The cytokine interleukin-1 (IL-1) is a major inflammatory hormone which activates a broad range of genes during inflammation. The signaling mechanisms triggered by IL-1 include activation of several distinct protein kinase systems. The stress-activated protein kinase (SAPK), also termed Jun N-terminal kinase (JNK), is activated particularly strongly by the cytokine. In an attempt to delineate its role in activation of gene expression by IL-1, we inhibited the IL-1-induced SAPK/JNK activity by stable overexpression of either a catalytically inactive mutant of SAPKβ (SAPKβ(K-R)) or antisense RNA to SAPKβ in human epidermal carcinoma cells. A detailed analysis of signal transduction in those cells showed that activation of neither NFκB nor p38 mitogen-activated protein kinase was affected, suggesting that we achieved specific blockade of the SAPK/JNK. In untransfected and vector-transfected KB cells, IL-1 induced a strong increase in expression of IL-6 and IL-8 mRNA, along with the synthesis of high amounts of the proteins. In two KB cell clones stably overexpressing the mutant SAPKβ(K-R), and three clones stably overexpressing antisense RNA to SAPKβ, expression of IL-6 and IL-8 in response to IL-1 was strongly reduced at both the mRNA and protein level. These data indicate that the SAPK/JNK pathway provides an indispensable signal for IL-1-induced expression of IL-6 and IL-8.

Interleukin-1 (IL-1) is the prototype inflammatory cytokine. It is produced in two forms (α and β) by activated monocytes/macrophages during acute or chronic inflammatory responses. It acts by inducing many genes, including cytokines (e.g. IL-2 and IL-6), chemokines (e.g. IL-8 and MCP-1), proteases (e.g. collagenase and stromelysin), adhesion molecules (e.g. ICAM-1 and E-selectin), and cyclooxygenase. Strength and duration of the expression of these genes is crucial for the intensity of an inflammatory process (reviewed in Ref. 1). Therefore much interest has focused on molecular mechanisms through which these genes are controlled by IL-1.

Two IL-1 receptors have been cloned (type I and type II) that are expressed on many different cell types (2). Only the type I receptor, heterodimerized to the IL-1 receptor accessory protein, is capable of signal transduction (3). Recently the understanding of IL-1 signaling pathways has been markedly increased by identification of novel molecules. IL-1 treatment of cells can activate at least four protein kinase cascades. One cascade involves association of an IL-1 receptor-associated protein kinase and TRAF6 with the IL-1 receptor complex (4, 5), leading to activation of an NFκB-inducing kinase (6), which activates the IκB kinase complex. Phosphorylated IκB is ubiquitinated, then degraded by the proteasome. This releases NFκB, a major transcription factor regulating IL-1 responsive genes, and allows it to translocate to the nucleus (7, 8).

The other cascades activated by IL-1 are those activating the three best known types of mitogen-activated protein kinase (MAPK), namely p42/p44 extracellular signal-regulated-protein kinase (ERK), p38 MAPK, and the stress-activated protein kinase that phosphorlates the N-terminal region of c-Jun (SAPK/JNK) (9). Although IL-1 has been shown to activate ERK in some cells (10, 11), it is a much more potent inducer of SAPK/JNK and p38 MAPK in cultured cells (12–14).

