Essential Role of p38 Mitogen-activated Protein Kinase in Contact Hypersensitivity*

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The present study was designed to elucidate the role of p38 mitogen-activated protein kinase (p38) in the pathogenesis of inflammation, using a mouse contact hypersensitivity (CHS) model induced by 2,4-dinitro-1-fluorobenzene (DNFB). Ear swelling was induced by challenge with DNFB, accompanied by infiltration of mononuclear cells, neutrophils, and eosinophils and a marked increase in mRNA levels of cytokines such as interleukin (IL)-2, interferon (IFN)-γ, IL-4, IL-5, IL-1β, IL-18, and tumor necrosis factor-α in the challenged ear skin. Both ear swelling and the number of infiltrated cells in DNFB-challenged ear skin were significantly inhibited by treatment with SB202190, a p38 inhibitor. Furthermore, the DNFB-induced expression of all cytokines except IL-4 was significantly inhibited by treatment with SB202190. Ribonuclease protection assay revealed that the mRNA levels of chemokines such as IP-10 and MCP-1 in ear skin were markedly increased at 24 h after challenge with DNFB. The induction of these chemokines was significantly inhibited by treatment with SB202190. In p38α+/− mice, both ear swelling and infiltration of cells induced by DNFB were reduced compared with those in wild-type mice. However, induction of cytokines by DNFB was also observed in p38α+/− mice, although the induction of IFN-γ, IL-5, and IL-18 was typically reduced compared with that in wild-type mice. Challenge with DNFB slightly induced IP-10 and MCP-1 mRNA in p38α+/− mice, with weaker signals than those in SB202190-treated wild-type mice. These results suggest that p38 plays a key role in CHS and is an important target for the treatment of CHS.

Mitogen-activated protein kinases (MAPKs) transduce a variety of extracellular signals to the transcriptional machinery via a cascade of protein phosphorylation. There are three genetically distinct MAPKs in mammals, consisting of extracellular signal-regulated kinase, c-Jun N-terminal kinase (JNK), and p38 MAPK (p38). All of three members are activated by dual phosphorylation of the conserved TXY motif and then phosphorylate their respective substrates on serine or threonine residues (1–3).

p38 was first identified as either a target for a group of anti-inflammatory drugs in human monocytes (cytokine-suppressive anti-inflammatory drug-binding protein (CSBP); see Ref. 4), a lipopolysaccharide-activated kinase in murine macrophage cell lines (p38; see Ref. 5), or a stress-responsive kinase that activates the protein kinase, MAPKAP kinase-2 reactivating kinase (IK; see Ref. 6). There are four mammalian isoforms of p38 (α, β, γ, and δ). Among them, p38α and -β are expressed relatively ubiquitously, as shown by Northern blot analysis of adult tissues (7), whereas p38γ is expressed only in skeletal muscle (8), and p38δ expression is limited to the kidney and lung (9). Recent reports (10, 11) demonstrated that targeted disruption of the p38α gene results in homozygous embryonic lethality because of defects in erythropoiesis or placental organogenesis.

Many groups have demonstrated that the p38 signaling pathway possibly controls inflammatory responses as follows. p38 has a role in transducing the mitogenic signal in T cells in response to interleukin (IL)-2 and IL-7 (12). p38 mediates lipopolysaccharide-stimulated monocyte production of IL-10, IL-1β, and tumor necrosis factor (TNF)-α (13). Interferon (IFN)-γ expression by T1 effector T cells is mediated by p38 (14). The production of IL-12 by macrophages and dendritic cells is reduced in M KK3 (a specific upstream MAPK kinase for p38) deficient mice (15). p38 regulates human T cell IL-5 synthesis and TNF-α mRNA stability (16, 17). These findings tempt us to think that targeting of p38 might be a suitable anti-cytokine strategy for inflammatory disease. Against this notion, it was reported that inhibition of p38 activity rather leads to induction of TNF-α and IL-6 in some cases (18, 19).

