Anti-Inflammatory Effects of a p38 Mitogen-Activated Protein Kinase Inhibitor During Human Endotoxemia


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Antimicrobial Effects of a p38 Mitogen-Activated Protein Kinase Inhibitor During Human Endotoxemia

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The p38 mitogen-activated protein kinase (MAPK) participates in intracellular signaling cascades resulting in inflammatory responses. Therefore, inhibition of the p38 MAPK pathway may form the basis of a new strategy for treatment of inflammatory diseases. However, p38 MAPK activation during systemic inflammation in humans has not yet been shown, and its functional significance in vivo remains unclear. Hence, we exposed 24 healthy male subjects to an i.v. dose of LPS (4 ng/kg), preceded 3 h earlier by orally administered 600 or 50 mg BIRB 796 BS (an in vitro p38 MAPK inhibitor) or placebo. Both doses of BIRB 796 BS significantly inhibited LPS-induced p38 MAPK activation in the leukocyte fraction of the volunteers. Cytokine production (TNF-α, IL-6, IL-10, and IL-1R antagonist) was strongly inhibited by both low and high dose p38 MAPK inhibitor. In addition, p38 MAPK inhibition diminished leukocyte responses, including neutrophilia, release of elastase/H9251, interleukins such as TNF-α, and IL-6, and the anti-inflammatory effects of the treatment. Therefore, inhibition of the p38 MAPK pathway may form the basis of a new therapeutic option in the treatment of inflammatory diseases. The Journal of Immunology, 2002, 168: 4070–4077.

Diseases such as rheumatoid arthritis and Crohn’s disease are characterized by chronic inflammation leading to destruction of normal tissue integrity. Mediators released during inflammatory diseases activate intracellular signaling cascades regulated by kinase and phosphatase enzymes (1). The mitogen-activated protein kinases (MAPKs)4 are part of such signaling cascades at which diverse extracellular stimuli converge to initiate inflammatory cellular responses. Several subgroups have been identified within the MAPK family, including the p42/44 extracellular signal-related kinases, c-Jun N-terminal kinase, and p38 MAPKs (2–5). The p38 MAPK has been implicated as an important regulator of the coordinated release of cytokines by immunocompetent cells and the functional response of neutrophils to inflammatory stimuli (6, 7). Many different stimuli can activate p38 MAPK. These include LPS and other bacterial products, cytokines such as TNF-α and IL-1, growth factors, and stresses such as heat shock, hypoxia, and ischemia/reperfusion (6, 7). In addition, p38 MAPK positively regulates a variety of genes involved in inflammation, such as TNF-α, IL-1, IL-6, IL-8, cyclooxygenase-2, and collagenase-1 and -3 (7).

Because of the broad proinflammatory role of p38 MAPK in several in vitro systems, inhibition of this pathway has been advocated as a novel therapeutic strategy for inflammatory diseases (8). However, the effect of p38 MAPK inhibition on in vivo models of inflammation has only been examined in a limited number of studies with equivocal results. The p38 MAPK inhibitors have been found to reduce LPS-induced TNF-α production in mice and rats (9–11). This result could not be duplicated with one of these inhibitors (SB203580) in mice despite almost completely abolishing the p38 MAPK activity in spleen cells harvested from these animals (12). Furthermore, inhibition of p38 MAPK was associated with a decrease in neutrophil recruitment and TNF-α release in bronchoalveolar lavage fluid in mice after intratracheal administration of LPS (13), but with elevated TNF-α concentrations in lungs during murine pneumococcal pneumonia and tuberculosis (12). In a murine model of peritonitis induced by cecal ligation and puncture, delayed administration of SB203580 improved survival and prevented the enhanced release of IL-10 by macrophages harvested from mice with peritonitis while concurrently improving the reduced IL-12 release by these cells (14).

