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Activation of 12-*O*-Tetradecanoylphorbol-13-acetate Response Element- and Dyad Symmetry Element-dependent Transcription by Interleukin-5 Is Mediated by Jun N-terminal Kinase/Stress-activated Protein Kinase Kinases*

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Interleukin-5 (IL-5) is one of the major regulators of eosinophilic granulocytes *in vivo*. IL-5 exerts its pleiotropic effects by binding to the IL-5 receptor, which is composed of an IL-5-specific α chain and a common βc chain shared with the receptors for IL-3 and granulocyte-macrophage colony-stimulating factor. Previous studies have shown that binding of IL-5 to its receptor triggers the activation of multiple signaling cascades, including the Ras/mitogen-activated protein kinase, the phosphatidyl -3′-kinase, and the Janus kinase/signal transducer and activator of transcription pathways. Here we describe that IL-5 activates the serine/threonine protein kinase Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK) pathway. We show that IL-5 activates TPA response element (TRE)-dependent transcription in transfection experiments. TRE activation by IL-5 is mediated by a region of the βc (577–581) that is also responsible for activation of JNK/SAPK and for activation of dyad symmetry element (DSE)-dependent transcription. Dominant-negative SAPK or ERK kinase-1 was used to demonstrate that JNK/SAPK activation is necessary for induction of DSE- and TRE-dependent transcription by IL-5, whereas extracellular signal-regulated kinase 2 was not essential for TRE- and DSE-dependent transcription. By contrast, IL-5-induced activation of the tyrosine kinase Janus kinase 2 seems to be a prerequisite for TRE- and DSE-dependent transcription. Taken together, we show for the first time that IL-5 activates kinases of the JNK/SAPK family, and that this activation is linked to IL-5-induced TRE- and DSE-dependent transcription.

Cytokines such as interleukin (IL)[1]-3, IL-5, and granulocyte-macrophage colony-stimulating factor (GM-CSF) play an important role in hematopoiesis (1–3). IL-3 and GM-CSF are broad specificity cytokines that have effects on multiple hematopoietic cell lineages (3, 4). By contrast, the actions of human IL-5 are restricted to the eosinophil and basophil lineages, because a functional IL-5 receptor (IL-5-R) is only expressed on these cell types (5, 6). IL-5 is essential for eosinophil differentiation (7, 8) and plays an important role in the functioning of mature eosinophils and basophils (9–13). The IL-5-R is a multimeric molecule composed of a unique α chain associated with a common βc chain, which is part of the receptors for IL-3 and GM-CSF (14). Because the βc chain is thought to play a major role in postreceptor signal transduction, it is not surprising that IL-3, IL-5, and GM-CSF exert multiple overlapping effects on cells that have all three receptor complexes (15).

Binding of IL-3, IL-5, or GM-CSF to their receptors leads to activation of multiple signal transduction pathways. Within seconds to minutes, multiple cellular proteins become phosphorylated on tyrosine residues, an event essential for most biological functions of these cytokines (12, 16). Because the α and βc chains of these receptors do not contain any enzymatic activity, cytoplasmic protein tyrosine kinases are likely to mediate this process. We and others have shown that IL-5 binding leads to rapid and transient activation of the Janus kinase JAK2 (17–19), one of the kinases involved in activation of STAT (signal transducer and activator of transcription) proteins (reviewed in Ref. 20). In addition, kinases such as Lyn, Syk, fyn, hck, and BTK are also activated by cytokines of this family (21–23). Activation of these signaling pathways is mediated by different functional domains in the βc chain. A membrane-proximal region containing box1, a motif found in multiple cytokine receptors, is necessary for JAK2 and STAT3 activation, induction of c-*myc* gene expression and cytokine-induced proliferation (23–27). A more distal region between amino acids 542 and 589 was shown to be involved in activation of the RAS/MAPK pathway mediated by Shc binding to the βc chain and for induction of transcription of the immediate-early genes c-*jun* and c-*fos* (24, 25, 28, 29) Moreover, this region was also implicated in activation of phosphatidylinositol 3′-kinase and p70S6 kinase by GM-CSF (25). Phosphorylation of tyrosine 577 of the βc chain, possibly by JAK2, was shown to be at least partially responsible for activation of MAPK and c-*fos,* although also other tyrosine residues are likely to play an important role (28, 29). In addition, the proline-rich region of the α chain was also shown to be involved in activation of different pathways, such as the JAK2/STAT pathway and cytokine-induced proliferation (27, 30, 31).

Immediate-early gene expression in response to a wide array of different extracellular stimuli is regulated by a limited

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[1] The abbreviations used are: IL, interleukin; GM-CSF, granulocyte-macrophage colony-stimulating factor; -R, receptor; JAK, janus kinase; STAT, signal transducer and activator of transcription; MAPK, mitogen-activated protein kinase; TRE, 12-*O*-tetradecanoylphorbol-13-acetate-response element; TNFα, tumor necrosis factor α; JNK, Jun N-terminal kinase; SAPK, stress-activated protein kinase; DSE, dyad symmetry element; SRE, serum response element; TCF, ternary complex factor; ERK, extracellular signal-regulated kinase; CAT, chloramphenicol acetyltransferase; GST, glutathione *S*-transferase; tk, thymidine kinase; AP-1, activator protein 1.

amount of *cis*-acting response elements in the promoters of the induced genes. Among these, the TPA-response element (TRE) binds transcription factors of the Jun/AP-1 family (reviewed in Refs. 32 and 33). This element mediates transcriptional activation by stimuli such as phorbol esters, UV light, tumor necrosis factor α (TNFα), IL-2, and stress induced by heat shock or protein synthesis inhibitors (33). Jun/AP-1 transcription factors are homo- or heterodimeric proteins that are activated by serine and threonine phosphorylation. The kinases that are responsible for these phosphorylations were described recently as a novel family of mitogen-activated protein kinases (MAPKs), the Jun N-terminal kinases (JNKs, also known as stress-activated protein kinases (SAPKs); reviewed in Refs. 34–36). The JNK/SAPKs themselves are activated by dual phosphorylation at conserved threonine and tyrosine residues, after which they phosphorylate AP-1 members such as c-Jun and ATF2 and stimulate their transcriptional activity (34–36).

