Inhibition of RICK/Nuclear Factor-κB and p38 Signaling Attenuates the Inflammatory Response in a Murine Model of Crohn Disease*

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Nuclear factor-κB (NF-κB) is the main target of anti-inflammatory therapies in human chronic inflammatory bowel diseases (IBD), Crohn disease, and ulcerative colitis. This study investigates the molecular anti-inflammatory mechanisms of SB203580, an inhibitor of the mitogen-activated protein kinase p38. The murine trinitrobenzene sulfonic acid (TNBS)-induced colitis was used as an established model of human Crohn disease. Here we show that SB203580 improved the clinical condition, reduced intestinal inflammation, and suppressed mRNA levels of pro-inflammatory cytokines elevated upon induction of colitis. Besides p38 kinase activity, the “classical” IkB-dependent NF-κB pathway was strongly up-regulated during colitis induction, whereas the “alternative” was not. SB203580 treatment resulted in a drastic down-regulation of p38 and NF-κB activity. The molecular analysis of NF-κB activation revealed that Rip-like interacting caspase-like apoptosis-regulatory protein kinase (RICK), a key component of a pathway leading to NF-κB induction, is also strongly inhibited by SB203580. In contrast, SB203580 had no effect on the colitis-induced activation of other potential NF-κB-activating kinases such as protein kinase Cθ (PKCθ), mixed lineage kinase 3, and the oncogene product Cot/TPL2. Thus, the inhibitory effect of SB203580 on NF-κB activation is to a large extent mediated by RICK inhibition. RICK is the effector kinase of the intracellular receptor of bacterial peptidoglycan NOD. Because bacterial products are suggested to be the key pathogenic agents triggering IBD, inhibition of the NOD/RICK pathway may serve as a novel target of future therapies in human IBD.

Crohn disease is characterized by chronic intestinal inflammation frequently relapsing with clinical manifestations including diarrhea, blood in the stool, abdominal pain, and weight loss. The etiology of Crohn disease is so far unknown (1) but epidemiological and linkage studies suggest a genetic predisposition of the patient and the involvement of environmental factors (1, 2). Furthermore, several observations strongly implicate a pathogenic role of the intestinal flora during the initiation process of the immunological dysregulation (3–5). At present, treatment of Crohn disease is limited to 5-aminosalicylates, corticosteroids, and immunosuppressants such as azathioprine or 6-mercaptopurine (1). Because of the lack of specific and curative treatments with limited toxicity or side effects, there is still a great demand for developing effective therapeutic approaches.

NF-κB1 is one of the most important transcription factors for the induction of genes mediating innate and adaptive immunity (6), and is also the key transcription factor for pro-inflammatory responses in inflammatory bowel disease (IBD) (7). The NF-κB system consists of homo- or heterodimers that are retained in the cytoplasm upon association with an inhibitory IκB protein (8). In the “classical” pathway of NF-κB activation, the IκB kinase complex (IKK) is activated and leads to phosphorylation and polyubiquitination of IκB thereby marking this NF-κB inhibitor for degradation in the 26 S proteasome. Protein kinase Cθ (PKCθ) and the MAP3K Cot/TPL2 and mixed lineage kinase 3 (MLK3) are important activators of the IKK complex and therefore of NF-κB (9–11). The recently described “alternative” activation pathway of NF-κB involves the processing of p100 to p52 (12–14).

One pathway critical for IBD and activation of NF-κB is dependent on NOD proteins (15, 16). NOD proteins are intracellular receptors for bacterial peptidoglycan and play a pivotal role in the innate immune system (17). Therefore, it is reasonable to suggest that NOD-mediated signal transduction pathways may serve as an initiation point for a variety of inflammatory responses in bacterial triggered “autoimmune” diseases. In fact, genome-wide screens have identified NOD2/caspase-activation recruitment domains (CARD15) as the first susceptibility gene for Crohn disease (15, 16). The pathogenic role of mutations within the NOD2 gene in patients with Crohn disease is supported by functional studies showing an inappropriate response to bacterial components with respect to NF-κB activation within the innate immune system (16). Those NOD2 mutations (i.e. the 3020insC mutant) lead to a truncated NOD2 protein because of a frameshift. This results in the inability to recognize bacterial lipopolysaccharide (LPS), an abolished NF-κB activation (16), and finally in a persistent intestinal inflammation because of the strongly reduced TNF-α-induced NOD2 gene transcription (2, 18). Furthermore, a direct link has been