Knowledge of the activation of p38 MAPK and its role in inflammation in humans in vivo is limited despite current interest in p38 MAPK inhibition in the treatment of human inflammatory disease. In addition, there are conflicting animal data. Therefore, in the present study we used the well-characterized model of human inflammation produced by i.v. injection of low dose LPS (15) to evaluate the activation of p38 MAPK and the effect of a new orally administered p38 MAPK inhibitor.

Materials and Methods

The p38 MAPK inhibitor

The p38 MAPK inhibitor used in this study (BIRB 796 BS) was developed by Boehringer Ingelheim Pharmaceuticals (Ridgefield, CT). BIRB 796 BS is 1-(5-tert-butyl-2-p-tolyl-2H-pyrazol-3-yl)-3-[(2-morpholin-4-yl-ethoxy)-...
naphthalen-1-yl]urea (empirical formula, C₉H₇N₃O₂; m.w., 527.6), a water-soluble, orally bioavailable molecule. Details about the structure and specificity of BIRB 796 BS for p38 MAPK will be published in a separate manuscript (16). BIRB 796 BS has a >230-fold selectivity for p38 MAPK compared with 12 other protein kinases studied. In contrast to other p38 MAPK inhibitors (e.g., SB203580), BIRB 796 BS prevents both the phosphorylation and kinetic activity of p38 MAPK by binding to a novel allosteric binding site as well as to the ATP pocket of p38 MAPK.

**Effect of BIRB 796 BS on p38 MAPK activation in vitro**

Blood from healthy volunteers was collected with 10 U/ml heparin (Leo Pharmaceutical Products, Weesp, The Netherlands). PBMCs were isolated by density gradient centrifugation on Ficoll-Paque (Pharmacia-LKB, Uppsala, Sweden) from blood diluted 1:1 with PBS. PBMCs were washed twice with PBS and resuspended in RPMI 1640 supplemented with 10% heat-inactivated FCS (both from Life Technologies, Grand Island, NY) at a concentration of ∼5 × 10⁶ cells/ml in 15-ml tubes (BD Biosciences, Franklin Lakes, NJ). After preincubation for 1 h with BIRB 796 BS (1, 10, 100, or 1000 nM) or DMSO as the solvent control, samples (containing ∼5 × 10⁶ cells each) were stimulated with LPS from *Escherichia coli* serotype 0111:B4 (25 ng/ml; Sigma-Aldrich, St. Louis, MO). After 15 min, 12 ml ice-cold PBS was added to each 15-ml tube, and cells were centrifuged at 400 × g for 5 min at 4°C. The cell pellets were lysed in 100 μl 3× SDS-sample buffer; this mixture was briefly sonicated twice (10 s each time) and boiled for 5 min, followed by brief centrifugation and storage at −20°C until further analysis. To ensure equal loading during Western blotting (see below), equal amounts of sample (25 μl; containing ∼1.25 × 10⁶ cells) were analyzed. Furthermore, after immunoblotting the blots were subjected to Amido Black staining to assess equal loading.

**Western blotting**

Samples mixed with SDS-sample buffer were loaded on 10% SDS-polyacrylamide gels and transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA). Subsequently, membranes were blocked in 5% BSA in PBS supplemented with 0.1% Tween 20 and washed in 0.2% BSA in PBS supplemented with 0.1% Tween 20. The extent of p38 MAPK phosphorylation was determined using Abs against phosphorylated (Thr180/Tyr182) p38 MAPK (40). A once-diluted slurry of agarose hydrazide-bound Abs to phospho-p38 MAPK (40) was separated chromatographically, followed by detection via the validated HPLC method with electrospray ionization mass spectrometry/mass spectrometry detection. Following the solid phase extraction of the sample obtained at −3 h was not adequately treated and was omitted from the analysis. All other samples were compared with one random sample set at an arbitrary value of 100 U for comparing p38 MAPK phosphorylation between subjects. In addition, a minimum measured activity was considered necessary for use as an internal reference (i.e., ≥2 U on a 2-D digital gray scale). Two subjects failed to meet this requirement (one from the low dose and one from the high dose BIRB 796 BS-treated volunteers), possibly due to low protein yield in these samples, and were not included in the analysis.