In the present study, we used a murine contact hypersensitivity (CHS) model induced by 2,4-dinitro-1-fluorobenzene (DNFB) to investigate the role of p38 in inflammatory disease. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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The abbreviations used are: MAPK, mitogen-activated protein kinase; CHS, contact hypersensitivity; DNFB, dinitro-1-fluorobenzene; IL, interleukin; IFN, interferon; TNF, tumor necrosis factor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; JNK, c-Jun N-terminal kinase; RT, reverse transcriptase; MIP, macrophage inflammatory protein; IP-10, IFN-γ inducible protein-10; EC, epidermal cell; PBS, phosphate-buffered saline; WT, wild type; ANOVA, analysis of variance; LNC, lymph node cells.
First, we elucidated the inhibitory effect of SB202190, a p38 inhibitor, on DNF-induced CHS. Second, we investigated how DNF induces CHS in p38α heterozygous mice.

**EXPERIMENTAL PROCEDURES**

**Experimental Animals**—The use of animal in all our experiments was in accordance with guidelines of Chiba University for animal care. Female mice heterozygous for targeted disruption of the p38α gene (10) were crossed with C57BL/6J male mice (Saitama Experimental Animal Supply Co.) to generate p38α-/+ and p38α +/+ (wild-type; Wt) mice. Offspring (>6 generations) were genotyped by PCR analysis of tail-derived DNA. Multiplex PCR with three primers per reaction was used.

**Histological Analysis**—The ears were removed and immersed in 10% formaldehyde in 0.1 M sodium phosphate buffer (pH 7.4) for 5 min followed by digestion with a thickness gauge (Ozaki Manufacturing Co.). The ears were taken from Wt, p38α-/- mice and p38α +/+ littermate mice challenged with DNFB and subjected to RNA preparation and RT-PCR for detection of each cytokine.

**TABLE I**

| Gene | Sequence (5’ to 3’) | A. temp. | Cycle no. | Size bp |
|------|---------------------|----------|-----------|---------|
| IL-2 | Sense | CAAGCTTCTTACAGCGGAG | 58 | 33 | 370 |
|     | Antisense | TCCACACAGTTGGTGCAC |          |       |
| IFN-γ | Sense | ATCTTTGAGACCCCTTGACT | 60 | 33 | 529 |
|     | Antisense | CGACTCTTCTTTCGCTTTC |          |       |
| IL-4 | Sense | AAGGCCAAGAGACTTTGATG | 60 | 35 | 454 |
|     | Antisense | ATGTTGCCTGTAATCACTGAG |          |       |
| IL-5 | Sense | ATTTGCTCTGATGGTTTAC | 60 | 33 | 733 |
|     | Antisense | ATGTTGATTACACCGGAGAAGT |          |       |
| IL-1β | Sense | TGGCAAGCTACCTGTGTTT | 60 | 35 | 525 |
|     | Antisense | AGCTTTGCTCTGTGTTG |          |       |
| TNF-α | Sense | CCTGCCCTTCATGGCTTTG | 60 | 35 | 992 |
|     | Antisense | ACACATCTTATCCCTACAGA |          |       |
| IL-18 | Sense | ACTGTACAAACCGGAGAATACGG | 60 | 33 | 319 |
|     | Antisense | TCCATCTTGTGTTGTTG |          |       |
| GAPDH | Sense | ATATCTTGCTGTGATGAT | 60 | 25 | 262 |
|     | Antisense | TCCACACACCTGTGCTTA |          |       |
measured (control) and 12, 24, and 48 h later, the thickness of the ears was measured in control, SB202190(−); closed bars, SB202190(+). SB202190 was topically applied to sensitized mice 30 min prior to challenge. Data presented are means ± S.D. of 12–18 mice. #, significantly different from control with p < 0.01 (ANOVA with Bonferroni method). *, significantly different from SB202190-untreated value for each period with p < 0.01 (Student’s t test for unpaired values).

