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chapter 7

Dichotomal role of inhibition of p38 MAPK with SB 203580 in experimental colitis.

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Abstract

Background: Crohn’s disease is characterised by a chronic relapsing inflammation of the bowel, in which pro-inflammatory cytokines play an important perpetuating role. Mitogen-activated protein kinase p38 (p38 MAPK) has been established as a major regulator of the inflammatory response, especially with regard to production of pro-inflammatory cytokines, but its role in inflammatory bowel disease remains unexplored. In this paper we describe the effects of a specific p38 MAPK inhibitor, SB 203580, in trinitrobenzene sulphonic acid (TNBS) induced colitis in mice. Results: SB 203580 had a dichotomal effect in TNBS mice. The weight loss of TNBS mice treated with SB 203580 was significantly worse and the weight of the colon weight upon sacrifice was significantly increased in MAPK inhibitor treated TNBS mice (229.2 mg and 289.1 mg resp.). However, the total number of cells in caudal lymph node decreased to $188.8 \times 10^4$ cells in SB 203580 treated TNBS mice compared to $334 \times 10^4$ cells in vehicle treated mice. CD3/CD28 double stimulated caudal lymph node cells of SB 203580 treated mice showed decreased IFNγ production, but increased TNFα production. The concentration of IL-12p70 in colon homogenates was significantly decreased in SB 203580 treated mice, whereas concentrations of IL-12p40, TNFα and IL-10 were similar in vehicle or SB 203580 treated TNBS mice. Conclusion: Our results reveal a dichotomy in p38 MAPK action during experimental colitis.
Introduction

Crohn’s disease is a complex multi-factorial disorder characterised by cytokine-driven T lymphocyte-dependent relapsing inflammation of the intestinal mucosa. The mitogen-activated protein kinase (MAPK) family of proteins is principal regulator of gene expression, and critically controls transcription of a number of cytokine genes. P38 MAPK is particularly involved in the inflammatory process, inflammatory stimuli being strong activators of this kinase and its activation being required for inflammatory gene transcription in vitro. The effects of p38 MAPK in inflammation are probably mediated by direct phosphorylation and activation of several transcription factors (e.g. ATF2) and downstream protein kinases (e.g. MAP kinase activated protein kinase 2 -MAPKAP2- and 3). These downstream kinases regulate cell growth and differentiation and cell death. The p38 MAPK signalling cascade has a pivotal role in the regulation of transcriptional activation of cytokine genes including IL-1β, TNFα, IL-6 and IFNγ.

In murine T lymphocytes, a p38 MAPK inhibitor inhibited IFNγ but not IL-4 production and over-expression of dominant negative p38 MAPK results in selective impairment of Th1 response. Conversely, when highly expressed, constitutively activated MKK6 caused increased production of IFNγ. The p38 MAPK pathway is also important for other T-lymphocyte functions. Mice deficient in MKK3, an upstream kinase of p38 MAPK are defective in the production of IL-12 by antigen presenting cells and as a result their CD4 positive T lymphocytes do not produce IFNγ. In addition, activation of p38 MAPK was suggested to be necessary for T cell proliferation, although this was not confirmed in other reports. Finally, p38 MAPK seems to be involved in positive and negative selection of T cells in the thymus.

The functional importance of p38 MAPK activation has been studied in several animal models, by using a specific inhibitor. SB 203580 is a selective inhibitor of p38 MAPK that inhibits the catalytic activity of p38 MAPK by competitive binding in the ATP pocket and has been shown to inhibit p38 MAPK in vivo. Treatment with SB 203580 reduced mortality in a murine model of endotoxin-induced shock and had therapeutic activity in collagen induced arthritis in mice. Several other inhibitors of p38 MAPK are reported to have efficacy including allergic pulmonary disease, LPS-induced lung inflammation, inflammatory angiogenesis, rat-adjuvant induced arthritis and pancreatitis-associated pulmonary injury. In these diseases treatment with p38 MAPK inhibitors attenuated both
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p38 activation and disease severity. Although it has been suggested that p38 MAPK may be involved in the regulation of intestinal inflammation and the importance of p38 MAPK for cytokine production and T lymphocyte activation, the function of p38 MAPK in inflammatory bowel disease is unknown. In this study we explored the effect the specific p38 MAPK inhibitor, SB 203580, in trinitrobenzene sulphonic acid (TNBS) induced colitis, a murine model of Crohn’s disease. The results reveal an unexpected dichotomy in the role of this kinase in inflammatory bowel disease.

