p38 Mitogen-activated Protein Kinase Regulates Interleukin-4-induced Gene Expression by Stimulating STAT6-mediated Transcription*

Received for publication, February 12, 2002, and in revised form, July 31, 2002
Published, JBC Papers in Press, August 2, 2002, DOI 10.1074/jbc.M201427200

Marko Pesu‡§, Saara Aittomäki‡, Kati Takaluoma‡, Anssi Lagerstedt‡, and Olli Silvennoinen‡§

From the ‡Institute of Medical Technology, University of Tampere, FIN-33014 Tampere, Finland, the §Department of Clinical Microbiology, Tampere University Hospital, FIN-33521 Tampere, Finland, and the ¶Department of Pathology, University of Tampere, FIN-33014 Tampere, Finland

STAT6 functions as a critical mediator of IL-4-stimulated gene activation, and the function of STAT6 is regulated by both tyrosine and serine kinase activities. Here we analyzed the role of serine phosphorylation in regulation of STAT6-mediated transcription. Optimal transcriptional response of IL-4-inducible promoters requires costimulatory signals through CD40-stimulated intracellular kinases such as p38 MAPK. We found that the p38 MAPK inhibitor SB202190 as well as the dominant negative p38 MAPK inhibited interleukin (IL)-4 regulated expression of CD23 in Ramos B cells. IL-4 stimulation did not stimulate p38 MAPK activity, but inhibition of p38 MAPK activity directly correlated with inhibition of IL-4-induced gene activation. Dissection of individual response elements on IL-4-regulated promoters showed that C/EBPβ-mediated transcription was insensitive to SB202190 treatment in B cells whereas STAT6-mediated transcription was regulated by p38 MAPK. The IL-4-induced immediate activation events of STAT6 were not affected by p38 MAPK activity. Furthermore, phosphoamino acid analysis and phosphopeptide mapping indicated that STAT6 is not a direct substrate for p38 MAPK. Instead, p38 MAPK was found to directly regulate the activity of the transactivation domain of STAT6. These results show that, in addition to the well established proinflammatory effects, p38 MAPK also provides a costimulatory signal for IL-4-induced gene responses by directly stimulating the transcriptional activation of STAT6.

Interleukin-4 (IL-4)1 is a pleiotropic cytokine, which has an important function in regulation of Th2 cells and B cells during humoral immune responses. Optimal activation of B cells is dependent on two Th2-mediated stimuli, IL-4 and a second signal provided by CD40 ligand (CD40L) (1). IL-4, together with CD40 ligand signals, stimulates proliferation of B cells, and induces expression of the low-affinity Fc receptor for IgE (CD23), major histocompatibility complex class II molecules, and stimulates transcription of unarranged immunoglobulin heavy-chain germline Igα and Igγ1 genes leading to class switching and IgE and IgG1 synthesis (2, 3).

The functional interactions of IL-4 are mediated through the IL-4 receptor (IL-4R) complex that activates the Jak1 and Jak3 tyrosine kinases leading to intracellular signal transduction (2). STAT6 functions as a crucial mediator of IL-4-specific gene responses, as attested by the similar phenotypes of the STAT6- and IL-4 knock-out mice presenting deficient Th2 differentiation and absence of IgE responses (4, 5). Recruitment of STAT6 to the tyrosine-phosphorylated IL-4 receptor α-chain initiates the activation cascade, and in the receptor complex STAT6 becomes phosphorylated on its C-terminal tyrosine residue by the Jak kinases. Tyrosine phosphorylation causes STAT6 to dimerize and translocate to the nucleus where it binds to specific DNA elements on IL-4-responsive genes (2).

The promoter regions of IL-4 inducible genes contain several cis-acting elements and the cooperation between different transcription factors is critical for efficient regulation of gene transcription. For example, the promoters of human CD23 and Igε genes contain in addition to STAT6-binding sites, also response elements for CCAAT/enhancer-binding protein β (C/EBPβ) NF-κB, interferon regulatory factor-4, and B cell-specific activator protein (Pax5) (6, 7). STAT6 appears to be the only transcription factor that is functionally regulated by IL-4, whereas the other factors are either regulated by costimulatory signals as exemplified by CD40-mediated regulation of NF-κB or through transcriptional regulation (8, 9). STAT6 has been shown to cooperate with several transcription factors, but the mechanisms of cooperation between various transcription factors differ significantly. For example, C/EBPβ stabilizes the DNA binding of STAT6, whereas the effects of interferon regulatory factor-4 and NF-κB are directed to transcriptional activation and involve physical interaction with STAT6 (7, 10, 11).

STAT-mediated gene responses are modulated by additional signaling pathways, which provide important diversity and additional regulation to the biological effects of cytokines. It has become evident that STATs are subject to post-translational modification through serine phosphorylation. STAT1, STAT3, STAT4, STAT5, and STAT6 have been shown to be phosphorylated on serine residues in their transactivation domain (TAD) in response to various stimuli (12–14). The functional consequence of the serine phosphorylation modification...
is best characterized in STAT1 where phosphorylation of Ser-727 regulates the interaction with BRCa1 and mini-chromosome maintenance 5 coactivator and affects the specificity of target gene expression (15–17).

