p38 Mitogen-Activated Protein Kinase Inhibition Increases Cytokine Release by Macrophages In Vitro and During Infection In Vivo

Bernt van den Blink, Nicole P. Juffermans, Tessa ten Hove, Mark J. Schultz, Sander J. H. van Deventer, Tom van der Poll and Maikel P. Peppelenbosch

*J Immunol* 2001; 166:582-587; doi: 10.4049/jimmunol.166.1.582
http://www.jimmunol.org/content/166/1/582

References This article cites 24 articles, 16 of which you can access for free at: http://www.jimmunol.org/content/166/1/582.full#ref-list-1

Why *The JI*? Submit online.

- **Rapid Reviews! 30 days** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Fast Publication!** 4 weeks from acceptance to publication

*average

Subscription Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
p38 Mitogen-Activated Protein Kinase Inhibition Increases Cytokine Release by Macrophages In Vitro and During Infection In Vivo

Bernt van den Blink,1* Nicole P. Juffermans,*† Tessa ten Hove,* Mark J. Schultz,*† Sander J. H. van Deventer,* Tom van der Poll,*† and Maikel P. Peppelenbosch*

p38 mitogen-activated protein kinase (MAPK) has been suggested as a mediator of cytokine release and is currently being targeted for anti-inflammatory therapy. However, experimental data are contradictory and lack sufficient affirmation in vivo. We tested the effect of p38 MAPK inhibition in several cell types and in different murine models of infectious disease. We observed that most cell types react to p38 MAPK inhibition with diminished cytokine release, but that this treatment induced increased cytokine release in macrophages. Furthermore, we observed increased cytokine production in mouse models of pneumococcal pneumonia and tuberculosis accompanied by severely reduced bacterial clearance. This apparent inefficacy of p38 MAPK inhibition in reducing cytokine release in infectious disease, as well as its immune-compromising action, suggest that targeting p38 MAPK may not be a suitable anti-cytokine strategy in patients with such disease or at risk for infection. The Journal of Immunology, 2001, 166: 582–587.

P38 mitogen-activated protein kinases (MAPK)2 are 38-kDa intracellular signal transduction proteins comprising four variants: p38α, β, γ, and δ. Together with c-Jun amino-terminal kinase and p42/44 MAPK, p38 MAPK forms the MAPK family. MAPK are activated by phosphorylation of the Thr and Tyr in a Thr-X-Tyr motif by dual specificity MAPK kinases (MKK). A striking feature of p38 MAPK is its activation by a variety of inflammatory stimuli including cytokines and LPS (1–3), suggesting a role in inflammation. Insight into p38 MAPK action in inflammation has been gained by use of SB203580, a piri-dinyl imidazole derivative and a potent and specific inhibitor of p38 MAPK (4, 5). SB203580 binds to the ATP binding site, thus preventing phosphorylation of downstream targets including MAPK-activated protein kinase-2 and activating transcription factor-2 (ATF-2), though not preventing phosphorylation of p38 MAPK by its upstream activators, MKK3 and MKK6 (6). Although the exact function of p38 MAPK in inflammation remains ambiguous, studies using SB203580 have suggested involvement of this kinase in phospholipase A2 activation (7), ischemia induced apoptosis (8), NO synthase induction (9) and attenuating the role of p38 MAPK in several cell types important for cytokine production and in murine models of disease. To this end we investigated the effect of p38 MAPK inhibition in L929 fibrosarcoma cells and 4–4 murine macrophages as well as in freshly obtained, nontransformed, peritoneal macrophages and whole blood. In addition, we tested the effect of p38 MAPK inhibition on cytokine production in three well-established murine models of disease: an endotoxemia model and a pneumococcal pneumonia model that are representative for acute inflammatory disease, and a chronic inflammatory tuberculosis model. Using the endotoxemia model we were able to establish p38 MAPK activation in vivo and the efficacy of our strategy for inhibiting p38 MAPK (daily i.p. injection with SB203580). Our results show a dual role for p38 MAPK. Whereas inhibition of p38 MAPK results in decreased levels of cytokines in L929 cells and whole blood, 4–4 macrophages and freshly obtained peritoneal macrophages show enhanced cytokine production when p38 MAPK enzymatic activity is impaired. This dichotomy in the effect of p38 MAPK inhibition on cytokine production is also reflected in in vivo experiments: in both the pneumococcal pneumonia and tuberculosis model we observed increased TNF-α levels when mice were subjected to p38 MAPK inhibition, but this was not observed in endotoxin-challenged