RESULTS

Effect of SB202190, a Specific p38 Inhibitor, on CHS Response to DNFB—As shown in Fig. 1, DNFB induced ear swelling in mice in a time-dependent manner. The DNFB-induced ear swelling in SB202190-treated mice was significantly reduced compared with that in SB202190-untreated mice (p < 0.05). Histological analysis showed spongiosis in the challenged ear skin 24 h after challenge. Additionally, marked infiltration of inflammatory cells in the epidermis and dermis was observed in the challenged ear skin 24 and 48 h after challenge (Fig. 2, A–D). Treatment of mice with SB202190 decreased DNFB-induced pathophysiological parameters such as spongiosis and infiltration of inflammatory cells in the epidermis and dermis (Fig. 2, E–H). To characterize the inflammatory cells, sections of ear skin 24 h after challenge were examined under high power field. The infiltrated inflammatory cells were mainly mononuclear leukocytes, and significant migration of neutrophils and eosinophils was also observed (Fig. 3A). As shown in Fig. 3B, treatment of mice with SB202190 markedly reduced the number of infiltrated mononuclear cells, neutrophils, and eosinophils induced by DNFB in the challenged ear skin (p < 0.01).

Effect of SB202190 on Cytokine Expression in Challenged Ear Skin—The effect of SB202190 on DNFB-induced cytokine mRNA profiles in ear skin was elucidated. As shown in Fig. 4, mRNA of all of cytokines was rarely detected in the control ear skin from both SB202190-untreated and treated mice. Induction of IL-2, IFN-γ, IL-5, IL-1β, IL-18, and IL-18 mRNA in ear skin challenged with DNFB was observed 12 and 24 h after challenge. Induction of IL-4 and TNF-α mRNA in ear skin challenged with DNFB was also observed 24 h after challenge. In SB202190-treated mice, the induction of cytokine mRNA of IL-2, IFN-γ, IL-5, IL-1β, IL-18, and TNF-α in the challenged ear skin was markedly suppressed compared with that in SB202190-untreated mice. On the contrary, DNFB challenge-induced IL-4 mRNA induction was not affected by SB202190 treatment.

Ear Swelling, Expression of Cytokines, and Infiltration of Cells in p38α +/- Mice—To elucidate how DNFB-induced CHS progresses in p38α +/- mice, we investigated ear swelling, expression of cytokines, and infiltration of inflammatory cells in DNFB-challenged ear skin of p38α +/- mice. In p38α +/- mice, DNFB-induced ear swelling was significantly reduced 24 h after challenge with DNFB compared with Wt mice (Fig. 5A). As shown in Fig. 5B, the mRNA levels of IL-2, IFN-γ, IL-4, IL-5, IL-1β, IL-18, and TNF-α were increased in the ear skin of p38α +/- mice 24 h after challenge with DNFB. The induction of IFN-γ, IL-5, and IL-18 mRNA by DNFB was suppressed in p38α +/- mice compared with Wt mice. On the other hand, the induction of IL-2, IL-4, IL-1β, and TNF-α mRNA by DNFB was similar in p38α +/- and Wt mice. The number of infiltrating mononuclear cells, neutrophils, and eosinophils in the challenged ear skin was calculated in p38α +/- mice and Wt mice with or without SB202190 treatment. As shown in Fig. 5C, the number of infiltrated mononuclear cells, neutrophils, and eosinophils induced by DNFB in the challenged ear skin of p38α +/- mice was significantly reduced compared with that in Wt mice and was similar to that in SB202190-treated mice (p < 0.01).

Chemokine Expression in Challenged Ear Skin—To elucidate whether the expression of chemokines was affected by DNFB, an RNase protection assay for a series of chemokines was performed with total RNA samples from Wt, SB202190-treated Wt, and p38α +/- mice. As shown in Fig. 6, low expression of MIP-1β and MIP-1α mRNA and high expression of MIP-2 mRNA were observed in control ear skin of Wt mice. Challenge with DNFB markedly increased the expression of IP-10 and MCP-1 mRNA. This typical expression of IP-10 and MCP-1 mRNA induced by DNFB was significantly inhibited by treat-
The expression of MIP-1α, MIP-1β, and MIP-2 mRNA observed in control ear skin of Wt mice with or without SB202190 treatment was not detected in control ear skin of p38α/−/− mice, although expression of internal controls, L32 and GAPDH mRNA, was clearly demonstrated. Challenge with DNFB induced IP-10, MCP-1, and MIP-2 mRNA in ear skin of p38α/−/− mice with very weak signals.