The p38 MAPK enzymatic activity was measured using a kinase assay (New England Biolabs). White blood cell lysates were prepared as described above. A once-diluted slurry of agarse hydradize-bound Abs to phosphorylated (Thr180/Tyr182) p38 MAPK (40 μl) was used to selectively immunoprecipitate active p38 MAPK from the cell lysates by gently shaking overnight at 4°C. To assure equal loading, a fixed amount of lysate was used per sample (∼80 μg in 340 μl cell lysis buffer). The immunoprecipitate was washed twice with 500 μl ice-cold cell lysis buffer and twice with 500 μl ice-cold kinase buffer (25 mM Tris (pH 7.5), 5 mM β-glycerophosphate, 2 mM DTT, 0.1 mM Na₃VO₄, and 10 mM MgCl₂) at 4°C. The kinase reactions were conducted in the presence of 200 μM ATP and 2 μg activating transcription factor-2 (ATF-2) fusion protein at 30°C for 30 min. After the reaction had been terminated by the addition of 3× SDS-sample buffer, the mixture was boiled for 5 min, followed by brief centrifugation. The kinase reaction product was analyzed by SDS-PAGE and Phosphoimager. The autoradiograph was quantitated using Phosphorimager software (Molecular Dynamics, Sunnyvale, CA).

**LPS administration to humans in vivo**

The study was performed as a randomized, double-blind, placebo-controlled experiment. The study was approved by the institutional scientific and ethics committees, and written informed consent was obtained from each subject before the start of the study. Twenty-four healthy male volunteers (mean age, 22 years; range, 19–29 years) participated in the investigation. All subjects were in good health as documented by history, physical examination, electrocardiogram, and routine laboratory screening. Tests for HIV and hepatitis B and C were negative. The participants did not use any medication. All participants were nonsmokers. The subjects fasted overnight before LPS administration. On the study day, two i.v. cannulas were inserted, one for LPS administration and one for blood collection. Blood from healthy volunteers was collected with 10 U/ml heparin (Leo Pharmaceutical Products, Weesp, The Netherlands). After preincubation for 1 h with BIRB 796 BS (1, 10, 100, or 1000 nM) or DMSO as the solvent control, samples (containing 1.25 × 10⁶ cells) were analyzed. Furthermore, after immunoblotting the blots were subjected to Amido Black staining to assess equal loading.

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**Other assays**

Cytokine concentrations were determined in EDTA-anti-coagulated plasma by specific ELISAs according to the manufacturers’ instructions (with detection limits). These ELISAs were as follows: TNF-α (2.8 pg/ml), IL-6 (1.2 pg/ml), and IL-10 (2.4 pg/ml) (all from Central Laboratory of The Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands), and IL-1R antagonist (IL-1ra; 410 pg/ml; R&D Systems, Minneapolis, MN). Elastase-α1-antitrypsin complexes in EDTA plasma were measured with an ELISA modified from a previously described RIA procedure (12). Briefly, ELISA plates (Maxisorb; Nunc, Roskilde, Denmark) were coated with polyclonal rabbit Abs against human elastase and incubated with the samples to be tested. Bound complexes were detected by incubation with biotinylated mAbs against complexed α1-antitrypsin and...
FACS analysis