Departments of *Experimental Internal Medicine, and † Infectious Diseases, Tropical Medicine, and AIDS, Academic Medical Center, Amsterdam, The Netherlands

Received for publication May 15, 2000. Accepted for publication October 9, 2000.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Address correspondence and reprint requests to Dr. Bernt van den Blink, Academic Medical Center, Laboratory for Experimental Internal Medicine, G2-130, Meibergdreef 9, Amsterdam, NL-1105 AZ, The Netherlands. E-mail address: B.vandenBlink@amc.uva.nl

2 Abbreviations used in this paper: MAPK, mitogen-activated protein kinase; MKK, MAPK kinases; ATF-2, activating transcription factor-2; HKSP, heat-killed Streptococcus pneumoniae; rTNF, human rTNF-α; BAL, bronchoalveolar lavage; b.w., body weight.

Copyright © 2001 by The American Association of Immunologists

0022-1767/01/$02.00
mice. Furthermore, in these experiments, we observed that in vivo p38 MAPK inhibition correlated with enhanced bacterial outgrowth. Hence, these data indicate a critical role for p38 MAPK in bacterial clearance, but also show that the effect of p38 MAPK inhibition on cytokine production maybe cell type specific.

Materials and Methods

Cells and reagents

Murine macrophages (4-4 clone, which were previously shown to be a suitable model for studying macrophage function in vivo, as these cells are phenotypically and functionally different from primary isolated mature macrophages (16, 17)), whole blood obtained from healthy volunteers and freshly obtained murine peritoneal macrophages were cultured in RPMI 1640 supplemented with 10% FCS, 2 mM l-glutamine, and antibiotic-antimyotic. To isolate fresh murine macrophages, the peritoneal cavity was lavaged with RPMI 1640. Cells were placed in culture flasks and, 2 h after isolation, nonadherent cells were removed. L929 fibrosarcoma cells were cultured in DMEM supplemented with 10% FCS, 2 mM l-glutamine, and antibiotic-antimyotic. Cultures were routinely checked for Mycoplasma infection. DMEM, FCS, and antibiotic-antimyotic were obtained from Life Technologies (Grand Island, NY) and RPMI 1640 was obtained from Bio-Whittaker Europe (Verviers, Belgium). SB203580 (4-(4-fluorophenyl)-2- (4-pyridyl)-imidazole) was obtained from Alexis Biochemicals (Leiden, The Netherlands). SB203580 stock solution, dissolved in DMSO at 20 mM, was stored at −20°C. In cell culture, SB203580 was used at 1–10 μM; the DMSO concentration was never higher than 0.05%. Cells were incubated with SB203580 for 1.5 h before stimulation. Stimulation was performed with either LPS (Escherichia coli 011:B4; Sigma, St. Louis, MO; various concentrations), heat-killed Streptococcus pneumoniae (HKSP; 2 × 108 bacteria/ml), human rTNF-α; Knoll, Ludwigshaven, Germany; 50 ng/ml), or lipoteichoic acid (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands). All animal experiments were approved by the Institutional Animal Care and Use Committee of the Academic Medical Center. Experimental groups consisted of eight mice. For all in vivo experiments, mice were subjected to 1 μM SB203580 per kilogram body weight (b.w.) by i.p. injection in 1 ml sterile saline or 1 ml solvent control (DMSO) in 1 ml sterile saline. Controls. One hour before inducing disease SB203580 was administered, then given once daily, for up to 2 wk if appropriate. No apparent toxicity was observed.