Expression of Cytokine mRNA in Infiltrated CD4+ Cells—The profiles of cytokines mRNA in CD4+ cells infiltrated into ear skin of Wt, SB202190-treated Wt, and p38α/−/− mice were elucidated 24 h after DNFB challenge. As shown in Fig. 7A, mRNA of cytokines such as Th1-like cytokines, IFN-γ and IL-2, and Th2-like cytokines, IL-4 and IL-5, was clearly detected in the infiltrated CD4+ cells from Wt mice. The expression of IL-2, IL-4, and IL-5 mRNA in infiltrated CD4+ cells from SB202190-treated mice was also observed and similar to that from Wt mice, although the expression of IFN-γ mRNA was significantly decreased by SB202190 treatment. In p38α/−/− mice, the expression of IFN-γ and IL-5 mRNA in infiltrated CD4+ skin of p38α/−/− mice, although expression of internal controls, L32 and GAPDH mRNA, was clearly demonstrated.

Challenge with DNFB induced ear swelling response in p38α/−/− mice (closed bars) and Wt littermates (open bars). Data presented are means ± S.D. of 8–10 mice. *, significantly different from value for Wt with p < 0.01 (Student’s t test for unpaired values).

Fig. 3. Effect of SB202190 on histopathological findings of 24 h CHS ear skin reaction induced by DNFB challenge. A, marked infiltration of mononuclear cells (•), neutrophils (†), and eosinophils (#) was observed in the dermis of DNFB-challenged ear skin in SB202190-untreated mice (panel a) but was not typically observed in SB202190-treated mice (panel b). Bar represents 50 μm. B, effect of SB202190 on cellular distribution of challenged skin. Bars represent number of mononuclear cells, neutrophils, and eosinophils that infiltrated the challenged ear skin of SB202190-untreated mice (open bars) and SB202190-treated mice (closed bars). Data presented are means ± S.D. of 12 mice. *, significantly different from SB202190-untreated values with p < 0.01 (Student’s t test for unpaired values).

Fig. 4. Effect of SB202190 on expression of cytokine mRNA in DNFB-challenged ear skin. Total RNA was prepared from the ear of control mice and challenged ear with or without SB202190 treatment at various times (12 and 24 h) after challenge. RT-PCR was performed under the experimental conditions defined in Table I. PCR product samples were subjected to 1.2% agarose gel electrophoresis and visualized by staining with ethidium bromide. Left and right panels represent the data in SB202190-untreated mice and SB202190-treated mice, respectively. Similar results were confirmed in three independent experiments. Lane C, control.
cells was typically suppressed compared with that in Wt mice. On the other hand, the expression of IL-2 and IL-4 mRNA was similar in p38α +/- and Wt mice (Fig. 7B).

Adaptive Transfer—To elucidate whether the reduction of CHS response to DNFB in p38α +/- mice takes place at the sensitization or elicitation phase, adoptive transfer experiments were performed. Ear swelling was measured 24 h after the challenge. As shown in Fig. 8, DNFB induced ear swelling in Wt mice after the injection of lymph node cells (LNC) from the sensitized Wt mice. Also in case of injecting LNC from the sensitized p38α +/- mice, ear swelling was induced even though LNC from the sensitized Wt mice were injected into the ears of Wt mice, DNFB induced ear swelling. Even though LNC from the sensitized Wt or p38α +/- mice were injected into ears of Wt mice, application of DNFB-free A/O solution alone on the ears did not induce ear swelling. Without the injection of LNC, DNFB induced no ear swelling in naive Wt mice.

DISCUSSION

The present study was designed to elucidate whether p38 is predominantly involved in the progression of CHS as a model of inflammatory disease and whether topical application of a p38 inhibitor has therapeutic utility in CHS. Here we showed that topical treatment with SB202190 significantly reduced both ear swelling and the histopathological findings induced by DNFB, indicating that p38 plays a critical role in CHS (Figs. 1–3).