The serine-phosphorylation sites of STAT1, STAT3, and STAT4 contain a conserved PMSP or PSP motif that confers the consensus mitogen-activated protein (MAP) kinase phosphorylation site, and thereby the role of MAP family kinases in regulation of STAT phosphorylation has been extensively studied. Extracellular signal-regulated kinases (ERK) are mediating the serine phosphorylation of STAT3 upon epidermal growth factor stimulation but ERKs are not involved in cytokine-induced phosphorylation of STATs (18). Recently, much attention has been focused on the role of p38 MAPK in the regulation of STATs. p38 MAPK has been shown to mediate the lipopolysaccharide and cellular stress-induced phosphorylation of Ser-727 of STAT1, and the IL-12-induced phosphorylation of Ser-727 in STAT1 (21, 22). The IL-6-induced transcriptional activation of STAT3 has also been shown to be dependent on IL-6-stimulated p38 MAPK activity in hepatocytes (23).

We have recently shown that STAT6-mediated gene activation is regulated through as yet unidentified serine kinase(s) (13). Engagement of CD40 surface antigen leads to rapid activation of several tyrosine and serine/threonine kinases (24–26), and provides costimulatory signals for STAT6-mediated transcription. p38 MAPK is readily activated through CD40, and thereby we wanted to analyze the role of p38 MAPK in IL-4-induced transcription. Our results indicate a direct role for p38 MAPK in regulation of STAT6-mediated transcriptional activation.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Transfections**—All the cell lines were obtained from American Type Culture Collection, Manassas, VA. HepG2 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and antibiotics. Daudi and Ramos 2g6 B cells were grown in RPMI medium containing 10% fetal bovine serum and antibiotics. Transfections of HepG2 cells were done by the calcium phosphate coprecipitation method. Daudi cells were electroporated with a Bio-Rad gene pulser at 220 V/960 μF. For some experiments Daudi cells were also transfected with DEAE-dextran as previously described (27). Ramos cells were transfected with 40 μg of plasmid DNA by electroporation at 200 V/960 μF (28).

**Antibodies**—α-Phosphotyrosine antibody (clone 4G10) was from Upstate Biotechnology (Lake Placid, NY) and mouse monoclonal antibody against human CD40 was from Immunotech (Marseille, France). α-STAT6 (M-20), α-p38 (N-20), and α-CREBβ (C-19) antibodies were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). α-Phospho-p38 antibody was from New England Biolabs Inc. (Beverly, MA).

**Immunoprecipitation and Western Blotting**—Cells were lysed in Triton lysis buffer (50 mm Tris-HCl, pH 7.5, 10% glycerol, 150 mm NaCl, 1 mm EDTA, 1% Triton X-100, 50 mm NaF, 1 mm Na3VO4), supplemented with phenylmethylsulfonyl fluoride and aprotonin, and immunoprecipitations from equal protein amounts were carried out as previously described (29). Protein concentrations of the lysates were measured using the Bio-Rad protein assay system. Immunoprecipitates were separated by SDS-PAGE and transferred onto nitrocellulose membrane. Proteins were visualized by autoradiography.

**Mobility Shift Assay**—Daudi cells were suspended in RPMI + 10% fetal bovine serum and stained for 30 min at 4°C with 20 μl of fluorescein isothiocyanate-conjugated mouse α-human-CD23 antibody (Pharmingen, San Diego, CA) or with 20 μl of PE-conjugated mouse α-human-CD23 antibody (BD Biosciences). Unspecific staining was monitored with isotype-matched control antibodies, fluorescein isothiocyanate-conjugated mouse α-human-CD64 antibody (Immunotech, Marseille, France) and PE-conjugated mouse α-human-CD13 antibody (BD Biosciences). Cells were washed twice with phosphate-buffered saline and analyzed with FACSScan (BD Biosciences).

**RESULTS**

**p38 Mitogen-activated Protein Kinase Activity Is Required for Stimulation of CD23 Expression in Ramos B Cells**—We have previously shown that STAT6-mediated transcription is regulated by Ser/Thr kinases (13). CD40 cell surface molecules mediate important costimulatory signals for IL-4-induced gene responses (33, 34), and activate protein kinases including p38 MAPK (24–26). These findings together with recent results...
about the role of p38 MAPK in regulation of cytokine signaling led us to investigate the role of p38 MAPK in IL-4-induced gene activation. We first tested the effect of p38 MAPK inhibitors on IL-4/CD40-induced expression of CD23 on highly IL-4-responsive Ramos 2g6 B cells. Cell-permeable pyridinyl imidazole compounds SB202190 and SB203580 are specific inhibitors of isoforms of p38 MAPK and they do not inhibit kinase activity of other mitogen-activated protein kinases such as ERKs and c-Jun NH2-terminal kinases (35).