Pneumococcal pneumonia was induced as described (18). Briefly S. pneumoniae serotype 3 was obtained from American Type Culture Collection (ATCC 6303; Manassas, VA). Pneumococci were grown for 15 h. One hour after intranasal inoculation was conducted by placing 105 viable bacteria of medium. Mice were lightly anesthetized by placing two 0.5 ml aliquots of sterile isotonic saline. Lavage fluid (0.8 ml) was retrieved per mouse and spun at 750 × g for 5 min at 4°C, and supernatants were frozen at −20°C until measurements were performed. Pneumococcal counts. The inoculum was diluted 1:1 with lysis buffer (150 mM NaCl, 15 mM Tris, 1 mM MgCl2, 1 mM CaCl2, 1% Triton X-100, and protease inhibitors) for 30 min at 4°C. Homogenates were then spun at 1500 × g at 4°C for 15 min, to remove cell debris, after which the supernatants were stored at −20°C until cytokine measurements. For BAL, the trachea was exposed trough a midline incision and cannulated with a sterile 22-gauge Abbocath-T catheter (Abbott, Sligo, Ireland). BAL was performed by instilling two 0.5 ml aliquots of sterile isotonic saline. Lavage fluid (0.8 ml) was retrieved per mouse and spun at 750 × g for 5 min at 4°C, and supernatants were frozen at −20°C until measurements were performed. Pneumococcal numbers were counted on sheep-blood agar plates. Mice were sacrificed 0, 15, 30, 60, or 90 min after LPS injection. Blood was removed through cardiac puncture and spun at 7000 × g; supernatants were collected and stored at −20°C for later cytokine sampling with ELISA. All cultures were routinely checked for antibiotic-antimyotic. Cultures were frozen in liquid N2, then stored at −80°C until further processing.

Western blot analysis

Spleens were thawed at 4°C and passed through a 40-μm strainer, which was then rinsed with ice-cold PBS. Cells were spun down and resuspended in ice-cold cell lysis buffer (20 mM Tris (pH 7.5), 150 Mm NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerolphosphate, 1 mM NaVO3, 1 μM leupeptin and 1 mM Pefabloc). Samples were sonicated four times for 5 s on ice and spun at 7000 × g for 10 min at 4°C. Protein concentration in the clear supernatant was determined according to Bradford, the supernatant was then stored at −80°C. Approximately 3 μg splenic cell lysate was mixed with 4 × SDS-sample buffer, and the mixture was boiled for 5 min followed by brief centrifugation. Samples were loaded on 10% SDS-polyacrylamide gels and transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA). To check for equal loading of protein in each lane, Coomassie brilliant blue staining of the membrane was performed. Subsequently, membranes were blocked in 2% nonfat dry milk in PBS supplemented with 0.1% Tween 20 and washed in 0.2% nonfat dry milk in PBS supplemented with 0.1% Tween20. The extent of p38 MAPK activation was determined using Abs against phosphorylated (Thr180/Tyr 182) p38 MAPK, purchased from Cell Signaling Technology (Danvers, MA). To check for equal loading of protein in each lane, Coomassie brilliant blue staining of the membrane was performed. Subsequently, membranes were blocked in 2% nonfat dry milk in PBS supplemented with 0.1% Tween 20 and washed in 0.2% nonfat dry milk in PBS supplemented with 0.1% Tween20. The extent of p38 MAPK activation was determined usingAbs against phosphorylated (Thr180/Tyr 182) p38 MAPK, used in a 1:1000 dilution overnight. After three washes with 10 min secondary Abs, membranes were washed with HRP-conjugated goat anti-rabbit IgG in a 1:2000 dilution. After enhanced chemoluminescence using Lumilight substrate, Ab binding was visualized using a Lumi-imager. The membranes were then stripped with strip buffer (62.5 mM Tris-HCl (pH 6.8), 2% SDS, and 100 mM 2-ME) and reprobed with Ab for p38 MAPK (New England Biolabs) by a similar procedure.