Leukocyte counts and differentials were assessed in EDTA-anti-coagulated blood using a Stekker analyzer (counter STKS; Coulter, Bedfordshire, U.K.). Expression of CD11b (Mac-1) and L-selectin (CD62L) on circulating granulocytes was determined in heparinized blood obtained at −3, 0, 2, 4, 6, and 24 h relative to LPS injection. All blood samples were placed on ice immediately after blood drawing. After lysis of erythrocytes in isotonic NH4Cl solution (155 mM NH4Cl, 10 mM KHCO3, and 0.1 mM EDTA, pH 7.4) for 10 min, samples were centrifuged at 400 × g for 5 min. The remaining cells were washed and subsequently kept in PBS containing 0.5% BSA, 1.5 mM sodium azide, and 0.35 mM EDTA at a final concentration of 5 × 106 cells/ml. All procedures were performed at 4°C. The following Abs were used: FITC-labeled mouse anti-human L-selectin (Immunotech, Marseilles, France) and PE-labeled mouse anti-human CD11b (Immunotech). All FACS reagents were used in concentrations recommended by the manufacturer. To correct for nonspecific staining, all analyses were also conducted with the appropriate control Abs (FITC- and PE-labeled murine IgG1 (CLB, Amsterdam, The Netherlands)). At least 10,000 granulocytes were counted in each assay. Mean cell fluorescence of forward and side angle scatter-gated granulocytes was assessed using a FACScan flow cytometer (BD Biosciences, Mountain View, CA). Data are presented as the difference between mean cell fluorescence intensities of specifically and nonspecifically stained cells.

Statistical analysis

All laboratory-based values are given as the mean ± SEM. Differences in results among the three treatment groups were tested by repeated measurements analysis of variance. A value of p < 0.05 was considered to represent a statistically significant difference.

Results

**BIRB 796 BS is a potent p38 MAPK inhibitor in vitro**

To assess the effect of BIRB 796 BS on p38 MAPK activity, we stimulated PBMCs with LPS in the presence of increasing concentrations of BIRB 796 BS or diluent (Fig. 1). BIRB 796 BS inhibited LPS-induced phosphorylation of p38 MAPK in a dose-related fashion.

**BIRB 796 BS inhibits LPS-induced p38 MAPK activation in vivo**

Plasma levels of BIRB 796 BS determined after oral ingestion of the p38 MAPK inhibitor peaked at 0.5–2 h after LPS injection (low dose group, 0.74 ± 0.25 μM; high dose group, 7.38 ± 1.64 μM; i.e., within the same range as the concentrations used in the in vitro experiment). Although there is abundant evidence that active p38 MAPK is involved in LPS-induced cytokine production in vitro (13, 18, 19), it is not known whether p38 MAPK has a similar role in humans in vivo. To investigate the activation of p38 MAPK in human endotoxemia and the effectiveness of BIRB 796 BS in inhibition of p38 MAPK phosphorylation, we measured p38 MAPK activation using phosphospecific Abs at various time points before and after LPS injection in healthy human subjects. As shown in Fig. 2A, administration of LPS resulted in p38 MAPK activation in subjects who did not receive BIRB 796 BS, peaking at 60 min. Both low dose and high dose BIRB 796 BS significantly inhibited p38 MAPK activation (both p < 0.05 vs placebo). To further establish the effectiveness of BIRB 796 BS in inhibiting p38 MAPK enzymatic activity in vivo, a kinase assay was performed on white blood cell lysates (one example of three is shown in Fig. 2B). In subjects who did not receive BIRB 796 BS, enhanced p38 MAPK enzymatic activity was observed 60 min after LPS administration relative to 0 h. In contrast, treatment with BIRB 796 BS almost completely prevented p38 MAPK activation at 60 min. These results are, to our knowledge, the first demonstration of p38 MAPK inhibition in humans in vivo.
of TNF-α, IL-6, IL-10, and IL-1ra (Fig. 4). All these cytokine responses were strongly inhibited by both doses of BIRB 796 BS (all $p < 0.05$ vs placebo, except for IL-1ra at the low dose). In addition, the inhibitory effect of BIRB 796 BS appeared dose dependent, although the low dose diminished cytokine release to a statistically significant extent. Thus, p38 MAPK activation is required for cytokine release during human inflammation.

