caused by the spirochete *B. burgdorferi* are mainly associated with consistent and strong inflammatory responses [3–7]. We argued that tetracyclines, beyond their antimicrobial properties, might attenuate the inflammatory effects elicited by *B. burgdorferi* spirochetes, their lipoproteins, or the bacterial debris left in the tissues after bacterial demise.

Previous studies have demonstrated that THP-1 cells respond to *B. burgdorferi* and their lipoproteins, eliciting such proinflammatory cytokines as TNF-α, IL-1β, and IL-6 [34, 35]. In light of these studies, we used the THP-1 monocytic cell line to examine the anti-inflammatory properties of tetracyclines. Both doxycycline and minocycline could significantly reduce the release of TNF-α, IL-6, and IL-8 in stimulated THP-1 cells. Microarray analysis verified that this down-regulation of inflammatory mediators was not part of a general shutdown of protein synthesis, because up to 1890 genes were significantly up-regulated on stimulation with sonicated *B. burgdorferi* for 12 h in the presence of doxycycline. Gene expression analysis also revealed the down-regulation of *NFkB* and *CHUK* after 2 and 12 h of stimulation, respectively. NF-κB is a transcription factor that regulates the expression of a large number of genes that are critical for the

**Figure 4.** DNA-binding activity of NF-κB in untreated and doxycycline- or minocycline-treated stimulated THP-1 cells. The activity of the NF-κB subunit p65 in THP-1 cells was measured using the TransAM ELISA Kit. Cells were incubated in medium alone or treated with doxycycline or minocycline at 0.005 or 0.01 mmol/L, followed by incubation with sonicated *Borrelia burgdorferi* (quantity equivalent to an MOI of 10) for 2 or 4 h. Shown are means ± SDs for triplicate specimens. *P < .05 and **P < .005, for the comparison between untreated and doxycycline- or minocycline-treated cells (differences were significant in a dose-dependent manner).

**Effect of doxycycline on the phosphorylation of IkBα in stimulated THP-1 cells.** Western blot analysis was performed to test the hypothesis that the phosphorylation of IkBα is affected by the down-regulation of the CHUK (IKKA) gene. IKKα

**Figure 5.** Western blot analysis showing the effects of doxycycline on IkBα phosphorylation. THP-1 cells were pretreated for 24 h with doxycycline at 0.01 mmol/L, washed, and incubated with fresh doxycycline, followed by stimulation with sonicated *Borrelia burgdorferi* for 15, 30, or 60 min. IkBα phosphorylation was determined by Western blot analysis using anti–phospho-IkBα antibody; β-tubulin was used as control for equal protein loading. Similar results were obtained in 3 independent experiments.
NF-κB activity is regulated by the IKKα and IKKβ kinase complex [38], which mediates the phosphorylation of IκB proteins, freeing NF-κB for translocation into the nucleus to function as a transcription factor. Previous studies have shown that *B. burgdorferi* and spirochetal lipoprotein can induce the production of NF-κB and cytokines in monocytes [39, 40]. Therefore, interfering with NF-κB activity could have critical effects on proinflammatory gene expression induced by *B. burgdorferi*. Recently, evidence has been presented [28] indicating that minocycline inhibits IκB degradation, preventing the translocation of NF-κB to the nucleus. Considering that most of the stimuli that activate NF-κB also activate IKK [41] and in view of our microarray analysis results, we hypothesized that doxycycline moderates the production of proinflammatory mediators through the IKK/NF-κB signaling pathway. We tested this hypothesis by determining the phosphorylation status of IκBα and quantifying functional NF-κB in cell or nuclear extracts of stimulated THP-1 cells after pretreatment with doxycycline or minocycline. IκBα, the best-characterized member of the IκB family, is a protein mainly regulated by phosphorylation [41]. Our results showed that doxycycline may interfere with phosphorylation processes and, consequently, with the degradation of the IκBα protein in THP-1 cells stimulated for 30 min with sonicated *B. burgdorferi*. We also demonstrated that NF-κB DNA-binding activity was reduced by both doxycycline and minocycline.