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been shown between NOD signaling and NF-kB activation (19).
Recently, DLG5 as a scaffolding protein involved in the mainte-
nance of epithelial integrity has been mapped as another candidate
susceptibility gene for Crohn disease. This function of DLG5 may be
another indication for the role of intestinal flora in IBD, because
a shreded epithelial bowel layer is a potential prerequisite for
bacterial triggered disease induction (20).

The mitogen-activated protein kinase (MAPK) p38 is another
crucial mediator of inflammation (21–24). p38 directly controls
the activity of several transcription factors relevant to the produc-
tion of pro-inflammatory cytokines, which are up-regulated
in IBD. Furthermore, p38 can modulate a number of
different steps in the inflammatory cascade including the tran-
scriptional regulation of the genes encoding the key cytokines of
IBD TNF-α, IL-1, and interferon γ in monocytes and lympho-
cytes, the degranulation of neutrophils, and the expression of
receptors essential for neutrophil activation and chemotaxis like
the CXC receptors (25–27). The expression of this receptor is
strongly inhibited by p38 MAPK inhibitors (28). Addition-
ally, upstream kinases of p38 like the MAPK kinase MKK3 as
well as downstream substrates such as the activator of tran-
scription factor 2 (ATF-2) or MAPK-activated protein kinase 2
are by itself potent regulators of genes for Th1-derived cyto-
kines like TNF-α, which are all up-regulated in Crohn disease
(26, 27, 29–31). In a murine knock-out model, elimination of
MAPK-activated protein kinase 2 led to a dramatic reduction of
TNF-α in response to lipopolysaccharide (32).

Because the p38 gene as well as other candidate genes has
been localized to the IBD susceptibility region 3, much atten-
tion has been focused on p38 as a key kinase maintaining
intestinal inflammation in IBD (21, 33, 34). p38 displays the
strongest activity compared with other MAPK within the in-
flamed intestinal mucosa of IBD patients (21, 35, 36). A recent
study investigating the efficiency of the combined p38 and
JNK inhibitor CNI-1493 in patients with Crohn disease dem-
onstrated a significant clinical improvement in moderate to
severe active disease (35).

In this study, we investigated the anti-inflammatory effects
of the p38 MAPK inhibitor SB203580 on signal transduction
pathways involving NF-κB and p38 in a murine model of Crohn
disease. We show that SB203580 has highly beneficial anti-
inflammatory effects resulting in a significant suppression of
the transcription of pro-inflammatory cytokines as well as the
induction of TNF-α and the classical but not the alternative
NF-κB activation pathway. The inhibition of NF-κB by
SB203580 is probably mediated by the blocked NOD/RICK
pathway. Thus, dual inhibition of p38 and NOD/RICK may lead to
new treatment options with very limited side effects in
chronic inflammatory conditions like human IBD.