Materials and methods

Mice and induction of colitis

All experiments were approved by the Animal Studies Ethics Committee of the University of Amsterdam, The Netherlands. BALB/c mice were obtained from Harlan Sprague Dawley Inc (Horst, the Netherlands). The mice were housed under standard conditions, and supplied with drinking water and food (AM-II 10mm, Hope Farms, Woerden, The Netherlands). Experiments were conducted in 8 and 10 weeks old female BALB/c mice. Colitis was induced by rectal administration of two doses (separated by a 7 day interval) of 2 mg 2,4,6-trinitrobenzene sulphonic acid (TNBS)(Sigma Chemical Co, St Louis, MO, USA) in 40% ethanol (Merck, Darmstadt, Germany), using a vinyl catheter that was positioned 3 centimetres from the anus (10 mice per group). During the instillation, the mice were anaesthetised using isoflurane (1-chloro-2, 2,2,-trifluoroethyl-isoflurane-difluoromethyl-ether, Abbott Laboratories Ltd., Queensborough, Kent, UK), and after the instillation they were kept vertically for 60 seconds. Control mice underwent identical procedures, but were instilled with physiological salt. All mice were sacrificed at 9 days following the first TNBS administration (i.e. 48 hours following the second TNBS challenge). Mice were treated daily by intra-peritoneal injection with 1µM SB 203580/ kg bodyweight in 1 ml sterile saline or 0.01 % DMSO in 1 ml sterile saline as a vehicle control.

In order to study the kinetics of p38 MAPK activity in TNBS colitis, TNBS-instilled mice were sacrificed at 0,1,3,5,8 and 9 days after the induction of colitis. Mice were either treated with vehicle or SB 203580.
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Assessment of inflammation

Body weights were recorded daily. Spleens, caudal lymph nodes and colons were harvested upon sacrifice. The colons were removed through a midline incision and opened longitudinally. After removal of faecal material, the wet weight of the distal 6cm was recorded and used as an index of disease-related intestinal wall thickening. Subsequently, the colons were longitudinally divided in two parts, one of which was used for histological assessment.

Histological analysis

The longitudinally divided colons were rolled up, fixed in 4% formalin and embedded in paraffin for routine histology. Two investigators who were blinded for the treatment allocation of the mice scored the following parameters: 1) percentage of area involved, 2) number of follicle aggregates, 3) oedema, 4) erosion/ulceration, 5) crypt loss and 6) infiltration of mono- and polymorphonuclear cells. The percentage of area involved and crypt loss was scored on a scale ranging from 0 to 4 as follows: 0, normal; 1, less than 10%; 2, 10%; 3, 10 to 50%; 4, more than 50%. Erosions were defined as 0 if the epithelium was intact, 1 for the involvement of the lamina propria, 2 ulcerations involving the submucosa, and 3 when ulcerations were transmural. The severity of the other parameters was scored on a scale 0 to 3 as follows: 0, absent; 1, weak; 2, moderate; 3, severe. This score ranges from 0 to a maximum of 26 points.

Cell culture and ELISA for cytokines

Caudal lymph node cell suspensions were prepared using filter cell strainers (Becton/Dickinson Labware, New Jersey, USA). Cells were suspended in the RPMI 1640 medium (BioWhittaker-Boehringer, Verviers, Belgium) containing 10% FCS and the antibiotic ciproxin (10 μg/ml) (Sigma Chemical Co., St. Louis, MO, USA). Cell suspensions were counted and 1 x 10^5 cells were incubated in 200 μl RPMI containing antibiotics and 10% foetal calf serum in triplicate wells. Cells were stimulated by pre-coating with anti-CD3
antibody (1:30 concentration; 145.2C11 clone) and soluble anti-CD28 antibody (1:1000 concentration; Pharmingen, San Diego, CA, USA). Supernatants were removed after 48 hours and IFN-γ and TNF-α (R&D systems, Abingdon, United Kingdom) concentrations measured by ELISA assay.