Doxycycline has been implicated in the inhibition of MAPK pathways in microglia in a stimulus-specific manner. Primary rat microglia stimulated with LPS or H2O2 showed enhanced phosphorylation of p38, ERK1/2, and JNK1/2. Minocycline (0.1 mmol/L) had an inhibitory effect on all 3 of these pathways when the stimulant was LPS but no effect at all when the stimulant was H2O2 [28]. When we examined the effect that doxycycline has on these pathways in THP-1 cells stimulated with sonicated *B. burgdorferi* by means of the IPA algorithm, only the JNK pathway was inhibited. Several explanations exist for this difference in outcome between the present study and that of Nikodemova et al. [28], including differences in response between human THP-1 monocytes and rat microglia; the use of a Toll-like receptor (TLR) 2-activating stimulus (sonicated *B. burgdorferi* [29]) as opposed to LPS, which binds to TLR4; and the use of a 10-fold-lower concentration of antibiotic (0.01 vs. 0.1 mmol/L). The JNK pathway was recently shown to mediate the production of TNF-α in murine macrophages stimulated with *B. burgdorferi* lysates [42]. Thus, inhibition of this pathway by doxycycline, as indicated by the IPA algorithm, may have further contributed to the observed down-regulation of inflammatory mediator production.

Microglia are the primary immune effector cells of the CNS and play an important role in the initiation of inflammatory responses [43, 44]. Inhibition of the activation of microglia and thereby of their release of proinflammatory and toxic molecules by means of tetracycline has been demonstrated in vitro [27, 45] and in other models of neurodegeneration [16–19, 22]. In the present study, we observed that doxycycline markedly attenuated the release of IL-6 and IL-8 elicited in primary microglia in response to L-OspA or to live or sonicated *B. burgdorferi* in a dose-dependent manner. Furthermore, we demonstrated that doxycycline also influences the production of IL-6 and IL-8 elicited by the same stimuli in primary astrocytes. Astrocytes are the major glial cell in the brain. They have been shown to contribute to CNS inflammatory activity by expressing inflammatory cytokines and chemokines [46]. Our results suggest that doxycycline, an antibiotic that crosses the blood-brain barrier and that is used in chemotherapy for Lyme disease [11], may affect 1 or more inflammatory pathways common to both cell types, thus inhibiting the ability of these cells—especially microglia—to produce proinflammatory mediators in response to *B. burgdorferi*. In fact, preliminary microarray analyses indicated that the expression of 460 genes (262 up-regulated and 198 down-regulated) was perturbed after 12 h of stimulation with sonicated *B. burgdorferi* on treatment with doxycycline. Among the significantly down-regulated genes were TNFA, IL1, IFNA, and IFNB (authors’ unpublished data). As previous studies have shown, minocycline is associated with the decrease of TNF-α and IL-1β expression in LPS-stimulated microglia [47, 48]. Here, our preliminary data suggest that doxycycline affects complex transcriptional processes involving the expression of proinflammatory cytokines, such as TNF-α.

Approximately 10% of patients with Lyme borreliosis who are treated for erythema migrans (the rash that heralds the infection in most patients) have subjective musculoskeletal, cognitive, or fatigue complaints at 12 months of follow-up; these symptoms correlate retrospectively with disseminated disease and a greater severity of illness at presentation [49]. The underlying mechanism for these subjective symptoms is unknown. These patients do not develop objective manifestations of late Lyme disease (e.g., Lyme arthritis) and lack evidence of persistence of infection by several microbiological testing methods. These facts argue against the notion that the cause of the subjective symptoms is residual *B. burgdorferi* infection [50]. In view of our present results, we submit that the beneficial effect of doxycycline in Lyme borreliosis therapy may not be confined to the antimicrobial properties of the antibiotic but may include an anti-inflammatory component. It follows that, if patients with posttreatment symptoms experience such a beneficial effect after retreatment with doxycycline, this effect may be due to the anti-inflammatory properties of the antibiotic.

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References