Ramos B cells were stimulated for 20 h with IL-4 in the presence or absence of different concentrations of SB202190, and the surface expression of CD23 was measured using FACScan. IL-4 stimulation resulted in robust induction of CD23 expression (Table I, A). αCD40 treatment alone did not increase CD23 expression, but further enhanced the IL-4-induced CD23 expression. B cells express low levels of CD23 without any apparent stimulation, and SB202190 inhibited both the basal and the induced expression of CD23 in a dose-dependent manner. Inhibition was evident already at 1 μM concentration of SB202190. The inhibitor did not affect the viability of Ramos B cells at any concentration used.

The role of p38 MAPK in regulation of CD23 expression was further investigated by using the dominant negative form of p38 MAPKs. Ramos B cells were transfected with the dominant negative p38 MAPKα (p38AF) or empty vector (pSG5), and the CD23 expression was analyzed by FACScan using PE-conjugated α-CD23 antibody. Despite the low transfection efficiency in Ramos cells, the ectopic expression of p38AF resulted in 25–35% inhibition of CD23 expression in three independent experiments (Table I, B).

These results suggested that p38 MAPK activity is regulating CD23 expression. However, the enhancing effect of CD40 ligation on IL-4-induced CD23 expression was rather modest, and next we analyzed the effect of IL-4 stimulation and CD40 engagement on p38 MAPK activation in B cells directly. Ramos 2g6 and Daudi B cells were pretreated with SB202190 or with vehicle before IL-4 and/or α-CD40 were added to the cultures for different times. Cell lysates were subjected to Western blotting with α-phospho-p38 MAPK antibody, which is specific for the Thr180/Tyr182-phosphorylated catalytically active form of p38 MAPK. Constitutive phosphorylation of p38 MAPK was readily detected in both Daudi and Ramos cells (Fig. 1). IL-4 stimulation (5 min to 24 h) did not activate p38 MAPK in any of the cell lines tested (Daudi, Ramos, HepG2, HeLa, and 293T) (Fig. 1, and data not shown). In Daudi cells cross-linking of CD40 resulted in rapid induction and prolonged activation (at least up to 6 h, Fig. 1B) of p38 MAPK, whereas in Ramos cells CD40 engagement only slightly increased (10–20%) p38 MAPK activity (Fig. 1B). SB202190 pretreatment markedly diminished the basal phosphorylation of p38 MAPK as well as the CD40-induced phosphorylation in both cell lines. Thus, in Ramos cells, CD23 expression directly correlated with the level of p38 MAPK activation (high basal activity, low α-CD40 induction).

**FIG. 1.** The effect of IL-4 and α-CD40 on phosphorylation of p38 MAPK in Ramos and Daudi B cells. Cells were serum-depleted overnight and left untreated or pretreated with SB202190 (20 μM) for 60 min. IL-4 (100 ng/ml) and α-CD40 antibody (1 μg/ml) were added to cultures for 5 or 15 min (A) or 6 h (B) as indicated. Cells were lysed in Triton X-lysis buffer and 20 μg of total cell lysate were separated on 10% SDS-PAGE. Proteins were transferred onto nitrocellulose filter, which was probed with α-phospho-p38 MAPK antibody. In the lower panels are shown the same filters probed with αp38 MAPK antibody.

### Table I

|                | SB202190 |       |       |
|----------------|----------|-------|-------|
|                | 1        | 5     | 10    |
| No stimulation | 29.4     | 17.5  | 10.4  | 9.8  |
| IL-4           | 189.4    | 68.5  | 14.9  | 12.0 |
| α-CD40         | 29.7     | 18.4  | 10.5  | 10.1 |
| IL-4 + α-CD40  | 211.0    | 75.0  | 16.7  | 11.7 |

**A** Ramos B cells were left untreated or treated with IL-4 (10 ng/mL) and/or α-CD40 antibody (1 mg/ml) for 20 h in the absence or presence of different concentrations of p38 MAPK inhibitor SB202190. (B) Ramos B cells were transfected by electroporation with the dominant negative p38 MAPKα (p38AF) or with empty vector (pSG5) and treated with IL-4 and α-CD40 antibody as in A. CD23 expression was analyzed by staining with fluorescein isothiocyanate-conjugated (A) or PE-conjugated (B) α-CD23 mAb. Fluorescence intensity was determined using FACScan, and the mean fluorescence intensities (MFI) are shown. MFI for isotype matched fluorescein isothiocyanate-conjugated negative control antibody was 5.6 and for PE-conjugated negative control antibody 4.0. Experiments were repeated three times with identical results.
IL-4 induced the activity of the C/EBPβ-STAT6-RE reporter in Daudi cells (Fig. 2A). The IL-4-induced activity was further stimulated via CD40 antigen, but CD40 signal alone did not induce any reporter activity. Pretreatment of Daudi cells with SB202190 inhibited both IL-4 and IL-4+α-CD40 enhanced reporter activities to the same extent. Similar inhibition was observed with another p38 MAPK inhibitor SB203580 (data not shown). Furthermore, the p38 MAPK inhibitor was found to decrease the IL-4-induced C/EBPβ-STAT6-RE reporter activity also in nonhematopoietic HepG2 cells in a dose-dependent manner (data not shown).