A central role of SAPK/JNK for IL-1 signaling is suggested by our finding that IL-1 activates it and its activator, MAPK kinase 7 (MKK7), in rabbit liver in vivo, without activating either p38 MAPK or ERK (15). Ten different SAPK isoforms derived from three different genes (called SAPKα, β, and γ in rat and JNK 2, 3, and 1 in man, respectively), which are highly conserved across species, have been cloned from vertebrate tissues (16, 17). The homology among SAPK/JNK isoforms is 80–90% on the protein level (18). We purified a 46-kDa form of SAPK/JNK activated by IL-1 from KB cells, and 50- and 55-kDa forms from rabbit liver. They accounted for essentially all of the biochemically detectable IL-1-activated JNK activity in liver and were both identified as SAPKα (JNK2) by amino acid sequencing a number of peptides. This suggested that SAPK/JNK isoforms might be expressed or activated in a tissue-specific manner (19). The functional consequence of general activation of SAPK/JNK, or of the individual isoforms, on the regulation of IL-1 responsive genes is unclear (20). SAPK/JNK have been shown to phosphorylate the proteins c-Jun and ATF-2, which are components of the dimeric transcription factor AP-1. These phosphorylations result in enhanced transcription of AP-1-dependent reporter genes (21–26). Besides phos-
phorylating the activation domains of the transcription factors, SAPK/JNK isoforms bind Jun and ATF-2 proteins with different affinities, a mechanism which could result in targeting them to different intracellular substrates and to exert distinct functions (18, 25, 27). Although AP-1 can be considered to be a principal target of SAPK/JNK, other transcription factors, like the ternary complex factors ELK-1 and SAP-1, are also substrates (28–31).

In an attempt to elucidate the function of IL-1-induced activation of SAPK/JNK in the human keratinocyte line KB, we stably overexpressed either an inactive mutant of the enzyme or antisense RNA. This allowed us to study the role of SAPK/JNK on endogenous gene expression. We investigated the role of SAPK/JNK in the regulation of IL-6 and IL-8, two cytokines which are highly inducible by IL-1 in keratinocytes. IL-6 is a multifunctional cytokine that promotes B-cell growth and differentiation and stimulates acute-phase protein synthesis in liver (32). IL-8 is a chemokine attracting and stimulating leukocytes at sites of inflammation (33). We report here that inhibition of SAPK/JNK activation in cells stimulated by IL-1 results in inhibition of IL-1-induced IL-6 and IL-8 gene expression.

**EXPERIMENTAL PROCEDURES**

**Cells and Materials**—KB cells were obtained from the American Type Culture Collection and cultured in Dulbecco’s modified Eagle’s medium in the presence of 10% fetal calf serum.

Human recombinant IL-1α was expressed in Escherichia coli and purified as described (12). E64 (trans-epoxysuccinyl-l-leucylami-d(4-guanidinobutane), pepstatin, leupeptin, phenylmethylsulfonyl fluoride, and all other chemicals were from Sigma, Deisenhofen, Germany. [γ-32P]ATP and [α-32P]dCTP were purchased from Hartmann Analytics, Braunschweig, Germany. Expression plasmids for GST–SAPKα and GST–SAPKβ were kind gifts of Dr. J. R. Woodgett. The Ontario Cancer Research Institute, Toronto, Canada. GST fusion proteins were expressed and purified from E. coli by standard methods. Recombinant bacterially expressed histidine (His)-epitope-tagged MAPK activated protein kinase-2 (MAPKAPK-2) was a kind gift of Dr. M. Gaestel (Max-Delbrueck-Center for Molecular Medicine, Berlin, Germany). The chicken anti-p54 SAPKβ antibodies were produced by immunizing chickens with recombinant GST p54 SAPKβ and immunoglobulins were purified from eggs. Besides the p54 SAPKβ (which is identical to human JNK3), the antibodies also recognize purified rabbit SAPKα and the human JNK1 and JNK2 isoforms in cells transiently transfected with the respective cDNA, and proteins of corresponding sizes in human mesangial cells (34).

Rabbit antisera to p38 MAPK was to synthesize in Escherichia coli (amino acids 346–360). Rabbit anti-chicken IgG coupled to horseradish peroxidase was from Sigma. cDNAs for IL-6 (1100 bp), IL-8 (700 bp), and glyceraldehyde-3-phosphate dehydrogenase (1400 bp) were amplified by reverse transcription (RT)-PCR. The cDNA encoding the open reading frame (ORF) of p54 SAPKβ was cloned into the expression vector pCI (Promega, Madison, WI) and transfected into KB cells by the DEAE-Dextran method. cDNAs for IL-6 (1100 bp), IL-8 (700 bp), and glyceraldehyde-3-phosphate dehydrogenase (1400 bp) were amplified by reverse transcription (RT)-PCR. The cDNA encoding the open reading frame (ORF) of p54 SAPKβ was cloned into the expression vector pCI (Promega, Madison, WI) and transfected into KB cells by the DEAE-Dextran method.