Colon homogenates

Colon was harvested and homogenates were made with a tissue homogeniser in 9 volumes of Greenburger lysis buffer (300 mM NaCl, 15 mM Tris-HCl, 2mM MgCl₂, 2mM Triton (X-100), Pepstatin A, Leupeptin, Aprotinine (all 20 ng/ml), pH 7.4) Tissue was lysed for 30 minutes on ice followed by two times centrifugation (10 min., 14,000g, 4°C). Homogenates were stored on −20°C until use. TNFα and IL-10 (both R&D), and IL-12p70 and IL-12p40 (both Pharmingen) concentrations were measured by ELISA assay.

p38 MAPK activity assay and Western Blotting

Colon homogenates were made using a tissue homogeniser in 9 volumes of ice-cold cell lysis buffer (20mM Tris (pH7.5), 150Mm NaCl, 1mM EDTA, 1mM EGTA, 1% Triton, 2,5 mM sodium pyrophosphate, 1 mM β-Glycerolphosphate, 1mM Na₃VO₄ 1 μg/ml Leupeptin and 1mM Pefabloc (Merck, Darmstadt, Germany)). Samples were sonicated 4 x 5 seconds on ice and spun at 7000 x g for 10 min at 4°C. Protein content in the clear supernatant was determined using a bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL), using BSA as the standard, and the supernatant was stored at −80 °C.

Approximately 250μg of protein was used to measure p38 MAPK enzymatic activity using the p38 MAPK assay kit purchased from Cell Signaling, Beverly, MA. A once diluted slurry of agarose hydrazide-bound antibodies to phosphorylated (Thr180/Tyr182) p38 MAPK (40μl) was utilized to selectively immunoprecipitate active p38 MAPK from the colon cell lysate (in 200μl cell lysis buffer) by gently shaking overnight at 4 °C. The immunoprecipitate was washed twice with 500 μl of ice cold cell lysis buffer and twice with 500 ml of ice cold kinase buffer (25 mM Tris, pH 7.5, 5 mM β-glycerolphosphate, 2 mM DTT, 0.1 mM Na₃VO₄, 10
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mM MgCl₂) at 4 °C. The kinase reactions were carried out in the presence of 200 μM ATP and 2 μg of ATF-2 fusion protein at 30 °C for 30 min. After the reaction had been terminated by the addition of 3x SDS-sample buffer, the mixture was boiled for 5 min followed by brief centrifugation. ATF-2 phosphorylation was selectively measured by Western immunoblotting. Samples were loaded on 10% SDS-polyacrylamide gels and transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA). Subsequently, membranes were blocked in 5% non-fat dry milk in TBS supplemented with 0,1% Tween-20 and washed in TBS supplemented with 0,1% Tween-20. Membranes were incubated overnight using specific antibodies against phosphorylated (Thr⁷¹) ATF-2 in 5% BSA in TBS supplemented with 0,1% Tween-20. After three washes for 10 min secondary antibody incubation was performed for 1 hour with GAR-PO in a 1:2000 dilution. After enhanced chemoluminescence using Lumilight+ substrate (Boehringer Manheim, Germany), antibody binding was visualized using a Lumi-imager (Boehringer Manheim, Germany).

250μg of the colon cell lysate was suspended in a final volume of 80μl SDS sample buffer. 25μl was loaded on a SDS-polyacrylamide gel in order to measure p38 MAPK phosphorylation, using Western blotting as described previously. p38 MAPK phosphorylation was detected using antibodies against phosphorylated p38 MAPK (Cell Signaling, Beverly, MA).

Antibody binding was quantified using image analysis software (Boehringer Manheim, Germany) and samples were compared to a control sample set on a 100 arbitrary units.