To exclude the possibility that SB202190 would inhibit the IL-4-stimulated transcription by an unspecific, p38 MAPK-independent way, we studied the effect of overexpression of both dominant negative and wild type p38 MAPKα (p38AF and p38wt, respectively) in Daudi cells (Fig. 2, B and C). Overexpression of p38 MAPKα resulted in activation of the kinase as detected by Western blotting with phospho-p38 MAPK-specific antibody and in autokinase assay (data not shown). As expected, the ectopic expression of p38AF reduced and p38wt enhanced both IL-4- and IL-4+α-CD40-induced gene activation in Daudi cells (Fig. 2, B and C). The inhibitory effect of SB202190 (10 μM) was observed also in the p38wt-transfected Daudi B cells (Fig. 2C).

In HepG2 cells the C/EBPβ-STAT6 region in the human Igκ promoter has been shown to be the minimal IL-4 response element (37). To analyze the individual roles of these transcription factors, reporter constructs consisting of only C/EBPβ-RE or STAT6-RE were utilized. C/EBPβ-RE reporter was found to be constitutively active in both Daudi (Fig. 3A) and HepG2 cells (data not shown). Overexpression of p38 MAPKα did not stimulate the activity of C/EBPβ-RE in HepG2 cells (data not shown), and in Daudi cells SB202190 treatment did not reduce the activity of the C/EBPβ-RE reporter construct (Fig. 3A). The activity of C/EBPβ is regulated by p38 MAPK-induced serine phosphorylation in mouse hepatocytes (38). We analyzed the effects of IL-4- and α-CD40 stimulation, in the presence or absence of SB202190, on the total phosphorylation of C/EBPβ in B cells. Endogenous C/EBPβ was constitutively phosphorylated in Daudi B cells but no changes on phosphorylation of C/EBPβ were observed in response to any of the treatments (data not shown).

The reporter construct that consists of binding sites only for STAT6 is not functional in HepG2 cells (37) (data not shown). However, in Daudi B cells the STAT6-RE functioned similarly as the C/EBPβ-STAT6-RE, and the STAT6-RE reporter was activated by IL-4 and the activity was further enhanced via CD40 (Figs. 2A and 3B). The IL-4 and IL-4+α-CD40-induced activities of STAT6-RE were sensitive to SB202190 treatment. In conclusion, these results suggest that the target for p38 MAPK activity is on STAT6-mediated transcription.

STAT6 Is Not Phosphorylated by p38 MAPK—To analyze the mechanism of p38 MAPK-mediated regulation of STAT6 activation in more detail, we studied the effect of SB202190 on tyrosine phosphorylation and DNA binding of STAT6 in Daudi B cells. IL-4 stimulation caused rapid tyrosine phosphorylation and DNA binding of STAT6 (Fig. 4, A and B), whereas CD40 engagement alone did not activate STAT6. Pretreatment of the cells with SB202190 had no effect on the immediate activation events of STAT6 for up to 2 h (Fig. 4, A and B, and data not shown).

Previously we showed that STAT6 is subject to both constitutive and IL-4-induced serine phosphorylation (13). To investigate the possible role of p38 MAPK on phosphorylation of STAT6, overnight starved Daudi B cells were metabolically labeled with [32P]orthophosphate for 3 h in the presence or
absence of SB202190. Cells were stimulated with IL-4 and/or α-CD40 for 20 min, and STAT6 was immunoprecipitated and separated on SDS-PAGE. Proteins were transferred onto nitrocellulose filter and visualized by autoradiography. IL-4 stimulation induced phosphorylation of STAT6, which was not affected by SB202190 treatment. α-CD40 treatment did not have any effect on total phosphorylation of STAT6 (Fig. 5A). The band corresponding to STAT6 was excised and phosphorylation events were further studied in phosphoamino acid analysis and phosphopeptide mapping.

In unstimulated cells STAT6 was phosphorylated at low levels only on serine residues and IL-4 induced phosphorylation on both tyrosine and serine residues (Fig. 5B). No phosphorylation on threonine residues was detected. CD40 engagement did not have any marked effect on phosphorylation of STAT6. Also, pretreatment of Daudi cells with SB202190 did not inhibit tyrosine or serine phosphorylation of STAT6.

To confirm that phosphorylation of STAT6 was not affected by p38 MAPK, part of the purified STAT6 protein was used for tryptic phosphopeptide mapping (Fig. 5B). Two weakly phosphorylated peptides were detected in nonstimulated cells. IL-4 stimulation enhanced the phosphorylation of these peptides and induced three additional phosphopeptides. α-CD40 treatment alone or in combination with IL-4 did not change the phosphorylation pattern of STAT6. However, in this particular experiment a very faint phosphopeptide was induced by α-CD40, which was identified by the hot-sequencing method and mutagenesis to correspond to the tyrosine 641 phosphopeptide (data not shown). Previously longer α-CD40 treatment (60 min) has been reported to induce the tyrosine phosphorylation of STAT6 (39). Notably, in our experiments neither CD23 expression on Ramos cells nor STAT6-dependent reporter activities were stimulated via CD40. In two subsequent experiments we were unable to reproduce the induction of Tyr641 phosphorylation by α-CD40 treatment. SB202190 treatment did not change the phosphopeptide pattern of STAT6, and there were no consistent changes in the relative intensities of individual phosphopeptides. Thus, our results indicate that p38 MAPK does not mediate the phosphorylation of STAT6.