Inhibition of p38 MAPK reduces LPS-induced neutrophil activation

The p38 MAPK phosphorylation results in activation of several proinflammatory neutrophil functions in vitro (6, 7). Therefore, the effect of BIRB 796 BS on neutrophil activation induced by LPS in vivo was assessed by measuring neutrophil counts, release of elastase, and expression of CD11b and L-selectin (Fig. 5). LPS injection in subjects treated with placebo was associated with a biphasic change in neutrophil numbers in peripheral blood, characterized by initial neutropenia with a nadir at 1 h, followed by neutrophilia peaking at 8 h. LPS administration also induced a transient rise in the plasma concentrations of elastase-α1-antitrypsin complexes, reflecting neutrophil degranulation (17) and an up-regulation of CD11b at the surface of circulating granulocytes with a concurrent down-modulation of L-selectin, indicative of cellular activation (20). Whereas the lower BIRB 796 BS dose tended to attenuate these LPS-induced neutrophil responses ($p < 0.05$ for L-selectin; $p \geq 0.05$ for other parameters vs placebo), the higher dose of the p38 MAPK inhibitor resulted in significant reduction of all parameters of neutrophil activation (all $p < 0.05$ vs placebo), suggesting an essential role for p38 MAPK in mediating leukocyte responses in vivo.

BIRB 796 BS inhibits CRP release

To obtain insight into the role of p38 MAPK on more subacute consequences of LPS-induced inflammation, CRP concentrations were measured 3 h before and 24 h after LPS injection (Fig. 6). LPS induced a profound increase in the serum levels of this acute phase reactant, which was significantly lower in the group receiving BIRB 796 BS ($p < 0.05$ for both doses vs placebo).

Discussion

The present investigation examines the activation of p38 MAPK during an in vivo inflammatory response in humans along with the effect of a p38 MAPK inhibitor on this response. Intravenous injection of LPS elicited transient activation of p38 MAPK, followed

Table I. BIRB 796 BS reduces LPS-induced clinical symptoms

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Placebo ($n = 8$)</th>
<th>50 mg ($n = 8$)</th>
<th>600 mg ($n = 8$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total no.</td>
<td>S/mo/mi</td>
<td>Peaking time (h)</td>
</tr>
<tr>
<td>Headache</td>
<td>8</td>
<td>1/3/4</td>
<td>1.5</td>
</tr>
<tr>
<td>Chills</td>
<td>7</td>
<td>0/2/5</td>
<td>1.5</td>
</tr>
<tr>
<td>Myalgia</td>
<td>4</td>
<td>0/1/3</td>
<td>4</td>
</tr>
<tr>
<td>Nausea</td>
<td>5</td>
<td>0/2/3</td>
<td>2</td>
</tr>
<tr>
<td>Vomiting</td>
<td>3</td>
<td>1/1/1</td>
<td>3</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>2</td>
<td>0/2/0</td>
<td>3</td>
</tr>
<tr>
<td>Backache</td>
<td>1</td>
<td>0/0/1</td>
<td>4</td>
</tr>
</tbody>
</table>

*Subjects received an i.v. injection of LPS (4 ng/kg) at t = 0 h preceded by oral ingestion of placebo, 50 mg BIRB 796 BS, or 600 mg BIRB 796 BS.

**Total number of subjects suffering from the indicated symptom.

A further distinction was drawn between severe (s), moderate (m), and mild (mi) symptoms.

Peaking time refers to the maximum number of subjects suffering from the indicated symptom.

N/A, Not applicable.
by a characteristic inflammatory response, including the release of proinflammatory cytokines, neutrophilic leukocytosis, neutrophil activation, and acute phase protein release. Oral ingestion of BIRB 796 BS, a specific p38 MAPK inhibitor, attenuated these responses. These results not only identify p38 MAPK as a principal regulator of the inflammatory response in humans but also suggest that p38 MAPK inhibition may have significant potential for treating inflammatory disease.