**p38 MAPK Assay—**500 μg of whole cell extract protein was diluted in 500 μl of immunoprecipitation (IP) buffer (20 mM Tris, pH 7.3, 154 mM NaCl, 50 mM sodium fluoride, 1 mM Na3VO4, 50 mM sodium fluoride, 20 mM p-β-glycerophosphate, and freshly added 0.5 mM PMSF, 0.5 μg/ml pepstatin, 5 mM DTT, 400 mM 3-mercaptoethanol, 20 mM p-β-glycerophosphate). Cells were centrifuged for 5 min at 10,000 × g at 4 °C. The pellet was resuspended in buffer A + 0.1% Nonidet P-40 and vortexed. After centrifugation at 10,000 × g for 5 min supernatants were taken and stored at −80 °C.

**Preparation of Cytosolic and Nuclear Extracts—**KB cells were stimulated with IL-1, washed, and scraped in PBS at 4 °C as described above. Cells were resuspended in buffer A (10 mM HEPES, pH 7.9, 10 mM KCl, 1.5 mM MgCl2, 0.3 mM Na3VO4, and freshly added 200 μM leupeptin, 10 μM E64, 300 μM PMSF, 0.5 μg/ml pepstatin, 5 mM DTT, 400 mM 3-mercaptoethanol, 20 mM p-β-glycerophosphate). Cells were centrifuged for 5 min at 10,000 × g at 4 °C. The pellet was resuspended in buffer A + 0.1% Nonidet P-40 and vortexed. After centrifugation at 10,000 × g for 5 min supernatants were taken and stored at −80 °C.

**Preparation of Whole Cell Extracts—**Confluent KB cells were stimulated with IL-1 (10 ng/ml) added to the culture medium. After 15 min at 37 °C, the medium was removed, cells placed on ice, washed once in PBS, and scraped in PBS. Cells were collected at 500 × g for 5 min and lysed in whole cell lysis buffer (10 mM Tris, pH 7.05, 30 mM NaPP, 50 mM NaCl, 1% Triton X-100, 2 mM Na3VO4, 50 mM sodium fluoride, 20 mM p-β-glycerophosphate and freshly added 0.5 mM PMSF, 0.5 μg/ml pepstatin, 10 mM p-nitro-nitrophenyl phosphate, 400 mM 3-mercaptoethanol). After 10 min on ice, lysates were cleared by centrifugation at 10,000 × g for 15 min at 4 °C. Protein concentration of supernatants was determined by the method of Bradford and samples stored at −80 °C.

**Partial Purification and Assay for an Activator of SAPK/JNK—**KB cells were stimulated with IL-1, washed, and scraped in PBS at 4 °C as described above. Cells were resuspended in buffer A (10 mM HEPES, pH 7.9, 10 mM KCl, 1.5 mM MgCl2, 0.3 mM Na3VO4, and freshly added 200 μM leupeptin, 10 μM E64, 300 μM PMSF, 0.5 μg/ml pepstatin, 5 mM DTT, 400 mM 3-mercaptoethanol, 20 mM p-β-glycerophosphate). Cells were centrifuged for 5 min at 10,000 × g at 4 °C. The pellet was resuspended in buffer A + 0.1% Nonidet P-40 and vortexed. After centrifugation at 10,000 × g for 5 min supernatants were taken and stored at −80 °C. Protein concentration of supernatants was determined by the method of Bradford and samples stored at −80 °C.
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RESULTS