EXPERIMENTAL PROCEDURES

Animals and Experimental Design—Female BALB/c mice (6–8
weeks old) ~19–22 g in body weight were obtained from Harlan-
Winkelmann (Borchen, Germany) and given ad libitum access to water
and standard rodent food before and during the study. Mice were
weighted and randomized into groups of 7. Clinical assessments and
histological scoring of colitis were performed in a blinded fashion.
Experiments were performed after 3 days following arrival of the
animals. To induce colitis, 0.1 ml of a 2.5% (w/v) TNBS solution in 50%
ethanol (Sigma) was slowly administered into the colon of lightly anes-
thetized mice (Sigmar) by a thin catheter (PE-50, BD Biosciences, Heidelberg,
Germany) attached to a 0.5-ml syringe (37). The catheter tip was
inserted 5–6 cm proximal to the anal verge, and the mouse was held in
a vertical position for 1 min after instillation. Using this procedure,
>95% of the mice retained the TNBS-ethanol enema. However, if an
animal quickly (i.e. <10 min) excluded this solution, it was omitted from
the remainder of the study. Treatment with 5 μmol of
SB203508/kg of body weight in 200 μl of 0.9% NaCl solution was given
intraperitoneally twice daily beginning 12 h after TNBS administration
and continuing until death. For control experiments, bowel tissue was
analyzed from: 1) completely untreated animals, 2) animals receiving
vehicle injections (3% MeSO, without rectal challenge, or 3) mice
receiving SB203580 (Calbiochem, Schwalbach, Germany) injections in
the same manner but treated with 0.9% saline intracolonically instead
of TNBS.

Macroscopic Assessment and Histological Analysis of Colitis—Daily
weight, physical condition, stool consistency, and water/food consump-
tion were determined. Animals were sacrificed by CO2 inhalation at
days 1, 2, 3, 5, 7, and 10 (n = 7 in each group per day). After sacrifice,
the colon was quickly removed, opened longitudinally, and gently
cleared of stool. Macroscopic assessment of the disease activity was
scored according to a previously established scoring system (38) as
follows: 0: no ulcer, no inflammation; 1: no ulcer, local hyperemia; 2:
ulceration without hyperemia; 3: ulceration and inflammation at one
site only; 4: two or more sites of ulceration and inflammation; and 5:
ulceration extending more than 2 cm. Subsequently, samples of colonic
tissue were either fixed in 4% buffered formalin, embedded in paraffin,
and 4-μm thick serial step sections were stained with hematoxylin-
esosis (HE) or snap-frozen in liquid nitrogen in OCT embedding medium
and stored at ~80°C until usage. Microscopic scoring was performed
according to Elson et al. (39).

Blood Sampling—Blood (~0.4 ml) was drawn intracardially and
mixed with 50 μl of 0.5 M EDTA. Blood samples were subjected to
differential blood cell count analysis.

Extraction of Total RNA—A gut specimen obtained 5 cm proximal
to the anus was used for extraction of total RNA using a recently described
method (40). For each animal, 1 μg of total RNA was transcribed into
cDNA. Quantitative real-time reverse transcriptase-PCR was performed
in an iCycler (Bio-Rad). The 30-μl reaction mixture consisted of
15 μl of HotStarTaqTM Master Mix, 1.2 μl of the reverse transcriptase
reagent, 0.1 μl of SYBR Green (1:10000; Molecular Probes, Eugene, OR),
and 0.5 μM of the specific primers (TIB Molbiol, Berlin, Germany).
Initial denaturation and activation of Taq polymerase at 95°C for 15
min was followed by 40 cycles consisting of (i) denaturation at 94°C for
30 s, (ii) annealing at 60°C for 45 s for β-actin, TNF-α, interferon γ,
IL-2, IL-12p35, IL-18, and at 55°C for IL-10, and (iii) elongation at
72°C for 15 s. The fluorescence intensity of the double strand–specific
Taqman probe (TAMRA) on the analyte DNA was measured at the
end of each elongation step. Specific initial template mRNA amounts were calculated by determining the time point of a standard curve (artificial units). β-Actin mRNA amounts were used to normalize the cDNA contents of the different samples. In
addition, an aliquot of the PCR mixture was separated on a 1.8%
glycerol agarose gel stained with ethidium bromide.