p38 MAPK Regulates Directly the Transactivation Potential of STAT6 Transactivation Domain—The findings that phosphorylation of STAT6 was not regulated by p38 MAPK lead us to consider the possibility that p38 MAPK might affect the transactivation potential of STAT6. A fusion construct containing the yeast GAL4 DNA-binding domain and the TAD of STAT6 (GAL4-STAT6TAD) was used to directly test this possibility. The GAL4-STAT6TAD fusion construct has been shown to be constitutively active and to bind DNA independently of...
extracellular stimuli (30). GAL4-STAT6TAD and GAL4-binding sites containing reporter constructs were transfected into Daudi B cells together with the dominant negative form of p38 MAPK/H9251 (p38AF), wild type p38 MAPK/H9251 (p38wt), or empty pSG5 plasmid DNA (sg5). Co-transfection of p38AF and SB202190 (10^7 M) treatment diminished the reporter activity 50–80%, whereas expression of p38wt resulted in enhancement of gene activation (Fig. 6A). SB202190 treatment or expression of p38wt and p38AF constructs had no effect on the control GAL4 construct in B cells (data not shown).

Next we wanted to investigate whether the effects of p38 MAPKα are cell-type specific or whether it acts as an activator of STAT6TAD also in nonhematopoietic cells. Therefore, HepG2 cells were transfected with p38 MAPKα (p38AF), wild type p38 MAPKα (p38wt), or empty pSG5 plasmid DNA (sg5). Co-transfection of p38AF and SB202190 (10 µM) treatment diminished the reporter activity 50–80%, whereas expression of p38wt resulted in enhancement of gene activation (Fig. 6A). SB202190 treatment or expression of p38wt and p38AF constructs had no effect on the control GAL4 construct in B cells (data not shown).

Cytokine-induced gene responses are controlled by integration of signals from various signaling pathways on the promoter elements of target genes. Activation of STAT6 is dependent on tyrosine phosphorylation, but IL-4-dependent gene responses are modulated also through Ser/Thr kinase activity (2, 3, 13). In this study we provide new molecular insight into costimulatory signals for IL-4-induced gene responses, and demonstrate that STAT6-mediated transcription is directly regulated by p38 MAPK.

Since CD40 provides a costimulatory signal for IL-4 and promotes p38 MAPK activation, we sought to investigate the role of p38 MAPK on IL-4-induced gene responses. Our results demonstrate that p38 MAPK is regulating the STAT6-dependent gene responses. Somewhat surprisingly, inhibition of p38 MAPK, either by pharmacological inhibitors or by dominant negative p38 MAPK, inhibited both the basal as well as the IL-4/CD40-induced expression of CD23. IL-4 did not induce p38 MAPK activation in B cells, which is in accordance with previous studies performed in other hematopoietic cells (40). CD40 engagement resulted in rapid activation of p38 MAPK in Daudi cells that persisted for at least 6 h, and correlated directly with enhancement of IL-4-induced reporter gene activation. In Ramos 2g6 cells CD40 induced only modest activation of p38 MAPK, which is in accordance with the low level of α-CD40 enhancement on CD23 expression in IL-4-treated Ramos cells. However, in all cell types tested we observed basal activation of p38 MAPK, which was inhibited by SB202190 treatment. The highest activity was observed in Ramos 2g6 cells that were initially characterized by their high IL-4-induced CD23 expression.  

DISCUSSION

Cytokine-induced gene responses are controlled by integration of signals from various signaling pathways on the promoter elements of target genes. Activation of STAT6 is dependent on tyrosine phosphorylation, but IL-4-dependent gene responses are modulated also through Ser/Thr kinase activity (2, 3, 13). In this study we provide new molecular insight into costimulatory signals for IL-4-induced gene responses, and demonstrate that STAT6-mediated transcription is directly regulated by p38 MAPK.