In vitro p38 MAPK assay

This assay was performed using the p38 MAPK assay kit purchased from New England Biolabs. Splenic cell lysates were prepared as described previously for immunoblottting. A once-diluted slurry of agarose hydrazide-bound Abs to phosphorylated (Thr180/Tyr 182) p38 MAPK (40 μl) was used to selectively immunoprecipitate active p38 MAPK from −100 μg splenic cell lysate (in 200 μl cell lysis buffer) by gently shaking overnight at 4°C. The immunoprecipitate was washed twice with 500 μl of ice cold lysis buffer and twice with 500 μl of ice cold kinase buffer (25 mM Tris (pH 7.5), 5 mM β-glycerolphosphate, 2 mM DTT, 0.1 mM Na3VO3, 10 mM MgCl2) at 4°C. The kinase reactions were conducted in the presence of 200 μM ATP and 2 μM ATP-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. ATP-2 phosphorylation was selectively measured by Western immunoblotting as described previously using specific Abs against phosphorylated (Thr180) ATF-2.

Cytokine determination

Cytokines were measured by using commercially available ELISAs as follow. According to the manufacturers recommendations: murine TNF-α (Genzyme, Cambridge, MA), IL-6 (PharMingen, San Diego, CA), and human TNF-α (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands).
Statistical analysis

Data were statistically analyzed by the Student’s t test unless otherwise mentioned. All data are given as the mean ± SEM.

Results

The role of p38 MAPK in the regulation of cytokine production in vitro

A large variety of inflammatory stimuli activate p38 MAPK and a role for p38 MAPK in the regulation of cytokine production has been suggested. The nature of this role is unclear. Most authors report reduced cytokine production upon p38 MAPK inhibition (e.g., Lee et al., Ref. 1), but enhanced cytokine release has also been reported (e.g., Zhang et al., Ref. 14). Therefore, we investigated the influence of the p38 MAPK inhibitor SB203580 on TNF-α-induced IL-6 production in L929 fibrosarcoma cells and on LPS- or HKSP-stimulated TNF-α and IL-6 production in 4-4 macrophages. In agreement with Beyaert et al. (12), cells pretreated with SB203580 showed reduced IL-6 production upon stimulation with TNF-α (p < 0.01; Fig. 1A). In contrast, SB203580-pretreated 4-4 macrophages stimulated with HKSP showed enhanced IL-6 production (p < 0.05; Fig. 1B). 4-4 macrophages pretreated with SB203580 showed enhanced TNF-α production upon stimulation with LPS as compared with vehicle-treated cells (p < 0.05 when pooled experiments are tested with the Wilcoxon test; a representative example is shown in Fig. 1C). and also HKSP induced enhanced TNF-α production upon p38 MAPK inhibition in these cells (p < 0.01; Fig. 1D). Subsequently we decided to investigate the effects of p38 MAPK inhibition on cytokine release in non-transformed cells. LPS-stimulated whole blood showed inhibition of TNF-α production by SB203580 (10 μM SB203580, p < 0.01; 1 μM SB203580, p < 0.01; Fig. 2A) but peritoneal macrophages exhibited increased TNF-α levels upon p38 MAPK inhibition (10 μM SB203580, p < 0.01; 1 μM SB203580, p < 0.05; Fig. 2B). To further exclude the possibility that the differences in effect of p38

FIGURE 1. Effects of p38 MAPK inhibition on cytokine production in L929 cells and 4-4 macrophages. A, Effect of pretreatment with 10 μM SB203580 of L929 fibrosarcoma cells on TNF-α-induced IL-6 production, compared with vehicle (DMSO) control. B, Influence of pretreatment with SB203580 on HKSP induced IL-6 production in 4-4 macrophages, compared with DMSO control. C, Influence of pretreatment with SB203580 on 10 ng/ml LPS induced TNF-α production in 4-4 macrophages, compared with DMSO control. A representative example is shown. The statistical analysis employed for this experiment was Student’s t test. D, Influence of pretreatment with SB203580 on HKSP induced TNF-α production in 4-4 macrophages, compared with DMSO control. *p < 0.05; **p ≤ 0.01.