Another *cis*-acting element frequently found in promoters of immediate-early genes is the dyad symmetry element (DSE) or serum response element (SRE), which was first identified in the promoter of the c-*fos* proto-oncogene (reviewed in Ref. 37). The DSE/SRE binds multiple transcription factors, including the serum response factor and the ternary complex factor (TCF) ELK-1 (37, 38). Mitogenic stimulation leads to a rapid phosphorylation of both serum response factor and TCF/ELK-1, leading to enhanced DNA binding by serum response factor and, more importantly, enhanced transcriptional activation by TCF/ELK-1 (37, 38). In contrast to phosphorylation of Jun proteins, phosphorylation of TCF/ELK-1 is likely to be mediated by the classical MAPKs ERK1 and ERK2 (38–40), although in was more recently shown that the SRE can also be activated in response to JNK/SAPKs (41, 42).

In this report, we have investigated the effects of IL-5 on TRE- and DSE-mediated transcription. Here we show that IL-5 efficiently activates TRE- and DSE-containing promoters. We present evidence that this is likely to be mediated by activation of JNK/SAPKs. Moreover, we identify the regions in the IL-5-R α and βc chains responsible for these effects. Finally, we suggest that activation of JAK2 by IL-5 precedes and is necessary for activation of JNK/SAPKs and TRE- and DSE-dependent transcription.

MATERIALS AND METHODS

Cell Culture, Reagents, and Antibodies—Rat-1, P19EC, and COS-1 cells were cultured in Dulbecco's modified Eagle's medium (Life Technologies, Inc.) supplemented with 8% heat-inactivated fetal calf serum. Human TF-1 and mouse BaF3 cells were cultured in RPMI 1640 (Life Technologies, Inc.) supplemented with 10% heat-inactivated fetal clone I serum (Hyclone), 50 μm β-mercapthoethanol, and human IL-5 (20 pM for TF1) or mouse IL-3 (0.1 nM for BaF3). Human IL-5 (hIL-5) was a kind gift of Dr. D. Fattah (Glaxo Wellcome, Stevenage, United Kingdom), whereas mouse IL-3 was produced in COS cells by transfecting an expression vector containing the murine IL-3 cDNA. The 12Ca5 antibody to the HA-tag was a generous gift from Marc van Dijk. The anti-JNK/SAPK antibody (SC-572) was purchased from Santa Cruz Biotechnology, Inc., and the anti-Jun antibody (Ab-1) was obtained from Oncogene Science. The MEK inhibitor PD098059 (43) was a generous gift from David Dudley and Alan Saltiel. The JAK2 inhibitor AG490 (44) was a generous gift from Alexander Levitzki.

Plasmid Construction—pSGhIL-5Rα was constructed by inserting the cDNA for the human IL-5α receptor into the *Not*I/*Kpn*I sites of pSG513, whereas pSGhIL-5Rβ was constructed by inserting the cDNA for the human βc subunit into the *Eco*RI sites of pSG513 as described previously (27). The expression vectors encoding βc mutants 763, 627, 542, 517, 456, δI/II, and δII and α mutants 405, 390, and 366 are described elsewhere (27). Other βc mutants were generated by progressive deletions using βc 763 and the erase-a-base kit (Promega Corp.). Stop codons were introduced by ligating in an oligonucleotide containing stop codons in all reading frames, and the identity of the different mutants was verified by dideoxy-sequencing using T7 polymerase (Pharmacia). 3xTREtkCAT, 3xDSEtkCAT (45), and the p54SAPK/JNK expression vector (46) are described elsewhere. Dominant-negative SEK (SEK-AL, serine 254 to alanine and threonine 258 to leucine; Refs. 46 and 47) and HA-tagged ERK2 (48) were described previously. 3xTREtataCAT contains three copies of the collagenase TRE coupled to a synthetic minimal promoter.

Transient Transfections—For transfection experiments, COS-1 cells were subcultured in six-well dishes (Costar), and 3 h later, the cells were transfected with 10–20 μg of supercoiled plasmid DNA as described previously (27, 49). Rat-1 and P19EC cells were transfected 16 h after splitting. Following 16–20 h exposure to the calcium-phosphate precipitate, medium was refreshed, and cells were incubated for 16 h with IL-5 (0.5 pM). Rat-1 cells were incubated in medium containing 0.5% fetal calf serum 8 h prior to the addition of IL-5. Transfected cells were subsequently harvested for CAT assays or Immuno-kinase assays. CAT assays were performed as follows. Cells were lyzed by repeated freeze-thawing in 250 mM Tris, pH 7.4, 25 mM EDTA. Twenty-five μg of cellular extract were then incubated in a total volume of 100 μl containing 250 mM Tris, pH 7.4, 2% glycerol, 0.3 mM butyryl coenzyme A, and 0.05 μCi of [^{14}C]chloramphenicol for 2 h at 37 °C. Reaction products were then extracted using 400 μl of xylene:pristane (1:2), and the percentage of acetylated products was then determined using liquid scintillation counting.