Since CD40 provides a costimulatory signal for IL-4 and promotes p38 MAPK activation, we sought to investigate the role of p38 MAPK on IL-4-induced gene responses. Our results demonstrate that p38 MAPK is regulating the STAT6-dependent gene responses. Somewhat surprisingly, inhibition of p38 MAPK, either by pharmacological inhibitors or by dominant negative p38 MAPK, inhibited both the basal as well as the IL-4/CD40-induced expression of CD23. IL-4 did not induce p38 MAPK activation in B cells, which is in accordance with previous studies performed in other hematopoietic cells (40). CD40 engagement resulted in rapid activation of p38 MAPK in Daudi cells that persisted for at least 6 h, and correlated directly with enhancement of IL-4-induced reporter gene activation. In Ramos 2g6 cells CD40 induced only modest activation of p38 MAPK, which is in accordance with the low level of α-CD40 enhancement on CD23 expression in IL-4-treated Ramos cells. However, in all cell types tested we observed basal activation of p38 MAPK, which was inhibited by SB202190 treatment. The highest activity was observed in Ramos 2g6 cells that were initially characterized by their high IL-4-induced CD23 expres-
showed that C/EBPβ MAPK inhibitors inhibited these activities. Our results also indicated with 0.5–5.0 μg of GAL4-RE, CMV-β-galactosidase, GAL4-STAT6TAD, p38 MAPK (p38 wt, 4 μg) as indicated. pSG5 plasmid (sg5) was used to make DNA amounts equal. Two hours after transfection cells were left untreated (black bars) or treated with SB202190 (10 μM) (open bars) for 24 h as indicated. B, HepG2 cells were transfected as indicated with 0.5–3.0 μg of GAL4-RE, CMV-β-galactosidase, GAL4-STAT6TAD, p38 MAPKα (p38 wt), MKK6b(E), and pSG5 plasmids by using the calcium phosphate coprecipitation method. Cells were lysed 48 h after transfection. The luciferase activities were normalized against β-galactosidase values and pSG5-transfected cells were given an arbitrary value of 100, and the other values are shown in proportion to this as percentage of activity (%). The means of three independent experiments with standard deviations are shown.

FIG. 6. p38 MAPK regulates the TAD of STAT6. A, Daudi B cells were transfected by electroporation with GAL4-RE (8.0 μg), EBB-β-galactosidase (8.0 μg), dominant negative p38 MAPKα (p38AF, 12 μg), and wild type p38 MAPKα (p38 wt, 4 μg) as indicated. pSG5 plasmid (sg5) was used to make DNA amounts equal. Two hours after transfection cells were left untreated (black bars) or treated with SB202190 (10 μM) (open bars) for 24 h as indicated. B, HepG2 cells were transfected as indicated with 0.5–5.0 μg of GAL4-RE, CMV-β-galactosidase, GAL4-STAT6TAD, p38 MAPKα (p38 wt), MKK6b(E), and pSG5 plasmids by using the calcium phosphate coprecipitation method. Cells were lysed 48 h after transfection. The luciferase activities were normalized against β-galactosidase values and pSG5-transfected cells were given an arbitrary value of 100, and the other values are shown in proportion to this as percentage of activity (%). The means of three independent experiments with standard deviations are shown.

sition (41). Thus, our results suggest that the level of p38 MAPK activity is critically regulating CD23 expression.

We dissected the role of p38 MAPK on different response elements on IL-4-regulated promoter, and the STAT6-binding element was identified as a direct target for p38 MAPK activity. The STAT6-RE was activated by IL-4 stimulation and further stimulated in the presence of α-CD40 whereas p38 MAPK inhibitors inhibited these activities. Our results also showed that C/EBPβ activity was not regulated by p38 MAPK and that C/EBPβ did not appear to be a substrate for p38 MAPK kinase activity in B cells. These results also demonstrated that p38 MAPK is not a general regulator of transcription and the effect of p38 MAPK is STAT6-dependent. Interestingly, whereas C/EBPβ is required for STAT6-mediated transactivation in HepG2 cells, this factor is not required for STAT6 activity in B cells. This finding suggests that B cells express either a transcription factor or a coactivator that is critically required for STAT6-mediated transcription.

Several lines of evidence indicated that p38 MAPK is not directly regulating the phosphorylation of STAT6. The SB202190 inhibitor did not have any effect on tyrosine phosphorylation or DNA binding of STAT6. Phosphoamino acid and phosphopeptide analysis indicated that CD40 engagement or SB202190 treatment are not regulating the phosphorylation events of STAT6. In phosphoamino acid analysis or in phosphopeptide mapping we could not detect any repeatable changes in the phosphorylation pattern of STAT6 upon p38 MAPK activating (α-CD40) or inactivating (SB202190) signals. However, the methodology used for analysis of protein phosphorylation is not absolutely quantitative, and thus we cannot strictly exclude the existence of a minor p38 MAPK-regulated phosphorylation event in STAT6. Furthermore, the TAD of STAT6 does not contain the conserved MAPK Ser-phosphorylation motif found in STAT1, STAT3, and STAT4. Taken together these results strongly suggest that STAT6 is not a direct target for p38 MAPK kinase activity. The kinase responsible for STAT6 phosphorylation is currently unknown but the kinase is insensitive to H7, wortmannin, and SB202190 Ser/Thr kinase inhibitors (13, 14). IL-4-induced serine phosphorylation has been reported to occur in the C-terminal TAD (14) but the exact Ser residue(s) has not been identified. In STAT1 Ser277 phosphorylation regulates the interaction with transcriptional coregulators mini-chromosome maintenance 5 and BRCA1 (15, 17) and it is possible that the phosphorylation-mediated increase in negative net charge may promote protein interactions in STAT6TAD as well.