The CHS model employed in the present study is based on chronic delayed type hypersensitivity (type IV hypersensitivity), because ear swelling and infiltration became worse and showed a maximum response 24 h after challenge. In the same CHS model, it has been demonstrated previously (23) that expression of a Th1-like cytokine, IFN-γ, in the skin lesion is essential for the formation of CHS by analysis using an anti-IFN-γ antibody that neutralizes the bioactivity of IFN-γ. The Th1-like cytokine, IFN-γ, plays an important role in cell-mediated immunity and chronic inflammation (24). Thus, the effect of SB202190 on IFN-γ expression in the skin lesion is primarily important for understanding the mechanism of SB202190-suppressed CHS. As expected, DNFB-induced IFN-γ expression was significantly inhibited by treatment of sensitized mice with SB202190 (Fig. 4). The inhibitory effect of SB202190 on IFN-γ mRNA expression in the present study is consistent with the previous in vitro finding that expression of IFN-γ by Th1 effector cells is mediated by p38 (14). IL-12, which was identified as an inducer of Th1-specific immune response, is known to promote IFN-γ production, and the p40 subunit is especially induced in CHS (25, 26). Likewise, neutralization of IL-12 prevents DNFB-induced CHS (27). Furthermore, the p38-signaling pathway is crucial for IL-12 production (15). However, IL-12 p40 mRNA was not detectable in control or challenged ear skin by RT-PCR with 40 cycles of amplification, although the primers used could detect IL-12 p40 mRNA in other tissues (data not shown). This might be explained by the previous investigation that IL-12 within the epidermis was detectable in hu-
man skin but not murine skin (27). Then, we investigated the expression of IL-18, another strong cofactor for Th1 cell development, in DNFB-challenged ear skin, because functional expression of IL-18 by murine keratinocytes has been reported (28). The expression of IL-18 mRNA was increased in DNFB-challenged ear skin and was significantly inhibited by SB202190 (Fig. 4). As IL-18 was initially identified as an IFN-γ inducing factor and stimulated IFN-γ production in a p38 signal-dependent manner (29, 30), the suppression of IL-18 production by SB202190 in the ear skin lesion may synergistically cause a decrease in expression of IFN-γ mRNA. On the other hand, Th1-like cytokine IL-2 also plays an important role in cell-mediated immunity and chronic inflammation through T cell growth and activation (24). The up-regulation of IL-2 mRNA in the DNFB-challenged ear skin was significantly inhibited by SB202190 (Fig. 4). This result is supported by the previous in vitro studies showing that p38 regulates the IL-2 gene (31).

In addition to Th1-like cytokines, recent studies demonstrated that Th2-like cytokines IL-4 and IL-5 in the skin lesion promptly contribute to inflammation not only in atopic dermatitis but also in DNFB-induced CHS (32–34). The expression levels of Th2-like cytokines IL-4 and IL-5 mRNA were also increased in DNFB-challenged ear skin in the present study. SB202190 fully inhibited DNFB-induced IL-5 mRNA expression but hardly affected DNFB-induced IL-4 mRNA (Fig. 4). IL-5 plays an important role in eosinophil development and differentiation, and the overexpression of IL-5 exacerbates DNFB-induced dermatitis in conjunction with extensive infiltration of eosinophils (34). Furthermore, IL-5 synthesis was selectively suppressed by a p38 inhibitor in human T cells (16), and a p38 inhibitor, SB239063, significantly reduced eosinophil infiltration in an airway inflammatory model (35). Thus, in our CHS model, p38 may also contribute to eosinophil infiltration through regulation of IL-5 production. On the other hand, our finding that SB202190 hardly affected DNFB-induced IL-4 mRNA in vivo was consistent with the in vitro finding that a p38 inhibitor, SB203580, had no inhibitory effect on IL-4 production by Th2 CD4+ T cells (14).

Local release of IL-1β and TNF-α is also critical for the optimal development of CHS (36, 37). SB202190 was first characterized as a potent inhibitor of IL-1β and TNF-α through translational depression (4). Thus, as expected, the expression levels of IL-1β and TNF-α mRNA induced in DNFB-challenged ear skin were significantly inhibited by treatment with SB202190 (Fig. 4). Taken together with the inhibitory effect of SB202190 on expression of other cytokines such as Th1 and Th2-like cytokines, p38 participates in the development of CHS at least by mediating cytokine expression.