Stable Overexpression of SAPKb(K-R) Mutant or Antisense RNA to SAPKb in KB Cells—In our efforts to identify a functional role of SAPK/JNK in IL-1-induced gene expression, we followed approaches that have been used successfully to demonstrate a role for the p42/p44 MAPK cascade in growth factor-regulated fibroblast proliferation; i.e. stable overexpression of MAPKK mutants (38) and overexpression of ERK mutants and ERK antisense RNA (39). We constructed a mutant rat p54 SAPKb cDNA, in which a lysine residue critical for kinase activity was replaced by arginine. This lysine residue is highly conserved in the protein kinase family (40) and is involved in ATP binding of the p42 MAPK (ERK2) as shown by structural studies (41). The open reading frame of the SAPKb(K-R) mutant was placed 5’ to the hemagglutinin (HA)-tag sequence under the control of a cytomegalovirus promoter. KB cells were stably transfected with the construct or the vector alone. Cell clones were analyzed by Western blotting using chicken egg antibodies, that were raised against bacterially expressed full-length GST-p54 SAPKb protein. Thereby we identified six KB cell clones overexpressing the SAPKb mutant. Western blot detection of two of these is shown in Fig. 1A. Both clones strongly overexpressed the transfected protein, compared with the endogenous SAPK/JNK. Identity of the HA-SAPKb(K-R) band was confirmed by detection of a product of identical size with anti-HA tag antibodies (not shown).

As expected from their high homology to each other, different SAPK/JNK isoforms are recognized by the chicken anti-SAPKb antibodies (see “Experimental Procedures”). In the KB cells they detected several endogenous JNK isoforms which appear as two doublet bands of about 45 to 48 kDa and 54 to 57 kDa. As detailed by Gupta et al. (18), the JNK 1 and JNK 2 genes each can give rise to differently spliced transcripts which encode proteins of 46 and 55 kDa. The JNK 3 gene transcripts apparently are translated into two doublets of 45–48 kDa and 54–57 kDa, due to partial usage of an additional upstream-located start codon (18). Thus while it is not possible to unequivocally assign each product to a specific isoform, the observed pattern is in close accordance with that reported in Ref. 18. All four bands were confirmed to represent different SAPK...
isoforms, since we observed protein kinases of corresponding sizes in in-gel kinase assays with GST-Jun as substrate. Three proteins of higher Mr on SDS-PAGE were not related to SAPK activity and represent proteins reacting nonspecifically with the chicken egg immunoglobulin.

On the cDNA level sequence comparison between the 1.3-kilobase open reading frames of SAPKβ and those of JNK1, JNK2, and JNK3 revealed a homology of 78, 74, and 91%, respectively. Therefore, overexpression of the full-length open reading frame in antisense orientation was likely to hybridize to all JNK mRNAs present in KB cells and inhibit their translation. In fact, suppression of similarly related proteins has been demonstrated in a study where a 1.75-kilobase antisense fragment of the ERK1 gene inhibited both, the expression of ERK1 and ERK2 isoforms (39). The homology of ERK1 and ERK2 is comparable to that between the JNK isoforms. We isolated several clones stably overexpressing antisense RNA. Endogenous SAPK/JNK protein levels in three of those clones and in untransfected KB cells were compared by Western blot. The bands corresponding to endogenous JNK isoforms were all significantly reduced in all three clones (Fig. 1B). The abundance of three cross-reacting proteins of higher molecular Mr on SDS-PAGE was not reduced. According to densitometric quantitation the amounts JNK proteins in the three different clones were 24, 54, and 28% of the amount in control cells.