Gel Retardation Assay—Nuclear extracts were prepared from unstimulated and IL-5-stimulated cells following a procedure described previously (27, 49). Oligonucleotides were labeled by filling in the cohesive ends with [α-^{32}P]dCTP using Klenow fragment of DNA polymerase I. Gel retardation assays were carried out according to published procedures with slight modifications. Briefly, nuclear extracts (10 μg) were incubated in a final volume of 20 μl, containing 10 mM HEPES, pH 7.8, 50 mM KCl, 1 mM EDTA, 5 mM $MgCl_2$, 10% (v/v) glycerol, 5 mM dithiothreitol, 2 μg poly(dI-dC) (Pharmacia Biotech Inc.), 20 μg of bovine serum albumin, and 1.0 ng of ^{32}P-labeled collagenase TRE oligonucleotide for 20 min at room temperature. Complexes were then separated though nondenaturing 5% polyacrylamide gels and visualized by autoradiography.

Immunoprecipitation and Immune-Kinase Assays—For metabolic labeling experiments, cells were incubated for 3 h in phosphate-free medium containing 1 mCi/ml [^{32}P]orthophosphate. Cells were then stimulated with cytokines, and subsequently the cells were lyzed in RIPA buffer (20 mM Tris, pH 7.4, 150 mM NaCl, 1% Nonidet P-40, 0.1% SDS, 0.5% sodium deoxycholate, 5 mM EDTA, 1 mM Na_3VO_4, 10 mg/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride, and 1 mM leupeptin) for 15 min on ice. The lysate was centrifuged to remove DNA and cellular debris. The cell lysates were incubated with the anti-Jun polyclonal antibody for 2 h at 4 °C. Immune complexes were then precipitated with protein A-Sepharose for 30′ at 4 °C, washed five times with lysis buffer, and boiled in 1 × Laemmli's sample buffer. The proteins were separated on a 10% polyacrylamide gel. After electrophoresis, the gel was fixed (50% methanol, 10% acetic acid), dried, and exposed to X-ray films (Kodak) or analyzed using a phosphorimager (Applied Biosystems).

For immune-kinase assays, cells were lyzed in JNK/SAPK lysis buffer (20 mM Tris, pH 7.4, 10% glycerol, 1% TX-100, 2 mM EDTA, 2 mM EGTA, 50 mM β-glycerophosphate, 1 mM Na_3VO_4, 10 mg/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride, 1 mM leupeptin, and 1 mM benzamidine), and JNK/SAPK protein was precipitated using the 12Ca5 antibody (for transfected cells) or the JNK/SAPK polyclonal antibody (SC-572 for endogenous JNK/SAPK) and protein A-Sepharose beads. After extensive washing with lysis buffer, the beads were resuspended in 20 μl of kinase buffer (25 mM Hepes, pH 7.5, 20 mM $MgCl_2$, 20 mM β-glycerophosphate, and 2 mM dithiothreitol) containing 10 μg of GST-Jun and 10 μCi of [γ-^{32}P]ATP. The kinase reaction was performed for 20 min at 30 °C, after which the reaction was terminated by adding 1 × Laemmli's sample buffer. Proteins were then separated on 12% polyacrylamide gels, after which the gels were fixed, dried, and exposed to x-ray film. ERK2 immune-kinase assays were performed as described previously (48).

RESULTS

IL-5 Activates TRE-dependent Transcription—IL-5, the major cytokine involved in regulating eosinophil functions, was demonstrated previously to be able to activate different signaling pathways, including the RAS/MAPK pathway (50), the phosphatidylinositol 3′-kinase pathway (51), and the JAK/STAT pathway (17–19, 27). Activation of the Jun family of transcription factors by IL-5, however, was not studied before. We, therefore, set out to determine whether IL-5 was able to

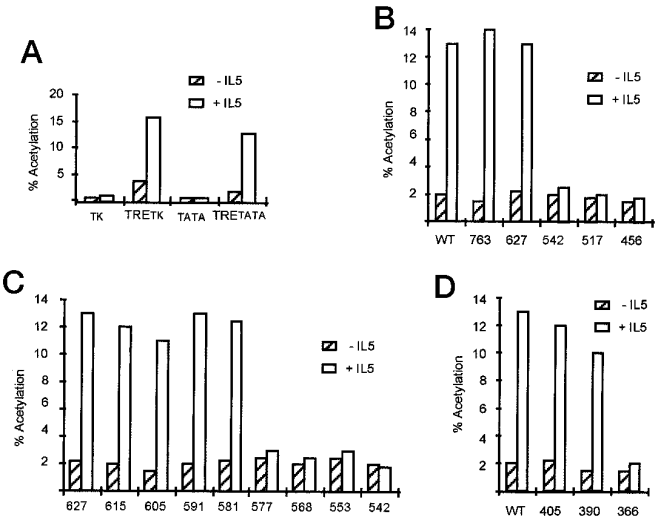

FIG. 1. **Activation of TRE-dependent transcription by IL-5.** *A,* Rat-1 cells were transfected with expression vectors encoding the IL-5 receptor α and βc chains and tk- or tata-CAT reporters with or without three copies of the collagenase TRE. Twenty-four h after transfection, cells were stimulated with IL-5 (0.5 nM) for 12 h, after which the cells were harvested, and CAT activity was determined. IL-5 treatment enhances CAT activity in cells expressing both TRE-containing reporters but not in the control cells. *B,* Rat 1 cells were transfected as described in *A* with the TREtataCAT reporter, the IL-5-R α plasmid, and different carboxyl-terminal deletions of the IL-5-R βc chain. IL-5 activation of TRE-dependent transcription is mediated by amino acids 542–627 of the IL-5-R βc chain. *C,* Rat-1 cells were transfected as in *B* with a large panel of progressive carboxyl-terminal deletions of the IL-5-R βc chain. It is clear that amino acids 577–581 are involved in TRE-dependent transcription induced by IL-5. *D,* Rat-1 cells were transfected with the TREtataCAT reporter, the wild-type IL-5-R βc chain and different progressive deletions of the IL-5-R α chain. This figure shows that amino acids 366–390 of the IL-5-R α chain are necessary for the IL-5-induced activation of TREtkCAT.

activate transcription of reporter constructs containing a TRE, the natural Jun binding site from the collagenase promoter (32). For this purpose, we transfected Rat-1 cells with the IL-5 R α and βc chains and different CAT reporter constructs. IL-5 was able to increase CAT activity in cells transfected with a reporter containing three copies of the TRE linked to the tk promoter (TREtkCAT) but failed to activate this reporter without TRE sites (Fig. 1*A*). This effect was not restricted to the tk promoter, because IL-5 also activated a CAT construct containing three TREs coupled to a synthetic minimal tata box (TRETATACAT) but not the enhancerless TATACAT reporter (Fig. 1*A*). Similar results were obtained in P19 EC and COS-1 cells (data not shown). These results strongly suggest that IL-5 can activate TRE-dependent transcription in these cell types.