**FIGURE 4.** BIRB 796 BS inhibits LPS-induced cytokine release. Subjects received an i.v. injection of LPS (4 ng/kg) at 0 h, preceded by oral ingestion of placebo (□), 50 mg BIRB 796 BS (○), or 600 mg BIRB 796 BS (▲) at −3 h. Data are the mean ± SEM. Both low and high dose BIRB 796 BS inhibited the release of all mediators shown (all p < 0.05 vs placebo), except for IL-1ra release in the low dose group (nonsignificant vs placebo).

**FIGURE 5.** BIRB 796 BS inhibits LPS-induced neutrophil responses. Subjects received an i.v. injection of LPS (4 ng/kg) at 0 h, preceded by oral ingestion of placebo (□), 50 mg BIRB 796 BS (○), or 600 mg BIRB 796 BS (▲) at −3 h. Data are the mean ± SEM. High dose BIRB 796 BS inhibited all neutrophil responses shown (all p < 0.05 vs placebo). Low dose BIRB 796 BS inhibited elastase release (p < 0.05 vs placebo). The other neutrophil responses shown were not inhibited by low dose BIRB 796 BS (nonsignificant vs placebo).
Intravenous administration of LPS induced activation of p38 MAPK in blood, peaking after 1 h, as demonstrated by two different methods, Western blotting with Abs against phosphorylated (Thr\(^{180}\)/Tyr\(^{182}\)) p38 MAPK and a kinase assay. Using similar methods, it has been previously demonstrated that there is transient activation of p38 MAPK in splenocytes of mice peaking within 15 min of i.p. injection of LPS (12). The later activation of p38 MAPK in the human model compared with the mouse may be related to differences in the route of LPS administration, the LPS dose administered, and the cell types analyzed.

The p38 MAPK activation was measured in lysates of all white blood cells obtained from whole blood, rather than in isolated cell fractions, because we argued that the isolation procedures might affect the activation status of p38 MAPK. Several papers have described the in vitro effects of LPS on different leukocyte cell fractions without showing apparent effects of the different separation methods on basal p38 MAPK activation. However, in contrast to these in vitro studies, in vivo endotoxemia studies do not allow cell separation before exposure to LPS. Furthermore, in our experience more elaborate sample handling can lead to activation of kinases (data not shown). Therefore, to accurately assess the activation state of p38 MAPK at several time points during human endotoxemia, we felt it warranted to process the in vivo stimulated cytokine network. The strong reduction in LPS-induced TNF-\(\alpha\) release with the p38 MAPK inhibitor BIRB 796 BS corresponds to the in vitro effect of p38 MAPK inhibition in stimulated monocyctic cells and neutrophils (4, 10, 13, 19, 21, 22). In addition, p38 MAPK inhibitors diminished LPS-induced TNF-\(\alpha\) production in mice and rats in vivo (9–11), and mice deficient in MAPK-activated protein kinase-2, a downstream substrate kinase for p38 MAPK, proved to be resistant to LPS-induced shock (23). A previous study in mice was not able to demonstrate an inhibitory effect of the p38 MAPK inhibitor SB203580 on TNF-\(\alpha\) release after i.p. administration of LPS (12). This may be the result not only of species differences, but also of differences in the mechanism of inhibition of the two drugs. In vitro data suggest that besides p38 MAPK other stress signaling pathways, e.g., MAPK family members p42/44 and c-Jun N-terminal kinase and the NF-\(\kappa\)B pathway, are involved in LPS-induced TNF-\(\alpha\) release (24, 25). Indeed, all three major MAPK family members and NF-\(\kappa\)B can be activated upon stimulation with LPS (26–28). Furthermore, the TNF-\(\alpha\) promoter has binding sites for NF-\(\kappa\)B as well as for transcription factors under control of the MAPK family (e.g., AP-1) (26, 29). Thus, full expression of the TNF gene seems to involve activation of several of the above-mentioned stress pathways. However, the relative importance of each of these pathways may vary under different conditions. Interestingly, deletion of one of the NF-\(\kappa\)B binding sites from the TNF promoter had little effect on LPS-induced TNF-\(\alpha\) production (29). We observed that inhibition of p38 MAPK decreased endotoxemia-induced plasma TNF-\(\alpha\) levels up to 97%. These data suggest that in the human endotoxemia model the p38 MAPK pathway has little redundancy with respect to TNF-\(\alpha\) release. The inhibition of cytokine release in subjects treated with BIRB 796 BS could have been related to the reduction in TNF-\(\alpha\) secondary to p38 MAPK inhibition. Elimination of endogenous TNF-\(\alpha\) activity with an anti-TNF Ab or a TNF receptor fusion protein in the human LPS model was accompanied by a marked reduction in the release of other cytokines and cytokine inhibitors, including IL-6, IL-10, and IL-1ra (30–33). In addition, anti-TNF administration resulted in reduced IL-10 release by LPS-stimulated monocytes, whereas p38 MAPK inhibition attenuated both TNF-\(\alpha\) and IL-10 production in this in vitro system (22). Thus, together with our in vivo findings these data indicate that p38 MAPK is involved in the production not only of proinflammatory cytokines, but also of anti-inflammatory cytokines. Yet the subjects treated with BIRB 796 BS demonstrated evidence of an overall anti-inflammatory effect, suggesting that p38 MAPK inhibition predominantly influences proinflammatory pathways.