The following primers were used for the reverse transcriptase-PCR
analysis: β-actin: forward primer, TGGAAATCTCTGGTGGATCCATG-
AAC and reverse primer, TAAAACGGAGCTGATACACGTGCC; TN-
F-α: forward primer, GGCAAGGCTCTTCTTGAGGATCAG and reverse
primer, ATACCTCGGAGGCTCTAGTGAATTG; interferon γ:
forward primer, AAGCGGTGACGATACATCAGTGTAG and reverse
primer, GTCGATCAGTTGGG; IL-2: forward primer, GTCAGTGA-
CCTACAGTGAGGCTCTAGG and reverse primer: GATGCA-
AATCGAAGATCAGTGCAGG; IL-10: forward primer, ACCCTGTGTA-
AGTGTACGCCCCAGCA and reverse primer, CTATGCTTGAATG-
AGATCCTA; IL-12p35: forward primer, GCAAGACTGACACAGTCC-
TGG and reverse primer, TGCATACGCTCTAGTTGAG; IL-18:
forward primer, GCAAGTTTGAGTTCACGCCCAGCA and reverse
primer, GTCCTAAGTGGATACAGAAGAAGCC.

Protein Extraction from Mice Gut—For total protein preparation, gut
specimens obtained 3–4 cm proximal from the anus were snap-frozen in
liquid nitrogen and stored at ~80°C until extraction of protein. Spec-
imens were homogenized in 1 ml of ice-cold lysis buffer containing 0.5%
Nonidet P-40, 0.5% Triton X-100, 100 mM NaCl, 50 mM Tris (pH 7.5),
5 mM EDTA, 0.1 mM Na2HPO4, 0.5% deoxycholic acid (Sigma), 20 mM NaF, 0.1 mM phenylmethylsulfonyl fluoride, 20 mM
glycerol 2-phosphate (Sigma), 1 mM Na3VO4, and protease inhibitor
mixture (Roche Applied Science). The lysate was centrifuged (14,000
×g at 4°C) for 15 min and the supernatant was separated into 2 fractions.
Total protein content was analyzed using the Advanced™ protein assay
(TOYOBO, Germany). Finally, samples were aliquoted and stored at ~80°C until usage.

For cellular subfractionation of proteins, tissue was carefully homog-
emized so that many viable single cells were obtained and re-suspended
in 1 ml of ice-cold TBS buffer, centrifuged (10 min, 800 × g at 4°C), and
washed in buffer A (10 mM HEPES, pH 7.9, 10% (v/v) glycerol, 10 mM KC1,
0.1 mM EDTA, 0.1 mM EGTA, 1 mM dithiothreitol, 0.5 mM phenylmeth-
ylsulfonyl fluoride, 1 mM Na3VO4, 10 mM NaF, protease inhibitor mix-

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RESULTS

SB203580 Represses TNBS-induced Colitis in Mice—Previous studies showed that rectal administration of TNBS induces colitis resembling human IBD (37, 42, 43) and this well-established murine model is commonly used to screen pharmacological agents (37, 42–46). Additionally, recently initiated clinical trials assessing MAPK inhibitors in vitro SB203580 were very successful in the treatment of patients with Crohn disease refractory to standard medication (35). Based on these studies, we explored the possibility that administration of SB203580 can reduce TNBS-induced intestinal inflammation of the murine colon. We found that BALB/c mice subjected to intrarectal TNBS administration reproducibly developed colitis with diarrhea accompanied by severe weight loss, multiple mucosal erosions, and ulcerative colitis with a maximum at 2–3 days following TNBS administration (Fig. 1A). In the treatment groups, SB203580 was administered to mice 12 h after TNBS/ethanol enema when the first signs of colitis were notable. The peak of clinical disease occurred always between days 2 and 3 but the recovery from colitis was markedly enhanced by SB203580 treatment as reflected by the clinical data and reduced macroscopic score (Fig. 1A and B). Control mice treated with rectal NaCl solution (n = 10) or intraperitoneal vehicle (3% MeSO) alone failed to develop wasting disease and appeared healthy. Analysis of weight loss and daily food and water consumption revealed a reduced intake of food from day 1 to 3 in non-treated versus the SB203580-treated mice. Thus, these mice also had a lower weight reduction (Fig. 1A). The daily assessment of weight and the evaluation of feeding behavior indicated no effect of SB203580 on food or water intake compared with controls (Fig. 1A). The colon of TNBS-treated mice removed at days 1, 2, 3, 5, 7, and 10 (n = 7/day) revealed...
macroscopic signs of inflammation strongly increased between days 1 and 2 and reached a maximum around day 3. SB203580 drastically down-regulated the mRNA levels of these cytokines (Fig. 3). The time course of these cytokines at the transcriptional level during increasing and resolving inflammation in bowel tissue of untreated and SB203580-treated animals is shown in Fig. 3. Collectively, these findings confirm an anti-inflammatory effect of SB203580 in murine TNBS-induced colitis. Control animals treated with SB203580 alone displayed the same mRNA levels as control animals receiving vehicle treatment only.