Previously, it was shown that activation of different signaling pathways can be assigned to different domains of the βc chain of the IL-5 and GM-CSF receptors (24–28). We, therefore, tested a previously described set of carboxyl-terminal deletions of the βc chain for IL-5-dependent activation of the TRETATACAT reporter. Deletion of the most carboxyl-terminal 254 amino acids (627) did not alter the effect of IL-5 on TRE-dependent CAT activity (Fig. 1*B*). However, further deletion of 85 amino acids (542) resulted in a complete loss of IL-5-induced TREtataCAT activity. This region (542–627) was described previously to be involved in activation of the RAS/MAP kinase pathway by GM-CSF (28). To be able to localize the residues of the βc chain involved in TRE activation more precisely, we generated a new set of βc chain mutants using S1 nuclease. As shown in Fig. 1*C,* deletion down to amino acid 581 did not significantly decrease IL-5-induced TREtataCAT activity. Interestingly, further deletion of 4 amino acids (577) resulted in a complete loss of IL-5-induced TRE activity (Fig. 1*C*), which was also observed in P19 EC cells (data not shown). The expression of all mutants was verified by Western blotting using a rabbit anti-βc antibody (data not shown). Therefore, the region of the βc chain around tyrosine 577 is likely to be involved in activation of TRE-dependent transcription upon IL-5 stimulation.

It was suggested previously that the βc chain of the IL-5-R is the major player in IL-5-induced signal transduction. We and others have, however, shown that the cytoplasmic region of the α chain is necessary for IL-5-induced JAK2 and STAT3 activity (27, 30, 31). To determine whether the α chain plays a role in IL-5-induced TRE activity, we transfected different carboxyl-terminal deletions of the α chain together with the wild-type βc chain and the TREtataCAT reporter into Rat-1 cells. As we have described for IL-5-induced STAT3 activity (27), the region between amino acids 366 and 390 of the α chain is also necessary for IL-5-induced TREtataCAT activity (Fig. 1*D*). Importantly, α366 was shown previously to be able to form a high affinity complex with the βc chain (30), showing that the failure of this mutant to activate TRE-dependent transcription is not trivial. Taken together, activation of TRE-dependent transcription by IL-5 is mediated by a region containing tyrosine 577 of the βc chain and the proline-rich region 366–390 of the α chain.

IL-5 Induces Hyperphosphorylation of c-Jun and Activation of JNK/SAPK—The activity of transcription factor Jun/AP-1 can be regulated at the DNA-binding level or the transcription activation level (reviewed in Refs. 32 and 33). To discriminate between these two possibilities, we examined Jun/AP-1 DNA binding activity in TF-1 cells stimulated with IL-5 or IL-3. For this purpose, we isolated nuclear extracts from these cells and assayed them for binding to the collagenase TRE in a band shift assay. As shown in Fig. 2*A,* neither IL-5 nor IL-3 significantly altered Jun/AP-1 binding activity in TF-1 cells or BaF3 cells stably transfected with the IL-5 receptor (data not shown). Therefore, it seemed likely that the observed activation of TRE-dependent transcription was caused by an IL-5-induced increase in the transcriptional activation potential of c-Jun. As was reported previously, phosphorylation of two residues near the amino terminus of c-Jun by kinases of the JNK/SAPK family are thought to regulate transcriptional activation by c-Jun (33). We thus set out to determine whether IL-5 treatment altered the phosphorylation status of c-Jun. Orthophosphate-labeled TF-1 cells were treated with IL-5 or the potent JNK/SAPK activators TNFα and cycloheximide, after which Jun phosphorylation was studied by immunoprecipitation. As was expected, we observed a dose-dependent increase in Jun phosphorylation by IL-5, albeit at lower levels than Jun phosphorylation obtained after TNFα or cycloheximide stimulation (Fig. 2*B, CH*).

To determine whether the observed increase in Jun phosphorylation upon IL-5 stimulation was caused by an increase in JNK/SAPK kinase activity, we performed an immune-complex kinase assay on IL-5-stimulated TF-1 cells. JNK/SAPK was precipitated from TF-1 lysates using a polyclonal antibody against JNK2 (recognizing JNK1, JNK2, and p54β), and JNK/SAPK activity was determined using purified GST-Jun fusion protein as a substrate. As shown in Fig. 2*C,* IL-5 treatment indeed caused a 3-fold enhancement in JNK/SAPK kinase activity, which again was lower than the activation obtained with cycloheximide (*CH*). To investigate a similar enhancement in JNK/SAPK kinase activity that was present in the cells that were used for assaying TRE activation, we transiently transfected an epitope-tagged version of p54SAPK/JNK together with the IL-5-R into Rat-1 and COS-1 cells. The activity of the