Stable Overexpression of SAPKβ(K-R) and SAPKβ Antisense RNA Inhibits IL-1-induced SAPK/JNK Activation.—We next investigated whether overexpression of the kinase-inactive SAPKβ had a dominant negative effect and interfered with SAPK/JNK activity induced by IL-1. Kinase activity was determined in vitro using GST-Jun (amino acids 1–135) as substrate. The Jun protein was then purified from the reaction mixture by adsorption to GSH beads. This assay allowed quantitative measurement of SAPK/JNK activity in the presence of both endogenous SAPK isoforms and the SAPKβ mutant. In whole cell lysates of clones overexpressing SAPKβ(K-R), IL-1-mediated stimulation of total SAPK/JNK activity was decreased by about 50% (Fig. 2A). A comparable degree of inhibition was observed when kinase activity was analyzed in cytosolic extracts of the cells (Fig. 2B). No apparent differences were observed in the kinetics of the IL-1 induced transient SAPK/JNK activation in clones 2 and 11 compared with untransfected or vector-transfected KB cells. After 60 min of IL-1 treatment Jun kinase activity was down-regulated irrespective of the presence of SAPKβ(K-R) (Fig. 2B). In agreement with this, the expression of MKP-1, a highly specific MAPK phosphatase implicated in down-regulation of SAPK/JNK (42), was induced by IL-1 within 15 min and sustained over several hours in KB cells as well as in the cells overexpressing SAPKβ(K-R) (data not shown).

SAPK/JNK activity was also impaired in cytosolic extracts of SAPKβ antisense RNA expressing clones. Impairment was even stronger than in the SAPKβ(K-R) expressing clones (Fig. 3, see also Table I). Quantitatively the degree of inhibition on protein and activity level by overexpression of SAPKβ(K-R) or SAPKβ antisense RNA is comparable with the results obtained

3 M. Kracht, data not shown.

Fig. 2. Inhibition of IL-1-induced SAPK/JNK activity by overexpression of SAPKβ(K-R). A, untransfected KB cells (KB, ●), vector transfected cells (KB-vector, ■), and clones overexpressing mutant SAPKβ (KB-SAPKβ(K-R) 2, ▲, and KB-SAPKβ(K-R) 11, ▼) were stimulated for 15 min with IL-1 (10 ng/ml) or left untreated and whole cell extracts prepared. SAPK/JNK activity was measured with GST-Jun (amino acids 1–135) as substrate by the in vitro kinase assay and quantified with a PhosphorImager as described under "Experimental Procedures." The fold increase in SAPK/JNK activation induced by IL-1 obtained from at least six independent experiments is shown. Error bars show standard error of the mean. B, the same cells as in A were stimulated for the indicated times with 10 ng/ml IL-1 or left untreated. Cytosolic extracts were prepared as described in detail under "Experimental Procedures" and SAPK/JNK activity determined. The GST-Jun phosphorylation was analyzed by autoradiography, and quantified with a PhosphorImager (C).
by interfering with the ERK MAPK cascade using the same approaches (38, 39, 43).

IL-1-induced Activation of SAPK Kinase (SAPKK) and p38 MAPK Is Unaltered in Cells Overexpressing SAPKβ(K-R) or SAPKβ Antisense RNA—Kinase-dead mutants have been widely used in transient co-transfection experiments and shown to act as dominant negative inhibitors. We were interested to know at which point of the SAPK cascade the SAPKβ(K-R) mutant was acting as an inhibitor. The mutated enzyme contains an intact regulatory domain and is phosphorylated by partially purified SAPKK in vitro. By interacting with SAPKK, large amounts of SAPKβ(K-R) might prevent its activation in response to IL-1. This prompted us to investigate the regulation of the SAPKK activity, which has not been unequivocally identified in KB cells but may correspond to MKK7 (15, 44, 45), in the cells overexpressing SAPKβ(K-R). We measured IL-1-induced SAPKK activation in a two-stage assay. Partially purified SAPKK was first incubated with recombinant GST-SAPKβ, the latter was then adsorbed to GSH-Sepharose beads and its activity measured on GST-Jun (1–135). Fig. 4 shows that in the two clones overexpressing SAPKβ(K-R) activation of the major IL-1-induced SAPKK was normal in comparison to untransfected or vector-transfected cells. Therefore the SAPKβ(K-R) mutant inhibits activation of the SAPK pathway downstream of SAPKK activation, presumably by competition with the endogenous SAPK/JNK for SAPKK.