Statistical analysis

Values are presented as mean and SEM per treatment group. Differences between groups were analysed using the non-parametric Mann-Whitney U test. Body weight changes in time were analysed by one-way analysis of variance. P<0.05 was considered significant. SPSS statistical software (SPSS inc., Chicago, USA) was used for the analyses.
Figure 1. SB 203580 inhibits p38 MAPK enzymatic activity in vivo. Colonic cell lysates were obtained from TNBS instilled mice treated with either vehicle (TNBS) or SB 203580 (TNBS + SB) at day 0, 1, 3, 5, 8, and 9 after the start of the experiment. The colonic cell lysate of a nil-treated control mouse (Co) was included in both experiments. (A) p38 MAPK enzymatic activity was determined by measuring phosphorylation of ATF-2 (pATF-2) in an in vitro kinase assay, using immunoprecipitated phosphorylated p38 MAPK from colonic cell lysate. Antibody binding was quantified using image analysis software, and expressed compared to a control (Co) that was set on a 100 arbitrary units. An immunoprecipitated control is shown (-), only ATF-2 and ATP were mixed, without adding immunoprecipitated p38 MAPK. In SB 203580-treated mice less enzymatic activity of p38 MAPK was detected. (B) p38 MAPK phosphorylation in colonic cell lysate was determined by Western Blotting, using specific antibodies against phosphorylated p38 MAPK. Antibody binding was quantified using image analysis software, and expressed compared to a control (Co) that was set on a 100 arbitrary units.

Results

*p38 MAPK is activated during TNBS colitis and inhibited by SB 203580.*

In order to investigate the kinetics of p38 MAPK phosphorylation and enzymatic activity, colons from mice subjected to TNBS colitis were harvested during a 9-day time period. In mice subjected to TNBS and treated with vehicle, enhanced p38 MAPK enzymatic activity was observed, most evident 5 days after induction of colitis compared to basal levels (figure 1a). Strikingly, after this clear activation at day 5 a reduction of p38 MAPK enzymatic
activity was observed. These data were confirmed by direct analysis of p38 MAPK phosphorylation status in colon homogenates, which paralleled the kinetics of the kinase assay (figure 1b). The function of p38 MAPK was further explored by analysing the effects of SB 203580, a selective p38 inhibitor, in TNBS colitis. Treatment with SB 203580 almost completely prevented p38 MAPK enzymatic activity in colonic cell lysates, induced by TNBS administration (figure 1a). Thus, SB 203580 effectively inhibits p38 MAPK at the concentrations used (1μM/ kg bodyweight/ day) in these mice. Analysis of p38 MAPK phosphorylation in the SB 203580 treated mice, showed no inhibition of the phosphorylation status (figure 1b). These data confirm previous reports that SB 203580 binds to the ATP binding site, thereby preventing enzymatic activity, though not preventing phosphorylation of p38 MAPK by its upstream activators\textsuperscript{205}.

![Graph](image)

**Figure 2.** Bodyweight was recorded daily from day 1 till day 10. The change in weight is expressed as percentage of the body weight from day 1 and data are mean (SEM) in saline (NaCl) instilled mice receiving vehicle (◆, n =10), saline instilled mice receiving SB 203580 (▲, n=10), TNBS mice receiving vehicle (◇, n=10) and TNBS mice receiving SB 203580 (Δ, n=7).

**Mice treated with SB 203580 showed significantly more wasting after induction of colitis**

Mice were intra-rectally installed with TNBS or saline on day 0 and day 7 and subsequently sacrificed on day 9. All ten TNBS mice receiving vehicle survived, but in the TNBS treated mice receiving SB 203580 3 mice died during the course of the experiment. All mice intra-rectally installed with 0.9% NaCl, treated either with vehicle or SB 203580, survived.

Body weights of all mice were recorded daily. The induction of colitis was paralleled with significant weight loss in both groups receiving TNBS (figure 2). Mice treated with SB 203580 lost more weight as compared to vehicle treated mice with colitis (p<0.05). Vehicle treated mice with colitis started recovering from their initial 15% weight loss at day 3, but in TNBS mice treated with SB 203580 bodyweight further declined to less then 80% of baseline.
weight. The mean bodyweight of the TNBS colitis mice treated with SB 203580 remained lower than the vehicle treated mice during the course of the experiment. Daily injection of SB 203580 in mice instilled with 0.9% NaCl did not induce weight loss, and body weight changes in these mice were similar to mice treated with vehicle. Hence the effects of SB 203580 on bodyweight changes during colitis are disease related and do not reflect a generalised effect on p38 MAPK inhibition on murine physiology.