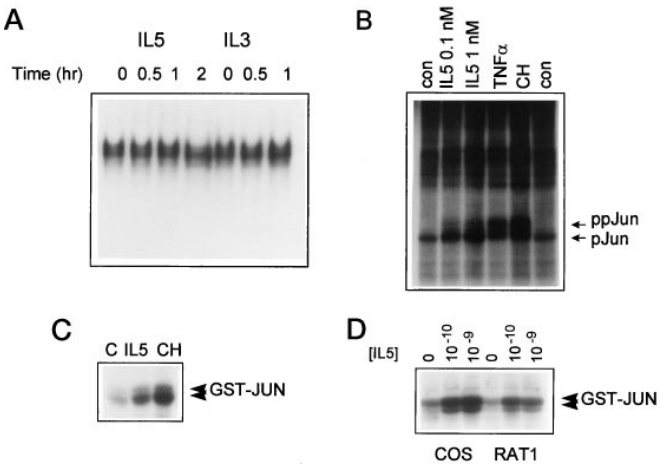

FIG. 2. **IL-5 induces hyperphosphorylation of c-Jun and activation of JNK/SAPK.** *A,* TF-1 cells were treated with IL-5 (0.5 nM) or IL-3 (0.5 nM) for different periods of time, after which nuclear extracts were prepared and assayed for Jun/AP-1 DNA binding activity in a bandshift assay using the collagenase TRE. Only the Jun/AP-1-DNA complex is shown. Neither IL-5 nor IL-3 modulate the binding activity of Jun/AP-1. *B,* TF-1 cells were labeled with [^{32}P]orthophosphate and subsequently stimulated with different cytokines for 20 min or with cycloheximide (*CH,* 200 μM) for 1 h. Cells were then lyzed, and c-Jun was immunoprecipitated. In unstimulated cells (*con*), c-Jun is already phosphorylated (*pJun*). However, IL-5 treatment causes a significant increase in c-Jun phosphorylation (*ppJun*). As controls, TNFα (1000 units/ml) and cycloheximide cause an even stronger enhancement in c-Jun phosphorylation. *C,* TF-1 cells were treated with IL-5 (0.5 nM) for 20 min or with cycloheximide (200 μM) for 60 min. JNK/SAPK kinase activity was then assayed using an immune-complex kinase assay with purified GST-Jun protein as a substrate. IL-5 enhances JNK/SAPK activity about 3-fold, whereas cycloheximide causes a 10-fold increase in GST-Jun phosphorylation. *D,* Cos-1 or Rat-1 cells were transfected with the IL-5-R α and βc chains and an expression vector encoding a tagged version of p54SAPK/JNK. Cells were treated with IL-5 in the indicated concentrations for 20 min, after which the kinase activity of the transfected p54SAPK/JNK was determined. IL-5 treatment leads to a significant increase in the activity of p54SAPK/JNK in both cell types.

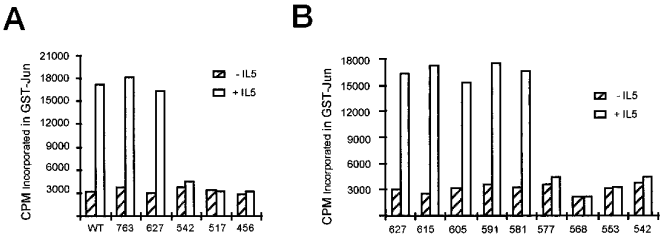

FIG. 3. **Activation of p54SAPK/JNK kinase activity in transfected cells is mediated by IL-5-R βc residues 581–577.** *A,* Rat-1 cells were transfected with the tagged p54SAPK/JNK, the IL-5-R α chain, and a general set of deletion mutants of the IL-5-R βc chain. Cells were treated with IL-5 (0.5 nM) for 20 min, after which the kinase activity of the tagged p54SAPK/JNK was determined and quantified. It is clear that amino acids 542–627 of the βc chain are necessary for the induction of p54SAPK/JNK activity by IL-5. *B,* Rat-1 cells were transfected as in *A* with a more detailed set of IL-5-R βc chain mutants. Amino acids 577–581 are involved in activation of p54SAPK/JNK by IL-5.

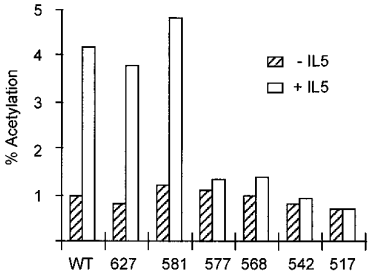

FIG. 4. **Activation of DSE-dependent transcription by IL-5.** Rat-1 cells were transfected with the IL-5-R α chain, a series of IL-5-R βc chain mutants, and a reporter containing three copies of the DSE from the human c-*fos* promoter coupled to tkCAT. Cells were treated with or without IL-5 (0.5 nM) for 12 h, after which CAT activity was determined. IL-5 treatment causes a 4-fold increase in DSE-dependent CAT activity, which is mediated by amino acids 577–581 of the IL-5-R βc chain.

tagged p54SAPK/JNK was then determined using an anti-epitope antibody in an immune-complex kinase assay. Fig. 2*D* shows that in both cell types, IL-5 significantly enhanced the activity of the transfected p54SAPK/JNK. Taken together, it seems likely that the observed activation of TRE-dependent transcription by IL-5 was caused by phosphorylation of c-Jun by JNK/SAPK.

To provide further evidence for this model, we tested the activity of the different βc chain mutants in the transient p54SAPK/JNK activation assay. Fig. 3*A* shows that the region between 542 and 627 is necessary for IL-5-induced enhancement of p54SAPK/JNK kinase activity. Moreover, as we described above for the activation of TRE-dependent transcription, mutant 581, but not 577, fully supported IL-5-induced p54SAPK/JNK activation (Fig. 3*B*). This again supports the hypothesis that IL-5-induced TRE activation is mediated by JNK/SAPK.