The p38 MAPK cascade is strongly stimulated by IL-1 in KB cells. p38 MAPK phosphorylates and activates MAPKAPK-2, which phosphorylates the small heat shock protein hsp27 (13, 46, 47). MAPKAPK-2 is a highly specific substrate for p38 MAPK and we made use of it to assess the activation state of p38 MAPK in the stably transfected cells. IL-1 activation of p38 MAPK in KB cells was not affected by overexpressing SAPKβ(K-R) (Fig. 5A), nor by overexpressing SAPKβ antisense RNA (Fig. 5B), suggesting that there was no interference with this pathway. The ERK MAPK cascade has been found by us to be activated in response to IL-1 in fibroblasts (11, 15) but not in KB cells (12) and was therefore not further investigated in this study.

Activation of NFkB in Clones Overexpressing SAPKβ(K-R) or SAPKβ Antisense RNA—Activation of NFkB is an important effect of IL-1 which has been demonstrated to be essential for IL-1-induced transcriptional regulation. This pathway involves activation of a kinase complex that phosphorylates the inhibitor IκB and is distinct from the known MAPK pathways (7, 8). The results of electrophoretic mobility shift assays showed rapid activation of NFkB in KB cells occurring within 15 to 30 min after IL-1 stimulation. That response was not affected by overexpression of the SAPKβ mutant or antisense RNA (Fig. 6). The data presented in Figs. 2–6 suggest specific inhibition of the SAPK/JNK cascade by expression of mutant SAPKβ or its antisense RNA without interference with other early signaling events induced by IL-1.

Impaired IL-1-induced Expression of IL-6 and IL-8 in KB Cells Overexpressing SAPKβ(K-R) or SAPKβ Antisense RNA—Untransfected and vector-transfected KB cells respond to IL-1 with a rapid induction of IL-6 and IL-8. Their mRNAs are increased from barely detectable levels to high amounts within 1 h, and remain elevated for more than 30 h (Fig. 7). Analysis of their RNAs in the SAPKβ(K-R) transfected clones revealed that this response was markedly impaired, resulting in much lower mRNA levels for both cytokines especially at times following their initial increase. Impairment of IL-6 and IL-8 induction was even more pronounced in the antisense-transfected clones. In those cells the amounts of both transcripts were low already in the early phase of IL-1 stimulation.

TABLE I

Comparison of KB cell clones overexpressing SAPKβ(K-R) or SAPKβ antisense RNA

Results (mean ± S.E.) of SAPK/JNK protein amounts (determined by Western blot, n = 4) and activity (determined as in Figs. 2 and 3, n = 6 to 9) and of IL-1-induced cytokine production (values from Fig. 8) are expressed in comparison to untransfected control cells (=100%).

| cDNA overexpressed          | Clone 2 | Clone 11 | Clone 64 | Clone 165 | Clone 240 |
|-----------------------------|---------|----------|----------|-----------|-----------|
| SAPK/JNK protein            | NE*     | NE       |          |           |           |
| SAPK/JNK activity           | 43 ± 14%| 45 ± 9%  | 26 ± 7%  | 33 ± 15%  | 31 ± 7%   |
| IL-6 synthesis              | 29 ± 6% | 34 ± 11% | 19 ± 3%  | 24 ± 5%   | 20 ± 9%   |
| IL-8 synthesis              | 39 ± 4% | 47 ± 3%  | 8 ± 2%   | 8 ± 2%    | 9 ± 4%    |

* NE, not examined.
The secretion of IL-6 and IL-8 by the transfected and control cells was analyzed after 24 h of IL-1 treatment by specific enzyme-linked immunosorbent assay. As shown in Fig. 8, IL-6 and IL-8 production was decreased in clones overexpressing SAPKβ(K-R), and in clones overexpressing SAPKβ antisense RNA. Of note, suppression was stronger in the latter clones, corresponding to a stronger decrease in their JNK activity (see also Table I). Thus the results obtained on the IL-6 and IL-8 protein level reflect the effects of SAPK inhibition obtained on the mRNA level and confirmed an impaired response to IL-1-induced IL-6 and IL-8 expression in cells manipulated to suppress SAPK/JNK activation.