Colon weights were significantly increased in MAPK inhibitor treated TNBS mice

The colon weight of saline-instilled mice treated with vehicle or SB 203580 was similar (81.7 ± 5.3 mg and 81.3 ± 6.5 mg respectively) (Figure 3). Due to the induction of colitis colon weight increased to 229.2 ± 22.2 mg in TNBS colitis, and treatment with SB 203580 caused a significantly further increase of the colon weight (289.1 ± 29.1 mg in SB 203580 treated mice) (p<0.05). TNBS administration caused a significant increase of the baseline colitis score, but no differences were observed between vehicle and SB 203580 treated TNBS mice. The colitis score of TNBS mice was comparable between vehicle and SB 203580 treated mice (mean total score 13.9 ± 1.1 and 13.3 ± 0.4 respectively) (Figure 4).
Figure 4. Haematoxylin-eosin staining of the colon of BALB/c mice treated with SB 203580 or vehicle (magnification 25 x object). (A) Colon of saline instilled mouse treated with vehicle. This picture shows normal architecture. (B) Colon of a saline instilled mouse treated with SB 203580, also showing normal architecture. (C) Colon of a TNBS instilled mouse treated with vehicle, common histological features including ulceration and influx of cells are seen. (D) Colon of a TNBS instilled mouse treated with SB 203580; common inflammatory features are also seen.

Cellularity of caudal lymph nodes in SB 203580 treated TNBS mice

The total number of cells in the caudal lymph nodes (CLN) that drain the inflamed colon increased in TNBS colitis from $65.7 \pm 17.1 \times 10^4$ cells (saline) to $334 \pm 36.7 \times 10^4$ cells (TNBS) (figure 5). Significantly less CLN cells were found in the SB 203580 treated mice with colitis compared to vehicle treated mice with colitis ($188.6 \pm 44.9 \times 10^4$ cells in SB treated TNBS mice, $p<0.05$). This effect of SB 203580 in mice control mice was not observed in saline-instilled mice ($52.1 \pm 11.2 \times 10^4$ cells). Apparently p38 MAPK is an important regulator of caudal lymph node cell number.
Cytokine production

Cytokine production upon activation of T cells in the caudal lymph node (CLN) was determined by CD3/CD28 double stimulation (figure 6). In TNBS colitis the IFNγ production of the CLN cells increased and SB 203580 treatment reduced IFNγ production from 981.3 ± 287.8 pg/ml (in vehicle treated TNBS mice) to 305.3 ± 83.01 pg/ml (figure 6a). In saline-instilled mice, IFNγ production of the CLN cells was similar in vehicle treated and SB 203580 treated mice (228.9 ± 60.8 pg/ml and 293.8 ± 116.7 pg/ml respectively). The TNFα production of stimulated CLN cells was increased in TNBS mice (figure 6b). Unexpectedly, treatment of TNBS mice with SB 203580 resulted in a higher of TNFα production as compared to vehicle-treated TNBS mice (375.3 ± 125.9 pg/ml in TNBS mice and 921.58 ± 351.2 pg/ml in TNBS mice treated with SB 203580). In saline-instilled mice treatment with SB 203580 only slightly increased the TNFα production by CLN cells (188.4 ± 100.6 pg/ml in SB 203580 treated mice and 108.3 ± 37 pg/ml in vehicle treated control mice).

Figure 5. Total number of cells present in the caudal lymph node upon sacrifice. Data are presented as mean (SEM) White bars represent the saline instilled mice treated with vehicle or SB203580 (both n=10), the black bars represent TNBS mice treated with vehicle (n=10) or SB203580 treated TNBS mice (n=7). * Significant difference.