It was reported that a high dose of SB202190 inhibits not only p38 but also JNK (38, 39). Moreover, JNK and p38 have a common upstream prerequisite, MEKK, as a MAPK kinase kinase, and are often activated in cells simultaneously (39). These findings suggest the possibility that SB202190 affects the expression of cytokines by inhibiting JNK activation. In order to rule out the inhibitory effect of SB202190 on JNK, we investigated JNK activation in DNFB-challenged ear skin of mice with or without SB202190 treatment. The time-dependent activation of JNK in DNFB-challenged ear skin was observed but not inhibited by SB202190 (data not shown).

In inflammatory cell lineages, differential expression and activation of the p38 MAPK family occur, and p38α is suggested to play a major role in the inflammatory response (40). This finding tempts us to consider the possibility that a decrease in p38α intrinsic activity affects generation of CHS. To address this possibility, we used p38α+/− mice in the present study, because targeted disruption of the p38α gene results in homozygous embryonic lethality (10, 11). In p38α+/− mice, DNFB-induced ear swelling was markedly suppressed compared with that in WT mice (Fig. 5A). On the contrary, the suppression profile of each cytokine expression in p38α+/− mice was not typical like that in SB202190-treated mice, except for the case of IFN-γ, IL-5, and IL-18 (Figs. 4 and 5B). The typical suppression of IFN-γ, IL-5, and IL-18 expression observed also in p38α+/− mice suggests that p38α among the p38 MAPK family is crucially involved in regulating IFN-γ, IL-5, and IL-18 expression. At the same time, however, the question arises why the cutaneous inflammatory reaction was markedly suppressed in DNFB-challenged ear skin of p38α+/− mice despite the smaller reduction of cytokine expression compared with that in SB202190-treated mice. Chemokines...
are generally known to mediate a selective inflammatory process in response to different stimuli (41). Indeed, the number of infiltrated mononuclear cells, neutrophils, and eosinophils induced by DNFB was markedly reduced in the challenged ear skin of p38α +/- mice compared with Wt mice, which was similar to the reduced number in SB202190-treated mice (Fig. 5C). We next investigated the expression of a series of chemokines in DNFB-challenged ear skin of p38α +/- mice and Wt mice with or without SB202190 treatment. Among the chemokines investigated in the present study, the expression levels of IP-10 and MCP-1 mRNA were selectively up-regulated to a marked extent in the DNFB-challenged ear skin, which was sensitive to treatment with SB202190. Rather surprisingly, in p38α +/- mice, the resting expression signals of chemokines observed in control ear skin of Wt mice with or without SB202190 treatment were not detected. Moreover, the DNFB-induced up-regulation of IP-10 and MCP-1 mRNA in ear skin of p38α +/- mice was much less marked than that in SB202190-treated mice (Fig. 6). The selective up-regulation of IP-10 and MCP-1 by DNFB and their suppression in SB202190-treated and p38α +/- mice suggest that at least IP-10 and MCP-1 may be involved in our CHS model. It has been suggested that eotaxin, one of the cysteine-cysteine chemokines, works along with IL-5 to elicit eosinophil infiltration (41). Although infiltration of eosinophils was observed in the present study, induction of eotaxin by DNFB was not detected under our experimental conditions. IP-10 was identified as the product of an IFN-γ-inducible gene and was expressed in a variety of cell types including mononuclear cells, keratinocytes, fibroblasts, endothelial cells, and T lymphocytes (42). Involvement of IP-10 in the regulation of leukocyte infiltration was clearly demonstrated in a graft model using IP-10-deficient mice (43). Also in the CHS model, regulation and participation of IP-10 during the elicitation phase were suggested (44). On the other hand, MCP-1 is a potent chemoattractant for monocytes, memory T lymphocytes, and natural killer cells (41). Neutralization of MCP-1 results in inhibition of T cell recruitment and inflammation in cutaneous delayed type hypersensitivity (45). Chemokines play an important role in the generation of inflammation in concert with cytokines by affecting their expression (32, 43, 46, 47). Thus, the marked decrease in expression of IP-10 and MCP-1 mRNA despite the modest suppression of cytokine expression may contribute to the suppression of the CHS response in p38α +/- mice.