The reporter gene and phosphorylation studies suggested that the effect of p38 MAPK is directed to the transcriptional activity of STAT6. In accordance with this hypothesis, the STAT6TAD construct was directly stimulated by p38 MAPK activation and inhibited by dominant negative p38 MAPK or SB202190. These results would be consistent with a role for p38 MAPK-mediated phosphorylation in regulation of interaction between STAT6TAD and transcriptional coregulators. The effect of p38 MAPK on NF-κB bears some resemblance to our findings regarding regulation of STAT6 activity (42). p38 MAPK has been shown to regulate the transcriptional activity, but not the activation events of NF-κB, by inducing the phosphorylation of TATA-binding protein and thereby facilitating the interaction between NF-κB and TATA-binding protein. We also investigated the possible role of p38 MAPK in regulation of the TBP-STAT6 interaction, but p38 MAPK did not have any apparent effect on TATA-binding protein phosphorylation in B cells (data not shown). The IFN-regulated STAT1 signaling also shows analogy to our findings (19, 21, 22). IFNα and IFNγ induce serine phosphorylation of STAT1 independently of p38 MAPK. However, p38 MAPK activity is required for IFNα/STAT1-mediated transcription of the interferon γ-activated site and interferon-stimulated response element promoters. The exact molecular mechanism by which STAT6 is connected to the basal transcriptional machinery is currently poorly understood. Several STATs, including STAT6, have been shown to interact with the general coactivator p300/CBP, but currently there is no information that would indicate that this interaction would be regulated by serine phosphorylation (43). However, it is likely that other transcriptional coregulators for STAT6 will be identified and p38 MAPK could be involved in regulation of these functional interactions. Furthermore, it is possible that in addition to the effects on transcriptional activation of STAT6, p38 MAPK may also regulate transcription by modulation of histone phosphorylation by the downstream kinase MSK-1 (44).

p38 MAPK is considered to be a proinflammatory regulator that is activated via Th1 class cytokines as well as several stress-induced factors. Our results now demonstrate that p38 MAPK activity is also a direct regulator of Th2 class cytokine-mediated responses and this effect is targeted to STAT6-mediated transcription. In addition, our results provide new molecular insight into CD40-mediated costimulatory functions for IL-4-induced gene responses. Recently, several reports have demonstrated an essential role for p38 MAPK in eosinophilic
inflammation (45, 46). Taken into account the effects of p38 MAPK on both NF-κB, as well as on STAT6-mediated responses, modulators of p38 MAPK kinase activity may prove to be effective drugs for allergic diseases and asthma.

Acknowledgments—We thank Paula Kosonen and Dr. Anri Tienhaara for technical assistance, Drs. B. Groner, V. M. Kahari, J. Han, and T. Kallunki for kindly providing reagents.