The p38 MAPK is considered important for many different proinflammatory neutrophil functions (13, 21, 34–39). Although the anti-inflammatory effects of p38 MAPK inhibitors on neutrophils are well known in vitro, little is known about the in vivo relevance of these findings. Inhibition of p38 MAPK has been reported to reduce neutrophil influx into bronchoalveolar lavage fluid after intratracheal administration of LPS in mice (13). Furthermore, local application of a p38 MAPK inhibitor in the lumen of an ileal loop before administration of Clostridium difficile toxin A at the same location has been associated with a strong reduction in both neutrophil recruitment and the severity of the resulting enteritis in mice (40). This study demonstrates that p38 MAPK inhibition attenuates the neutrophil response to i.v. LPS in humans in vivo and reduces the activation of neutrophils, as indicated by inhibition of degranulation, up-regulation of CD11b, and down-modulation of L-selectin. These data correspond with previous in vitro reports demonstrating that p38 MAPK inhibition reduces neutrophil degranulation (36, 37), the shedding of L-selectin (35),
and the up-regulation of CD11b (37, 38). BIRB 796 BS also reduced the incidence and severity of clinical symptoms and delayed the time point of maximal presentation, an effect that probably would have been more clear-cut if more subjects had been studied.

Intravenous injection of LPS induces a reproducible transient inflammatory state in normal subjects that is considered relevant for the investigation of pathophysiologic pathways operative in inflammatory conditions. As such, this model of inflammation in man offers an opportunity to obtain proof of the principle for the action of anti-inflammatory compounds. It should be noted that the human endotoxemia model is less suitable to investigate the efficacy of postponed treatment with an anti-inflammatory compound, because the inflammatory response to i.v. LPS is very rapid and transient. Nonetheless, the current findings establish that inhibition of p38 MAPK by the oral administration of BIRB 796 BS exerts anti-inflammatory effects during experimental endotoxemia. These effects are comparable to those seen in this model with anti-TNF Abs, TNFR fusion protein, and IL-10 (30, 33, 41, 42) drugs presently used clinically or in trials in the management of chronic inflammatory diseases such as rheumatoid arthritis and Crohn's disease (43–46). Clearly, the greatest advantages of compounds such as BIRB 796 BS would be their oral availability and lack of immunogenicity compared with the biological products. Taken together with (limited) animal data, these results provide hope for the future use of oral p38 MAPK inhibitors in patients with inflammatory diseases.

References