Previous studies (47-49) demonstrated that p38 is involved in the expression of chemokines. Our findings that not only resting expression levels of chemokines but also DNFB-induced expression levels of IP-10 and MCP-1 were low in p38α +/- mice suggest that p38α among the p38 MAPK family is critically involved in chemokine expression. We confirmed that the resting expression of chemokines observed in lung tissue of Wt mice was markedly reduced in p38α +/- mice.7 Thus, this phenomenon is not restricted to ear skin. Considering the results of DNFB-induced chemokine expression in the ear skin of p38α +/- mice, high activation of p38α is probably needed for induction of chemokines, and this step may be exclusively affected by even a 50% decrease in p38α expression. In case of SB202190-treated mice, activities of both the p38α and p38β isoforms were possibly inhibited by SB202190 (7). Nevertheless, the inducible expression of chemokines was observed, with stronger signals than those in p38α +/- mice. Regarding this point, further detailed study is needed.

The present study demonstrated that p38 is possibly involved in DNFB-induced CHS by regulating the local cytokine and chemokine networks. However, whether the reduction of the expression of cytokines and chemokines in the CHS sites of SB202190-treated Wt and p38α +/- mice was simply dependent on the decrease in these infiltrating immune cells is an important issue. To elucidate this point, we isolated the infiltrated CD4+ cells from the CHS sites of Wt, SB202190-treated Wt, and p38α +/- mice, and we then investigated the expression levels of Th1- and Th2-like cytokines mRNA in the cells because the infiltrated CD4+ cells are considered to be crucial for the formation of CHS in the present model (20, 23). In SB202190-treated mice, the expression levels of IL-2, IL-4, and IL-5 mRNA were similar to that in Wt mice. The expression of IFN-γ mRNA was reduced by SB202190 treatment with statistical significance but was not typical. On the contrary, the expression levels of IFN-γ and IL-5 mRNA were typically suppressed in p38α +/- mice, compared with those in Wt mice (Fig. 7, A and B). These results suggest that infiltrated CD4+ cells exhibit the typical defect in expression of cytokines such as IFN-γ and IL-5 in p38α +/- mice but not SB202190-treated mice. Considering that the expression profiles of Th1- and Th2-like cytokine mRNA in DNFB-challenged ear skin of p38α +/- mice was similar to those in the infiltrated CD4+ cells from p38α +/- mice, the decrease in intrinsic activity of p38α in infiltrating immune cells may lead the reduction of the expression of cytokines and chemokines in the CHS site of p38α +/- mice. On the other hand, in SB202190-treated mice, the decrease in infiltrating cells at least partly accounts for the reduction of the expression of cytokines and chemokines in the CHS. However, the suppression profile of each cytokine expression in the CHS site of SB202190-treated mice was typical compared with that of p38α +/- mice. Thus, in addition to the reduction in infiltrating cells, SB202190 treatment may directly affect the expression of cytokines in ECs. The impaired expression of cytokines such as IFN-γ and IL-5 observed in p38α +/- mice raises the question whether the reduction of CHS in p38α +/- mice was controlled at the sensitization phase or not. At least in case of SB202190-treated mice, SB202190 was applied on the sensitized Wt mice 30 min prior to challenge, indicating that the reduction of CHS in SB202190-treated mice was controlled at the elicitation phase. The adoptive transfer experiment clearly showed that LNC from p38α +/- mice potentially induced CHS in Wt mice like LNC from Wt mice did, suggesting that there is no impairment at the sensitization phase of p38α +/- mice (Fig. 8).

In conclusion, the present study demonstrated that p38 is possibly involved in DNFB-induced CHS at the elicitation phase by regulating the local cytokine and chemokine networks, and that topical application of a p38 inhibitor is useful for the treatment of a certain type of hapten-induced CHS.

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