FIGURE 2. Effects of p38 MAPK inhibition on cytokine release in LPS-stimulated whole blood and LPS-stimulated peritoneal macrophages. A, Whole blood was stimulated with LPS in increasing concentrations, TNF-α release was determined in the presence of 1 or 10 μM SB203580 or DMSO. B, Freshly obtained peritoneal macrophages were stimulated with increasing concentrations of LPS in the presence of 1 or 10 μM SB203580 or DMSO; the resulting TNF-α release was determined.
MAPK inhibition on cytokine production may be condition dependent rather than a cell type-specific effect, we stimulated 4–4 macrophages with rhTNF-α and lipoteichoic acid from S. aureus, and peritoneal macrophages with HKSP. The results are summarized in Table I and all show increased cytokine release upon p38 MAPK inhibition in macrophages. Apparently p38 MAPK has distinct functions in the regulation of cytokine release in different cell types, macrophages responding to p38 MAPK inhibition with increased cytokine production but other cell types showing diminished release.

**SB203580 inhibits p38 MAPK in murine endotoxemia**

The contrasting effect of p38 MAPK inhibition on cytokine production in different cell types prompted us to investigate the relevance of this phenomenon in in vivo models of disease. The ability of SB203580 to inhibit p38 MAPK enzymatic activity in vitro is well established (4) but its potency in vivo is less clear. To test the capacity of SB203580 to inhibit p38 MAPK in vivo, we used a murine endotoxemia model. As shown in Fig. 3, LPS-dependent p38 MAPK phosphorylation and enzymatic activity was detectable in spleen cells obtained from mice treated with solvent control. However, in SB203580-treated mice, almost no enzymatic activity of the kinase was detected although phosphorylation of the enzyme was much more pronounced, demonstrating that the inhibitor interfered with kinase activity itself but did not inhibit the signal transduction cascade leading to the phosphorylation of Thr\(^{180}\)/Tyr\(^{182}\), further supporting the specificity of this compound. However, despite the inhibition of p38 MAPK, no significant differences in the LPS-induced TNF-α production was measured in the plasma (Fig. 4A). These results are, to our knowledge, the first demonstration of p38 MAPK activation in an in vivo model of disease, but do not support a role for p38 MAPK in the regulation of endotoxemia-induced cytokine release.

**SB203580 enhances TNF-α levels in murine models of pneumococcal pneumonia and tuberculosis**

To further investigate the role of p38 MAPK in in vivo models of disease we tested SB203580 in murine pneumococcal pneumonia and tuberculosis. Induction of both diseases was associated with a marked increase in TNF-α levels in lung homogenates or BAL fluid, in parallel with a rise in the numbers of CFU in lung homogenates. TNF-α levels were enhanced in the SB203580-treated group compared to controls in both lung homogenates (\(p < 0.01\)) and BAL fluid (\(p < 0.05\)) from mice with pneumococcal pneumonia (Fig. 4, B and C) and the lung homogenates from mice with tuberculosis (\(p = 0.01\); Fig. 4D). In both the pneumococcal pneumonia model and the tuberculosis model the numbers of CFU in the SB203580-treated group were significantly higher (pneumococcal pneumonia, \(p < 0.01\); tuberculosis, \(p < 0.05\); Fig. 5). We conclude that the stimulation of cytokine release observed in p38 MAPK-inhibited macrophages in vitro is also reflected in increased cytokine production in vivo and is accompanied by severely reduced bacterial clearance, suggesting that p38 MAPK directed anti-cytokine strategy in infectious disease is not advisable.