**SB203580 Inhibits Activation of p38**—We visualized the activity of p38 MAPK in inflamed bowel tissue by immunofluorescence microscopy using phospho-specific antibodies. p38 was activated in lymphocytes and macrophages, the main cell types mediating early pro-inflammatory responses in human Crohn disease (Fig. 4A). The severity of inflammation detected by immunohistological analysis correlated with the macroscopic and histologic score in SB203580-treated and untreated animals. No lesions or significant p38 activity were observed in control mice.

Because SB203580 targets only the ATP-binding of p38, but does not interfere with p38 phosphorylation (47), we assessed the activity of p38 by immunoprecipitation using a p38-specific
antibody and bowel tissue protein extracts. Subsequently, we performed an \textit{in vitro} kinase assay using ATF-2 as substrate followed by immunoblotting with phospho-specific ATF-2 antibodies. A strong increase of p38 activity was detected after day 1 with a maximum between days 2 and 5. This activation was strongly down-regulated by SB203580 during the course of colonic inflammation (Fig. 4B).

\textbf{SB203580 Inhibits the Classical but Not the Alternative NF-κB Activation Pathway—}Because SB203580 inhibited the transcription of the pro-inflammatory cytokines in the bowel wall of mice with TNBS-induced colitis, we wanted to examine whether this effect is mediated by NF-κB, a key transcription factor in inflammatory processes. Cytoplasmic and nuclear extracts were prepared from bowel tissue. First, we analyzed the phosphorylation of IKK, IκBα degradation, and the nuclear translocation of p65 by immunoblot analysis. These parameters served as a readout for NF-κB activation. We found a peak of IκKα/β phosphorylation and of IκBα degradation at days 2 and 3 after the induction of colitis by TNBS administration (Fig. 5A). In addition, nuclear translocation of p65 showed in general the same kinetics as IκBα degradation (Fig. 5, A and B). However, the peak of p65 translocation is delayed compared with IκBα degradation. SB203580 almost completely inhibited IKK activation, IκBα degradation, and p65 translocation (Fig. 5, A and B). This might be an indication that SB203580 is not only specific for p38 but also affects NF-κB activation during intestinal inflammation via so far unknown targets.

To exclude the possibility that the inhibitory effect of SB203580 is because of a negative effect on other NF-κB activating pathways mediated by PKCδ, Cot/TPL2, or MLK3, which are known to phosphorylate and thereby induce the IKKs (9–11), we performed activation studies by immunoblot analysis using phospho-specific antibodies. All three kinases were activated by phosphorylation upon induction of the inflammatory process (Fig. 5C). Furthermore, this activation could not be suppressed by administration of SB203580 (Fig. 5C). Thus, based on our data, these signaling kinases are not involved in the SB203580-mediated down-regulation of NF-κB activity and the release of pro-inflammatory cytokines in TNBS-induced colitis in mice.

Additionally, we analyzed the alternative pathway of NF-κB activation involving p100 processing to active p52. Immunoblot analysis was performed using antibodies recognizing both, the p100 precursor as well as the p52 product. However, in contrast to the classical activation pathway of NF-κB, this pathway was not up-regulated during TNBS-induced colitis and hence, was not affected by SB203580 (data not shown).
RICK is a NF-κB Activating Kinase Inhibited by SB203580 during TNBS-induced Colitis—To analyze putative targets of SB203580, we studied molecular components regulated by NOD proteins that may exert a role in NF-κB activation. NOD as an intracellular receptor for bacterial peptidoglycan probably plays a crucial role in the pathogenesis of IBD by triggering intestinal inflammation (3–5, 17, 48). Initial studies have shown that NF-κB becomes activated following a transient expression of NOD1 and NOD2 in mammalian cells. This activation required the integrity of the CARD domains (41). RICK physically interacts with NOD1 through CARD-CARD interactions (47, 49) and has been shown to be the main effector kinase of NOD1. It also interacts with and regulates the IKK complex finally leading to NF-κB activation (47). Therefore, RICK is an interesting candidate to regulate NF-κB via the NOD signaling pathway.