Figure 6. Cytokine production by stimulated caudal lymph node cells. Caudal lymph node cells were stimulated with CD3/CD28 and IFNγ (A) and TNFα (B) production was measured after 48 hours. Data are presented as mean (SEM) White bars represent the saline instilled mice treated with vehicle or SB203580 (both n=10), the black bars represent TNBS mice treated with vehicle (n=10) or SB203580 treated TNBS mice (n=7). * Significant difference.
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<tr>
<th></th>
<th>TNBS</th>
<th>TNBS + SB 203580</th>
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<tr>
<td>IL-12p70</td>
<td>133.5 ± 8.5</td>
<td>91.2 ± 11.9 *</td>
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<tr>
<td>IL-12p40</td>
<td>1492 ± 112.8</td>
<td>1483 ± 124.7</td>
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<tr>
<td>TNFα</td>
<td>188.1 ± 8.5</td>
<td>179.6 ± 6.5</td>
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<tr>
<td>IL-10</td>
<td>470.9 ± 64.7</td>
<td>462.0 ± 42.8</td>
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Table 1. Cytokine concentrations in colon homogenates measured by ELISA. Data are presented as mean and SEM in pg/mg. *Significant difference (p<0.05). (TNBS mice n=10, TNBS + SB 203580, n=7)

In colon homogenates IL-12p70 and IL-12p40 concentrations were measured by ELISA (table 1). A significant reduction of IL-12p70 (p40/p35 heterodimer) concentrations was observed in the TNBS mice treated with SB 203580 (133.5 ± 8.5 pg/mg and 91.2 ± 11.9 pg/mg respectively.). No difference in total IL-12p40 concentrations was detected in the colon homogenates of TNBS mice (mean 1492 ± 112.8 pg/mg and 1483 ± 124.7 pg/mg resp. p<0.05). TNFα concentrations in colon homogenates were similar in TNBS mice treated with SB 203580 or vehicle (179.6 ± 6.5 pg/mg and 188.1 ± 8.5 pg/mg respectively.). In addition, no changes in IL-10 concentrations were detected in colon homogenates of TNBS mice treated either with vehicle or SB203580 (470.9 pg/mg ± 64.7 and 462.0 ± 42.8 pg/mg respectively).

Discussion

The present study was designed to explore the role of p38 MAPK in inflammatory bowel disease. To this end we pharmacologically inhibited p38 MAPK during TNBS colitis using the specific p38 MAPK inhibitor SB 203580. SB 203580 was previously shown to be a highly selective inhibitor of p38 MAPK and at a concentration of 1µM did not affect a wide range of other kinases, including p42 and p54 MAPK and phosphatases. In agreement with the reported p38 MAPK inhibitory activity of SB 203580 we showed a significant reduction in p38 MAPK activity in colonic cell lysates in mice treated with SB 203580. Furthermore, we found a transient activation of p38 MAPK in TNBS-instilled mice. Both the phosphorylation and the enzymatic activity were at a maximum 5 days after the start of the experiment.
Surprisingly, inhibition of p38MAPK with SB 203580 in mice with TNBS-induced colitis showed dual effects. Mice treated with SB 203580 lost more and had higher colon weights. However, we did find a reduction number of cells present in the CLN. It should be noted that this anti-proliferative response could be explained by an action specific for SB 203580 instead of p38 inhibition. Several reports describe the inhibition of T cell proliferation using the inhibitor SB 203580\textsuperscript{12,199,207}, however mice with dominant negative p38 and T cells treated with a different p38 MAPK inhibitor\textsuperscript{79}, CNI-1493, show normal T cell proliferation\textsuperscript{208}. On the other hand the reduction in cell number might be explained by the induction of apoptosis in the caudal lymph nodes of mice treated with SB 203580. However SB 203580 was reported to specifically inhibited the induction of T cell apoptosis\textsuperscript{114,207}. Therefore is seems that the reduction in cell number could be better explained by a diminished proliferation.

In line with previous reports stimulated caudal lymph node cells produced significantly less IFN\textgamma\textsuperscript{74,79,207,209}. Furthermore, treatment resulted in significantly lower IL-12p70 concentrations in the colon, whereas IL-12p40, TNF\alpha and IL-10 concentrations in the colon were unaffected. Interestingly, TNF\alpha production of the CLN cells was increased by SB 203580 treatment. Thus, despite reduction of IFN\textgamma and IL-12p70 production treatment with SB 203580 exacerbated disease.