Activation of DSE-dependent Transcription by IL-5—It was shown recently that besides c-Jun, JNK/SAPK also phosphorylates and activates p62TCF/ELK-1, one of the transcription factors regulating the activity of the dyad symmetry element (DSE) in the c-*fos* promoter (41, 42). Therefore, we set out to determine whether IL-5 will also activate DSE-dependent transcription in our transient system. A reporter construct containing three copies of the DSE coupled to tkCAT was transfected in Rat-1 cells together with the IL-5-R α and βc chains. Interestingly, IL-5 causes a 4-fold increase in DSE-dependent transcription in these cells (Fig. 4). Moreover, as we demonstrated for TRE and p54SAPK/JNK activation, βc 581 was able to activate DSEtkCAT activity, whereas βc 577 had lost this ability (Fig. 4).

ERK2 Is Not Involved in Activation of TRE- or DSE-dependent Transcription by IL-5—Activation of DSE-dependent transcription can also be accomplished by the RAS-ERK2 kinase pathway (40, 51). Moreover, it was shown recently that tyrosine 577 of the βc chain is involved in activation of this pathway by GM-CSF (28). We, therefore, determined whether activation of MAP kinase was involved in the observed regulation of TRE- and DSE-dependent transcription by IL-5. For this purpose, we used PD098059, a potent and selective inhibitor of the MAP kinase kinase MEK (43). PD098059 was indeed able to block ERK2/MAP kinase hyperphosphorylation induced by IL-5 in Rat-1 cells (Fig. 5*A*). Moreover, PD098059 completely blocks IL-5-induced ERK2/MAP kinase activity in Rat-1 cells (Fig. 5*B*). However, Fig. 6*A* shows that preincubation with PD098059 did not alter TRE-dependent CAT activity in IL-5-treated cells. Moreover, PD098059 pretreatment did also not influence IL-5-induced DSE activation (Fig. 6*B*) or IL-5-induced p54SAPK/JNK kinase activity (Fig. 6*C*). These results and those described previously strongly suggest that activation of TRE- and DSE-dependent transcription is mediated by JNK/SAPK but not by the ERK2/MAP kinase pathway.

To further investigate the role of JNK/SAPK in IL-5 induced TRE and DSE activation, we used a construct expressing dominant-negative SEK (SEK-AL, made by mutating serine 254 to alanine and threonine 258 to leucine; Refs. 46 and 47), an efficient repressor of JNK/SAPK activity. Indeed, when dominant-negative SEK was transfected in Rat-1 cells, IL-5-induced JNK/SAPK activity was strongly repressed (Fig. 7, *right panel*).

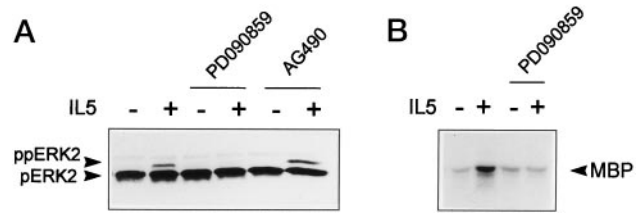

FIG. 5. **The MEK inhibitor PD098059 blocks IL-5-induced ERK2/MAP kinase activation in Rat-1 cells.** *A,* a Rat-1 clone stably expressing the IL-5Rα and β chains was treated with the MEK inhibitor PD098059 (50 μM), the JAK2 inhibitor AG490 (50 μM), or buffer alone for 30 min, after which IL-5 (0.5 nM) was added for another 5 min. Cells (200,000) were then lyzed in SDS sample buffer and analyzed by Western blotting. IL-5 treatment causes a significant phosphorylation (activation) of ERK2, which is fully inhibited by PD098059 but not by AG490. *B,* Rat-1 cells were transfected with the IL-5-R α and βc chains and an epitope-tagged version of ERK2. Thirty-six h after transfection, cells were treated with the MEK inhibitor PD098059 (50 μM) or buffer alone for 20 min, after which IL-5 (0.5 nM) was added for another 5 min. ERK2 kinase activity was measured using an immune-kinase assay using MBP as a substrate. IL-5-induced ERK2 kinase activity is completely blocked by pretreatment with PD098059.

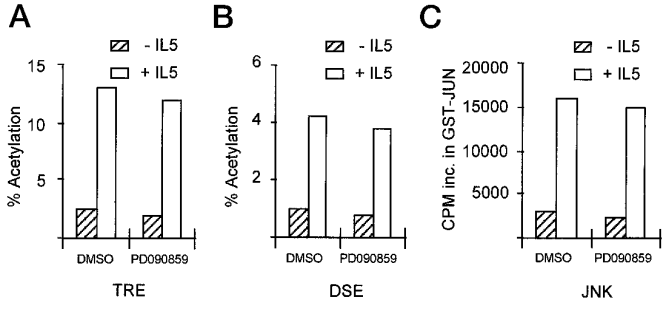

FIG. 6. **ERK2/MAP kinase is not involved in TRE, DSE, or p54SAPK/JNK activation by IL-5.** *A,* Rat-1 cells were transfected with the IL-5-R α and βc chains and the TREtataCAT reporter construct. Cells were then treated with the MEK inhibitor PD098059 (50 μM) for 1 h, after which IL-5 (0.5 nM) was added for another 12 h. Activation of MEK and MAP kinase is not necessary for activation of TRE-dependent CAT activity by IL-5. *B,* Rat-1 cells were transfected as in *A* but instead of the TREtataCAT, the DSEtkCAT construct was used as reporter. Again, MEK and MAP kinase do not seem to be involved in IL-5-induced DSE activation. *C,* Rat-1 cells were transfected with the IL-5-R α and βc chains and the tagged version of p54SAPK/JNK. Cells were treated as described in *A*. p54SAPK/JNK activity was then determined with an immune-complex kinase assay using GST-Jun as a substrate. Activation of p54SAPK/JNK by IL-5 occurs independently from MEK and MAP kinase activation.