**Discussion**

In several cell types, p38 MAPK inhibition was found to reduce cytokine release (1, 11, 12), although in mast cells the opposite effect was observed (14). In line with previous reports (1, 12), we observed that in TNF-α-stimulated L929 fibrosarcoma cells and in LPS-challenged whole blood p38 MAPK activity is required for cytokine production. Strikingly, however, we observed that inhibition of p38 MAPK stimulated cytokine production in a macrophage cell line as well as in nontransformed peritoneal macrophages. Hence we suggest that p38 MAPK acts in a cell-specific fashion, its inhibition increasing cytokine release in macrophages, while such inhibition reduces cytokine release in other cell types.

As p38 MAPK has been suggested to be a suitable target for in vivo anti-cytokine therapy in a number of inflammatory diseases, the possible cell type-specific effects of p38 MAPK inhibition make it important to assess the action of p38 MAPK inhibition on cytokine production during pathophysiology in vivo. Interestingly, p38 MAPK inhibition had no effect on plasma TNF-α levels of LPS-challenged mice. This observation may be explained in two

### Table I. Effect of p38 MAPK inhibition on cytokine release induced by different stimuli

<table>
<thead>
<tr>
<th>Cells</th>
<th>Stimulus</th>
<th>No.</th>
<th>Cytokine Measured</th>
<th>Cytokine Release (pg/ml) Concentration SB203580</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0 μM</td>
</tr>
<tr>
<td>4-4 macrophages</td>
<td>rhTNF-α</td>
<td>3</td>
<td>mTNF-α</td>
<td>343 ± 155</td>
</tr>
<tr>
<td>Peritoneal macrophages</td>
<td>LTA</td>
<td>2</td>
<td>IL-6</td>
<td>91 ± 11</td>
</tr>
<tr>
<td></td>
<td>HKSP</td>
<td>2</td>
<td>mTNF-α</td>
<td>1486 ± 545</td>
</tr>
</tbody>
</table>

*4-4 macrophages and freshly isolated peritoneal mouse macrophages were exposed to the stimuli indicated after preincubation with SB 203580. Subsequently cytokine production was measured with ELISA (see Materials and Methods).
ways: either different TNF-α production from different cell types cancel each other out, or p38 MAPK contributes less to the generation of TNF-α levels, induced by in vivo LPS challenge, than hitherto assumed. In both the murine pneumococcal pneumonia model and the tuberculosis model, higher levels of TNF-α were found in lung homogenates or BAL fluid from SB203580-treated mice. Macrophages are likely to play a role in cytokine production both in pneumococcal pneumonia as well as in tuberculosis (19, 20), although other cell types are obviously involved as well. Thus, the enhanced TNF-α levels observed in these pneumonia models, after p38 MAPK inhibition, may be an in vivo reflection of the increased cytokine production we encountered in p38 MAPK-inhibited macrophages in vitro. However, it should be stressed that our measurements cannot discriminate between different cell types. Thus it is possible that at the concentrations used, p38 MAPK in macrophages is not really inhibited in vivo. Hence the final proof of the notion that the enhanced cytokine levels observed in p38 MAPK-inhibited animals reflect cell type-specific effects of this inhibition, as opposed to a dose-response-specific effect for different cells under different conditions, awaits experiments in which p38 MAPK is cell type-specific knocked out. Until then, other possibilities should be kept in mind. For instance, the increase in cytokine production in SB203580-treated mice may have resulted from the observed increased bacterial burden in the p38 MAPK-inhibited group.

The reduced bacterial clearance and increased cytokine production encountered in p38 MAPK-inhibited mice strongly argue against a function of p38 MAPK inhibitors in therapeutic anti-cytokine strategy in infectious disease or in individuals at risk for infectious disease. As the main proposed beneficiaries of anti-p38 MAPK therapy are patients with autoimmune disease who typically are treated with immune-suppressive drugs; this is an important consideration.