RICK activity was assessed during the experimental colitis following immunoprecipitation of RICK from protein extracts derived from bowel tissue. RICK phosphorylates MBP in an in vitro kinase assay (Fig. 5D). Using this assay, we showed that RICK is dramatically activated during development of experimentally induced colitis. In contrast to the other NF-κB activating kinases analyzed (Fig. 5C), SB203580 treatment of the mice could almost completely inhibit the activation of RICK (Fig. 5D). Because this inhibitory effect was also achieved upon SB203580 addition directly to the cellular extracts of TNBS-treated mice, we postulate a direct suppressive effect of the SB203580 inhibitor on RICK activity (Fig. 5E). This indicates that RICK might be a crucial kinase involved in NF-κB activation in the course of IBD.

DISCUSSION

Because the exact molecular mechanisms of the reduced intestinal inflammation by p38 inhibitors are unsolved so far, we sought to analyze the impact of SB203580 on: 1) the transcription of key cytokines for the development of IBD, 2) NF-κB activation pathways, and 3) the NOD/RICK signal transduction pathway. The TNBS model of colitis in mice shares important similarities with human Crohn disease such as transmural inflammation, lymphocyte infiltration, Th1-dominated cytokine profile, and stricture formation (37, 42, 43). Thus, this model is very suitable to study anti-inflammatory agents during the course of developing and resolving inflammation.

We were able to demonstrate a clinical improvement of TNBS-induced colitis in mice treated with SB203580 as reflected in the clinical data, in the macroscopic and the histological disease score. This is in line with other studies using p38 inhibitors and different models of inflammation (e.g. animal models for arthritis, pulmonary inflammation, and other immune challenging procedures). In all cases, these inhibitors attenuated disease activity, mortality, or reduced pro-inflammatory cytokine concentrations (50, 51). However, a recent study did not show uniformly beneficial effects of SB203580 (52). This might be, at least in part, because of a different experimental design, like double TNBS enemas, and because of the inability of SB203580 to inhibit TNF-α synthesis at the rather low concentration used in this specific study. The findings of this study are not only in contrast to our but also to other in vitro studies using human monocytes stimulated with lipopolysaccharide, where SB203580 inhibited the production of IL-1 and TNF-α (53, 54). Furthermore, it is known that different BALB/c mice strains have variations in their susceptibility to chemically induced colitis (55).

TNF-α plays a central role in mucosal inflammation and is likely the key regulator of the inflammatory cascade in IBD (56). It is regulated by p38 (24) and AU-rich elements in the 3′-untranslated region of the TNF-α gene are necessary for p38-mediated regulation. Mice develop chronic inflammatory diseases when these AU-rich regions are deleted in the genome resulting in an increased TNF-α expression (57). p38 inhibitors in wild type mice can reduce the TNF-α synthesis, whereas AU-rich element-deficient mice did not respond to the drug (58). Our results clearly demonstrate that SB203580 down-regulates the expression of TNF-α as well as the expression of other pro-inflammatory cytokines (26, 30) confirming a general anti-inflammatory effect of SB203580 in the inflamed bowel tissue.