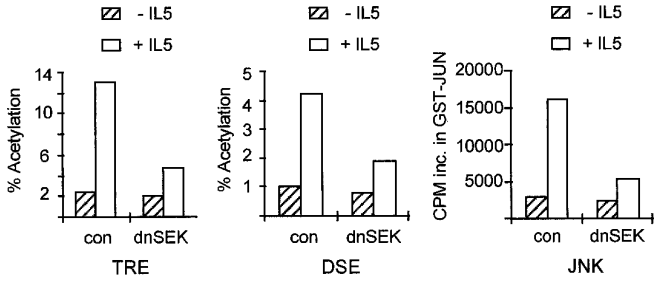

FIG. 7. **JNK/SAPK is involved in TRE- and DSE-dependent transcription by IL-5.** Rat-1 cells were transfected with the IL-5-R α and βc chains and the TREtataCAT reporter (*left*), the DSE reporter (*middle*), or the tagged version of p54SAPK/JNK (*right*) together with a dominant-negative SEK expression vector (*dnSEK*; SEK-AL, Refs. 46 and 47) or the insertless expression vector control (*con*). After 24 h, IL-5 (0.5 nM) was added for another 12 h. CAT activity and p54SAPK/JNK activity were determined as described above. It is clear that blocking p54SAPK/JNK activity by dominant-negative SEK also inhibits IL-5 induced TRE- and DSE-dependent transcription.

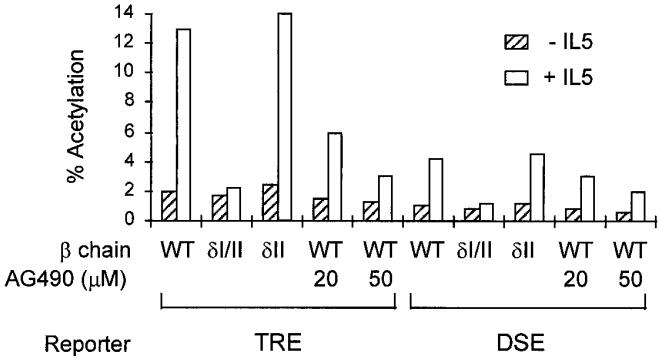

FIG. 8. **JAK2 is likely to be involved in TRE- and DSE-dependent transcription induced by IL-5.** Rat-1 cells were transfected with the IL-5-R α chain, the IL-5-R βc chain (*WT*), or mutants of βc from which either box1 and box2 (δI/II) or boxII alone (δII) were deleted. As reporters, TREtataCAT or DSEtkCAT were used. Some samples were incubated with the JAK2 inhibitor AG490 for 8 h prior to the addition of IL-5 (0.5 nM). Twelve h after IL-5 addition, CAT activity was determined. JAK2 activation seems to be involved in both TRE and DSE activation, because deletion of the JAK2 binding site of the βc chain (box1) or blocking JAK2 activity with AG490 inhibits both TRE and DSE activation by IL-5 at least partially.

Interestingly, dominant-negative SEK also reduces IL-5-induced TRE- and DSE-dependent transcription, suggesting that TRE and DSE activation by IL-5 are at least partially mediated by JNK/SAPK.

JAK2 Is Essential for IL-5-induced TRE- and DSE-dependent Transcription—We and others have shown previously that IL-5 causes a strong and rapid activation of the JAK2 tyrosine kinase in different IL-5-responsive cell types (17–19). Because IL-5 is known to induce tyrosine phosphorylation of the βc chain, a process that might well be mediated by JAK2 (52), we wanted to determine whether JAK2 activation by IL-5 is involved in the activation of TRE- and DSE-dependent transcription. For this purpose, we used two different approaches. (i) We transfected two different mutations of the βc chain, δI/II and δII, into Rat-1 cells and tested their activity on TRE- and DSE-containing reporters. Although both mutants are wild-type around tyrosine 577, mutant δI/II contains an internal deletion that removes both box1 and box2 from the full-length βc receptor, whereas δII is only lacking box2 in the full-length βc receptor. Deletion of both box1 and box2 completely blocked IL-5-induced TRE and DSE activation, whereas deletion of only box2 did not have any effect (Fig. 8). Interestingly, box1, but not box2, was shown previously to be involved in JAK2 binding and activation (19, 52). (ii) We used the tyrphostin AG490, which was shown previously to be an inhibitor of JAK2 (44). Fig. 8 shows that this inhibitor indeed causes a dose-dependent decrease in IL-5-induced TRE and DSE activity, although we failed to completely block these processes. Control experiments showed that AG490 caused a significant but not complete block in IL-5-induced JAK2 activation (data not shown). We, therefore, conclude that JAK2 is likely to be involved in IL-5-induced activation of TRE- and DSE-dependent transcription (Fig. 9).

DISCUSSION

IL-5 binding to its receptor results in the activation of multiple signaling pathways, including the RAS/MAPK pathway (50), the phosphatidylinositol 3′-kinase pathway (51) and the JAK/STAT pathway (17–19, 27). Here we show that IL-5 activates TRE- and DSE-dependent transcription via activation of JNK/SAPK. Moreover, we present evidence that activation of JAK2 is necessary for this process.