REFERENCES

1. Noelle, R. J., Roy, M., Shepherd, D. M., Stamenkovic, I., Ledbetter, J. A., and Greten, F. T. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 4341–4345
2. Nelms, K., Keegan, A. D., Zamorano, J., Ryan, J. J., and Paul, W. E. (1999) J. Immunol. 162, 2763–2767
3. Warren, W. D., and Berton, M. T. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 701–705
4. Warzynski, W., Jin, Y., Jernigan, J., and Taub, J. B. (1998) J. Biol. Chem. 273, 1866–1872
5. Shimoda, K., van Deursen, J., Sangster, M. Y., Sarawar, S. R., Carson, R. T., Tripp, R. A., Chu, C., Quelle, F. W., Nosaka, T., Vignali, D. A., Doherty, P. C., Gravell, G., Paul, W. E., and Iglewski, B. H. (2000) Blood 95, 4070–4075
6. Richards, M. L., and Katz, D. H. (1997) J. Immunol. 158, 263–272
7. Gupta, S., Jiang, M., Anthony, A., and Pernis, A. B. (1999) J. Immunol. 162, 2638–2642
8. Rebreanu, E., Seidah, N. G., and Skett, G. A. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 3395–3399
9. Iciek, L. A., Delphin, S. A., and Stavnezer, J. (1997) J. Immunol. 158, 4769–4779
10. Shen, C. H., and Stavnezer, J. (1998) Mol. Cell. Biol. 18, 3395–3404
11. Mikita, T., Kurama, M., and Schindler, U. (1998) Oncogene 17, 3404–3412
12. Foltz, I. N., Lee, J. C., Young, P. R., and Schrader, J. W. (1997) Mol. Immunol. 33, 1091–1095
13. Zauberman, A., Zipori, D., Krupsky, M., and Ben-Levy, R. (1999) Oncogene 18, 3886–3893
14. Faris, M., Gaskin, F., Parsons, J. T., and Fu, S. M. (1994) J. Exp. Med. 179, 1923–1931
15. Karkkainen, S. H., and Geha, R. S. (1997) Immunity 6, 379–387
16. Sutherland, C. L., Heath, A. W., Pelech, S. L., Young, P. R., and Gold, M. R. (1996) J. Immunol. 157, 3381–3390
17. Berberich, I., Shu, G. L., and Clark, E. A. (1994) J. Immunol. 153, 4357–4366
18. Yamashita, Y., Watanabe, S., Miyazato, A., Ohya, K., Hida, U., Shimada, K., Komatsu, N., Hatake, K., Miyura, Y., Ozawa, K., and Mano, H. (1998) Blood 91, 1496–1507
19. Silvennoinen, O., ——, and Kunis, T. E. (2000) J. Biol. Chem. 275, 38793–38798
20. Uddin, S., Lekmine, F., Sharma, N., Majchrzak, B., Mayer, I., Young, P. R., Bokoch, G. M., Fish, E. N., and Plataniotis, L. C. (2000) J. Biol. Chem. 275, 27634–27640
21. Zauberman, A., Zipori, D., Krupsky, M., and Ben-Levy, R. (1999) Oncogene 18, 3886–3893
22. Faris, M., Gaskin, F., Parsons, J. T., and Fu, S. M. (1994) J. Exp. Med. 179, 1923–1931
23. Karkkainen, S. H., and Geha, R. S. (1997) Immunity 6, 379–387
24. Sutherland, C. L., Heath, A. W., Pelech, S. L., Young, P. R., and Gold, M. R. (1996) J. Immunol. 157, 3381–3390
25. Berberich, I., Shu, G. L., and Clark, E. A. (1994) J. Immunol. 153, 4357–4366
26. Yamashita, Y., Watanabe, S., Miyazato, A., Ohya, K., Hida, U., Shimada, K., Komatsu, N., Hatake, K., Miyura, Y., Ozawa, K., and Mano, H. (1998) Blood 91, 1496–1507
27. Saharinen, P., Ekman, N., Sarvas, K., Parker, P., Alitalo, K., and Silvennoinen, O. (1997) Blood 90, 4341–4353
28. Morrigil, R., Berchtold, S., Friedrich, K., Standke, G. J., Kammer, W., Heim, M., Wissler, M., Stocklin, E., Gouilleux, F., and Groner, B. (1997) Mol. Cell. Biol. 17, 3663–3678
29. Raisinga, J., Whitmarsh, A. J., Barrett, T., Derijard, B., and Davis, R. J. (1996) Mol. Cell. Biol. 16, 1247–1255
30. Boyle, W. J., van der Geer, P., and Hunter, T. (1991) Methods Enzymol. 201, 110–149
31. Jabara, H. H., Fu, S. M., Geha, R. S., and Vercelli, D. (1990) J. Exp. Med. 172, 1861–1864
32. Paterson, R. L., Lack, G., De Meis, J. M., Delepois, G., Leung, D. Y., Finkel, T. H., and Gelfand, E. W. (1996) Eur. J. Immunol. 26, 1979–1984
33. Davies, S. P., Reddy, H., Caivano, M., and Cohen, P. (2000) Biochem. J. 351, 95–105
34. Crezton, A., Shu, G., Graves, J. D., Saklatvala, J., Krebs, E. G., and Clark, E. A. (1998) J. Immunol. 161, 3225–3236
35. Mikita, T., Campbell, D., Wu, P., Williamson, K., and Schindler, U. (1996) Mol. Cell. Biol. 16, 5811–5820
36. Buck, M., Poli, V., van der Geer, P., Chapgker, M., and Hunter, T. (1999) Mol. Cell 4, 1087–1092
37. Karras, J. G., Wang, Z., Hsu, L., Frank, D. A., and Rothstein, T. L. (1997) J. Immunol. 159, 4350–4355
38. Foltz, I. N., Lee, J. C., Young, P. R., and Schrader, J. W. (1997) J. Biol. Chem. 272, 2936–3001
39. Siegel, J. P., and Mostowski, H. S. (1990) J. Immunol. Methods 132, 287–295
40. Carter, A. B., Knudtson, K. L., Monick, M. M., and Hunninghake, G. W. (1999) J. Biol. Chem. 274, 30858–30863
41. McDonald, C., and Reich, N. C. (1999) J. Interferon Cytokine Res. 19, 711–722
42. Thomason, S., Clayton, A. L., Hazzalin, C. A., Rose, S., Barratt, M. J., and Mahadevan, L. C. (1999) EMBO J. 18, 4779–4793
43. Underwood, D. C., Osborn, R. R., Kotzer, C. J., Adams, J. L., Lee, J. C., Webb, E. F., Carpenter, D. C., Bochnowicz, S., Thomas, H. C., Hay, D. W., and Gravell, D. E. (2000) J. Pharmacol. Exp. Ther. 293, 261–288
44. Birrell, M., Hele, D., McCluskie, K., Webber, S., Foster, M., and Belvisi, M. G. (2000) Eur. Respir. J. 16, 947–950
45. Tanaka, M., Gupta, R., and Mayer, B. J. (1995) Mol. Cell. Biol. 15, 6829–6837
p38 Mitogen-activated Protein Kinase Regulates Interleukin-4-induced Gene Expression by Stimulating STAT6-mediated Transcription

Marko Pesu, Saara Aittomäki, Kati Takaluoma, Anssi Lagerstedt and Olli Silvennoinen

J. Biol. Chem. 2002, 277:38254-38261.
doi: 10.1074/jbc.M201427200 originally published online August 2, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M201427200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 47 references, 34 of which can be accessed free at http://www.jbc.org/content/277/41/38254.full.html#ref-list-1