We next wanted to know whether SB203580 had a suppressive effect on p38 as well as other pro-inflammatory pathways such as NF-κB, a potent transcription factor regulating cellular immune responses and inflammation (6). We observed that both p38 and NF-κB are dramatically activated during the development of TNBS-induced colitis and this induction was strongly inhibited by SB203580 treatment. A suppressive effect of SB203580 was only observed for the classical NF-κB activation pathway based on the IκB degradation by the 26 S proteasome (6). The more recently discovered so-called alternative NF-κB activation pathway characterized by NIK/IKKα-dependent processing of the p100 precursor to active p52 (12, 13) was not activated and, therefore, not affected by SB203580. This pathway was investigated, because p100 processing preferentially generates a different subset of NF-κB dimers, mainly p52/RelB. The two activation pathways of NF-κB might differentially influence inflammatory responses in IBD. Studies in transgenic mice suggest that the alternative activation pathway of NF-κB increases chemokine concentrations, and the susceptibility to inflammation in general (59, 60).

A fundamental finding of the current study is the suppression of the classical NF-κB activation by SB203580. It is very improbable that the inhibitory effect of SB203580 on this NF-κB activation pathway is indirect, e.g. mediated by the decrease of cytokines because of p38 inhibition. Cytokine synthesis is not regulated by p38 alone. NF-κB is always involved in the regulation of pro-inflammatory cytokine genes. The more likely explanation would be that SB203580 has a broader range of target protein kinases. The only known impact of p38 on NF-κB activity is by phosphorylation of p65/RelA, which increases the transcriptional activity of p65 containing NF-κB dimers (61). However, we also found that SB203580 blocks the nuclear translocation of p65, which is not known to be influenced by p38. Our data strongly suggest that SB203580 directly or indirectly suppresses kinases involved in NF-κB activation.

One strong candidate for such a novel target of SB203580-mediated inhibition during IBD development is the serine/threonine protein kinase RICK. We focused our studies on this kinase for three reasons. 1) As shown by a very recent proteome-wide study, RICK is a high affinity target of SB203580 (62). 2) RICK is known to interact with and activate IKKα (47). 3) RICK is a prominent effector kinase of NOD proteins (17), which, as mentioned above, are intracellular receptors of bacterial peptidoglycan and play a crucial role in IBD pathogenesis (15–17). RICK, like p38 and NF-κB was strongly activated in the course of experimentally induced colitis and this activation was also drastically reduced by SB203580 treatment of the mice. Our findings give support to the notion that a pathway triggered by bacteria, involving NOD proteins as well as RICK, and finally leading to NF-κB activation, plays a pivotal role in IBD pathogenesis. In vitro studies demonstrated a failure of NF-κB activation because of NOD2 mutations (16). However, to our knowledge evidence is still missing that patients carrying one of these NOD2 mutations have a decreased NF-κB activation in their bowel mucosa. Additionally, it is not unlikely that other NF-κB activating signal transduction pathways in addi-
FIG. 6. Schematic model of inflammatory pathways inhibited by SB203580. Rectal administration of TNBS induces colitis in mice. This triggers p38 MAPK activity via the upstream kinases MKK3/6 and NF-κB activation via the classical IκB-dependent pathway mediated by the IKK complex. RICK is a kinase upstream of IKK, which is able to activate the IKK complex. RICK activity is also induced upon colitis induction. SB203580 inhibits p38 MAPK as well as RICK activity. Suppression of RICK leads to down-regulation of NF-κB. The solid arrows indicate direct activation of downstream targets and the dotted arrows indicate indirect or not yet defined activation processes.

tion to the one analyzed in this study might also activate the underlying pathogenic immune response against the so far unidentified pathogen(s) in Crohn disease. This view is supported by epidemiological data showing that the majority of patients have no NOD2 mutations and virtually all patients with IBD exhibit strongly increased NF-κB activation patterns (63). Thus, because RICK is so far the only accepted effector of NF-κB activation via the classical IκB-dependent pathway mediated by the IKK complex, SB203580 was shown to significantly down-regulate NF-κB activation via the classical IκB-dependent pathway mediated by the IKK complex. RICK is a kinase upstream of IKK, which is able to activate the IKK complex. RICK activity is also induced upon colitis induction. SB203580 inhibits p38 MAPK as well as RICK activity. Suppression of RICK leads to down-regulation of NF-κB. The solid arrows indicate direct activation of downstream targets and the dotted arrows indicate indirect or not yet defined activation processes.

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