Immediate-early gene induction in response to growth factors and phorbol esters can be mediated by a DSE/SRE *cis*-acting elements present in the promoters of induced genes (reviewed in Ref. 37). The DSE/SRE binds serum response

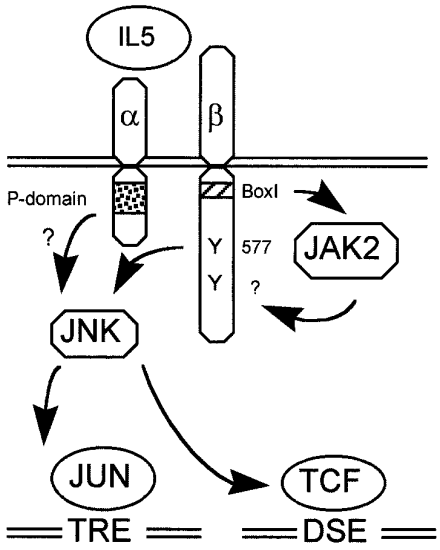

FIG. 9. **Putative model for IL-5-induced activation of TRE- and DSE-dependent transcription.** *α,* IL-5-R α chain; *β,* IL-5-R βc chain; *P-domain,* proline-rich domain; *Y,* tyrosine residue (potentially phosphorylated by JAK2). *Arrows* do not imply direct effects without intermediate proteins.

factor and proteins from the TCF family including Elk-1, SAP-1, and NET-1/ERP/SAP-2 (reviewed in Ref. 38). Upon growth factor signaling, Elk-1 is phosphorylated by the ERK group of MAP kinases, resulting in increased ternary complex formation as well as activation of the transcriptional activation domain of Elk-1 (38–40). Because IL-5 efficiently activates ERK2 (50)(Fig. 5), it is surprising that blocking this process with PD098059 does not influence IL-5-induced DSE activation (Fig. 6). At present, we can only speculate on this apparent contradiction. It is possible that phosphorylation and activation of Elk-1 by ERK2 is a cell type-specific process that involves components that might not be available in our IL-5-induced cellular system. However, because there are no examples of cell type-specific Elk-1 phosphorylation, it seems more likely that both ERK and JNK/SAPK are capable of activating Elk-1 in IL-5-stimulated cells. Activation of Elk-1 by JNK/SAPK is not unprecedented, because it was recently shown that JNK/SAPK activators, such as UV or IL-1, efficiently activate Elk-1 though phosphorylation on sites identical to those phosphorylated by ERK2 (41, 42). Blocking either the ERK or JNK/SAPK pathway, therefore, does not significantly alter IL-5-induced DSE activity because the other pathway is redundant. This redundancy was also suggested by Cano *et al.* (53), who showed that activation of a single MAPK subtype (ERK or JNK/SAPK) is sufficient to elicit a complete nuclear response. Further demonstration of this functional redundancy awaits the availability of an efficient JNK/SAPK inhibitor.

In agreement with our results, it was shown previously that box 1 and tyrosine 577 of the βc chain are involved in the activation of the c-*fos* promoter by GM-CSF (28, 52). However, mutation of tyrosine 577 (Y577F) in the context of the full-length βc chain resulted in only partial inhibition of c-*fos* induction by GM-CSF. This phenomenon was explained by the fact that other phosphorylated tyrosines might play a role in c-*fos* induction by GM-CSF (28). It was also suggested that the RAS/ERK cascade was essential for GM-CSF-induced c-*fos* expression, because dominant-negative RAS expression efficiently blocks c-*fos* induction by GM-CSF (28, 54). Our results with the MEK inhibitor PD098059 (Figs. 5 and 6) suggest that the ERK2/MAP kinase cascade is not involved in DSE (and c-*fos*) induction by IL-5. Although these results seem to be in contrast, they might be explained by the fact that activated RAS also stimulates the JNK/SAPK pathway (55, 56), although TNFα induction of JNK/SAPK occurs in a RAS-independent manner (56). Because PD098059 efficiently blocks ERK2 activation, but not RAS activation, the result with the dominant-negative RAS might be explained by inhibition of JNK/SAPK and, therefore, inhibition of GM-CSF-dependent c-*fos* expression.

Inhibition of IL-5-induced JAK2 activation by using βc chain mutants or the tyrphostin AG490 resulted in a decrease in IL-5-induced TRE and DSE activation (Fig. 8). Therefore, JAK2 activation seems to play a role in IL-5 signaling apart from STAT activation. In agreement with our results, Watanabe *et al.* (52) showed recently that blocking JAK2 with dominant-negative JAK2 forms inhibits c-*fos* promoter activation by GM-CSF. It is worthwhile to mention that JAK2 was also shown to be necessary for ERK2 activation by growth hormone (57). Because the βc chain is phosphorylated on multiple tyrosine residues after IL-5 stimulation, including tyrosine 577 (28), it seems likely that JAK2 is the kinase responsible for this phenomenon. Indeed, dominant-negative JAK2 expression resulted in abrogation of βc phosphorylation after GM-CSF addition (57). The phosphorylated tyrosine 577 might well be the binding site for adapter proteins such as Shc, Grb2, P80, and other proteins responsible for transmitting the IL-5/GM-CSF-induced signal from the βc chain through the cytoplasm (28, 29, 58). Verification of this model awaits the precise identification of βc residues that are phosphorylated by JAK2 and the characterization of proteins binding to these residues.

Although the cytoplasmic domain of the βc chain of the IL-5/IL-3/GM-CSF receptors is essential for all previously described intracellular signaling events, we have shown that amino acids 366–390 of the α chain are also necessary for IL-5-induced TRE and DSE activation (Fig. 1*D*). This is not trivial, because α366 is able to form a high affinity binding receptor with the βc chain (30). We have shown previously that this region is essential for IL-5-induced STAT3 activation (27). This region contains conserved proline residues that are also found in the IL-3-R α, GM-CSF-R α, prolactin receptor and growth hormone receptor. Site-directed mutagenesis showed that these prolines are essential for IL-5-induced proliferation and c-*jun* and c-*fos* induction as well as JAK2 activation (30, 31). Recent evidence suggests that the cytoplasmic domain of the α chain is necessary for the activation of a preformed βc dimeric complex (59). However, the molecular mechanism responsible for this process remains to be determined.

Taken together, we describe for the first time that besides ERK2, IL-5 also activates MAP kinases of the JNK/SAPK family. The underlying mechanism as well as the functional consequence of this process for IL-5-induced responses in eosinophils are objectives of future study.

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