Although stimulation of p38 MAPK is assumed to be a general response to inflammatory stimuli in vivo, actual activation of this kinase in a disease model had not yet been shown. To assess the role of p38 MAPK we used a murine model for endotoxemia since acute i.p. application of LPS should produce a rapid and synchronized activation of p38 MAPK. Indeed, we were able to demonstrate, using the endotoxemia model, in vivo activation of p38 MAPK. This was evident from both its enhanced phosphorylation state as well as by increased kinase activity when assayed by an in

FIGURE 4. Effect of pretreatment with SB203580 on TNF-α levels in murine models of disease. A, Plasma TNF-α levels at 90 min post LPS challenge. Data are pooled from three separate experiments (total of 13 mice per condition). TNF-α levels in lung homogenates (B) and, in a separate experiment, BAL fluid (C) from SB203580-treated mice with pneumococcal pneumonia were significantly enhanced at 24 h. D, TNF-α levels in lung homogenates from SB203580-treated mice with tuberculosis were significantly enhanced at 2 wk after induction of disease, compared with controls. *, p < 0.05; **, p ≤ 0.01.

FIGURE 5. Effects of SB203580 on number of CFU in mice with pneumococcal pneumonia and tuberculosis. Statistical analysis was performed with the Mann-Whitney test. A, Numbers of CFU in lungs from mice with pneumococcal pneumonia at 24 h. B, Numbers of CFU in lungs from mice with tuberculosis at 2 wk. *, p < 0.05; **, p ≤ 0.01.
vitro kinase assay. Furthermore, the latter assay showed the efficacy of i.p.-administered SB203580 to inhibit in vivo p38 MAPK activity. These experiments also provided further proof as to the specificity of the inhibitor; we observed enhanced levels of p38 MAPK phosphorylation in SB203580-treated animals, demonstrating that the inhibitor does not interfere with an upstream activator of this kinase. This SB203580-induced enhanced p38 MAPK phosphorylation may reflect the action of a p38 MAPK-dependent feedback mechanism, regulating the phosphorylation state of this kinase. If such a feedback mechanism involves the deactivation of p38 MAPK upstream activators, which may regulate other targets apart from p38 MAPK, inhibition of p38 MAPK enzymatic activity would result in sustained stimulation of such other targets. In turn, such target may mediate the enhanced cytokine release observed in our study. If this hypothesis is confirmed, this would imply that p38 MAPK inhibitors would be relatively ineffective for reducing cytokine release (at least in macrophages) but in contrast, inhibitors of its upstream activators would be suitable targets for anti-cytokine therapy.

Our results contradict a previous report in which a small number of mice was challenged with LPS and inhibition of TNF-α production was noted, although no actual inhibition of p38 MAPK activity was shown in this study (21). However, this effect of SB203580 was only observed in mice at concentrations in excess of 34 μM/kg b.w., whereas in the present study we have demonstrated complete inhibition of p38 MAPK enzymatic activity in vivo at a SB203580 concentration of 1 μM/kg b.w. As SB203580 has been reported to inhibit other kinases at concentration in excess of 10 μM (22) it is possible that inhibition of these kinases may explain the discrepancy between this earlier report and our study.

The most puzzling finding in the present study is the apparent cell-specific effect of p38 MAPK inhibition on cytokine release, enhancing cytokine production in peritoneal macrophages and a macrophage cell line but inhibiting this production in other cell types and whole blood. Recently it was reported that p38 MAPK inhibits TNF-α-induced inhibitory protein that dissociates with NF-κB phosphorylation and degradation (23, 24) and thus p38 MAPK may negatively regulate the NF-κB pathway, of which the involvement in cytokine production is undisputed. Thus the effects of p38 MAPK inhibition on cytokine release may result from negative signaling of p38 MAPK toward the NF-κB pathway. We are currently exploring this possibility.

Acknowledgments

We thank Adri Maas and Joost Daalhuisen for expert technical assistance in animal experiments. We are indebted to all of the volunteers for blood donations.

References