There are several explanations for this unexpected observation. We have recently reported that IFN\textgamma is not an important disease mediator in TNBS colitis, because mice that lack IFN\textgamma\textsuperscript{210} or its receptor\textsuperscript{211} are susceptible to TNBS colitis and, in fact, develop more severe disease. It was reported that IL-12 synthesis and specifically IL-12p40 synthesis is dependent on p38 MAPK activation\textsuperscript{69}. We found a specific reduction of IL-12p70 and no changes in IL-12p40 concentrations after SB 203580 treatment. The pathogenic importance of IL-12 in TNBS colitis is well known and IL-12 neutralising antibody treatment is highly protective in this model\textsuperscript{212}. It should be noted that the two IL-12 subunits, p35 and p40, have different biological functions. IL-12p35-deficient mice are protected against the development of TNBS-induced colitis, and in this situation, neutralisation of p40 restores the normal sensitivity to TNBS\textsuperscript{213}. Hence, IL-12p40 is protective, possibly through the activity of IL-12 homodimers, or dimerisation with another ligand. Against this background, the combination of a reduction of IL-12p70 without an effect on IL-12 p40 concentrations would be expected to be protective, but this was not substantiated in our study. The observed changes may have been too small to result in a therapeutic effect, or IL-12p35 may have formed heterodimers.
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with a ligand other than p40. It is known that heterodimers of IL-12p35 and Epstein-Barr virus-induced gene 3 (EBI3)\textsuperscript{214} have pro-inflammatory effects. The enhanced wasting observed during colitis in p38 MAPK inhibited animals coincided with enhanced TNFα production. In accordance with this finding, TNFα production of T lymphocytes was reported to be only partially dependent on p38 MAPK activation\textsuperscript{215}. Furthermore, we have recently shown that SB 203580 increases TNFα production by macrophages\textsuperscript{216} and increased TNFα production in mast cells has been reported\textsuperscript{217}. TNFα plays an important role in intestinal inflammation as affirmed in Crohn's disease\textsuperscript{191,205} and experimental colitis\textsuperscript{218}. Alternatively, it is possible that activation of p38 MAPK occurs downstream of the disease perpetuating signal transduction elements and that inhibition at this level does not affect disease severity. We consider it more likely that cells that play a protective role in intestinal inflammation also need p38 MAPK to function properly. T-regulatory cells (Tr1) that produce high amounts of IL-10 and are protective in experimental colitis in mice\textsuperscript{208}. Although we did not find a reduction of IL-10 concentration in colon homogenates of mice treated with SB203580, it was reported that SB 203580 suppresses IL-10 production of T cells\textsuperscript{212}, and thereby SB 203580 could inhibit the protective T cells present in the lamina propria.

Another explanation for our findings is that inhibition of p38 MAPK results in accumulation of upstream activators of p38 MAPK. SB 203580 binds to the ATP binding site, thus preventing phosphorylation of downstream targets including MAPKAP 2 and activation of transcription factor 2 (ATF2), though not preventing phosphorylation of p38 MAPK by its upstream activators M KK3 and M KK6\textsuperscript{206}. M KK3 and M KK6 are able to phosphorylate other downstream targets than p38 MAPK such as JNK1 and JNK2 pathway\textsuperscript{114}. Because JNK2 is involved in Th1 differentiation, p38 MAPK inhibition may paradoxically increase the severity of Th1 mediated diseases. It is however also possible that the observed effects are specific for the inhibitor or the species used in these experiments and studies with other p38 MAPK inhibitors are needed to elucidate this.

In summary, treatment with the p38 MAPK inhibitor SB 203580 does not ameliorate TNBS colitis, though it does prevent IFNγ and IL12-p70 production. This indicates that p38 MAPK might have a broader role in the mucosal immune response and is not only responsible for the production of pro-inflammatory cytokines but might also be involved in counter-regulatory responses.