DISCUSSION

A prominent early effect of IL-1 is activation of SAPK/JNK. Due to the lack of cell permeable specific inhibitors to the enzymes there is still very little information about their function in vivo. Mice bearing null mutations at all three loci could resolve this issue. Unless a particular SAPK/JNK isofrom is essential for a particular process, the existence of so many isofroms might compensate for deficits resulting from null mutations at only one or two loci. Consequently attempts have been made to inhibit the SAPK pathway at the level of the upstream activator(s). Partial inhibition of SAPK/JNK activation by stable overexpression of an inactive MKK4/SEK1 mutant in mouse fibroblasts resulted in enhanced thermotolerance and higher resistance to cytotoxic agents (48). Two recent studies (49, 50) reported MKK4/SEK1 knockouts by gene disruption in mouse embryonic stem cells. Homozygous mutant cells showed a complete inhibition of SAPK/JNK activation in response to anisomycin and heat shock, whereas osmotic stress and UV light still activated the cascade (49, 50), results which are consistent with the existence of additional activators of the SAPK/JNK pathway, such as the recently cloned MKK7 (44, 51). These important experiments therefore do not eliminate the need to examine SAPK/JNK function with regard to stimulus and cell type in vivo.

In this paper we present for the first time evidence for a role of SAPK in IL-1-induced expression of endogenous genes. By stably overexpressing an inactive mutant of SAPKβ in human KB cells we achieved partial inhibition of the SAPK/JNK pathway. Using an alternative approach we strongly inhibited the SAPK/JNK pathway by overexpressing SAPKβ antisense RNA.

We investigated early IL-1-induced signaling events, because one major concern regarding stable overexpression of MAPK mutants in cells is the possible interference with related signaling pathways. According to our previous results IL-1 did not significantly activate the ERK MAPK pathway in KB cells (12), implying that it is not involved in IL-1 effects in these cells. However, IL-1 strongly activated the p38 MAPK (13). The p38 MAPK was likely to be affected, for following reasons. First, SAPK/JNK and p38 MAPK are activated by the same stressful stimuli and inflammatory cytokines. Second, MKK4/SEK1 and MKK7, the only enzymes that activate SAPK/JNK, have also been shown to activate p38 MAPK when overexpressed, suggesting that they can act as common activators of the two MAPK cascades (24, 44, 52). Large amounts of a SAPK mutant may bind to these upstream activators and interfere with their activation by a MAPKK kinase. Third, SAPK/JNK and p38 MAPK phosphorylate common substrates, like ATF-2, a component of AP-1 (22, 53). Phosphorylation and activation of such substrates might be prevented by an excess of a SAPK/JNK mutant.

We found that the SAPKβ(K-R) mutant, although strongly overexpressed, did not interfere with activation of its activator. The most likely explanation for the inhibition of IL-1-induced SAPK/JNK activation is therefore competition of the SAPKβ(K-R) mutant with the endogenous kinases for the activating SAPKK. A competitive inhibitory mechanism would imply that much higher overexpression might be required for full blockade of IL-1 induced SAPK/JNK. We also found that overexpression of SAPKβ(K-R) did not affect p38 MAPK activation, concluding that it did not compete with p38 MAPK for a common activator of both enzymes.

The inhibition of IL-1-induced SAPK/JNK activity by antisense RNA occurs by a mechanism completely different from that of the mutant protein. This approach therefore represented an independent means of confirming the cellular phenotype resulting from overexpression of SAPKβ(K-R). If each approach resulted in inhibition of SAPK/JNK activation they should each cause similar downstream effects. IL-1 activated NFκB and p38 MAPK normally in cells overexpressing either SAPKβ(K-R) or the antisense RNA. Since inhibition of SAPK/
JNK was seemingly specific, we made use of the cell lines to assess the importance of the enzymes for expression of cytokine genes in response to IL-1. We examined gene expression of IL-6 and IL-8, two important mediators generated by IL-1. Both were strongly suppressed at mRNA and protein levels, suggesting that SAPK/JNK activation is crucial for the IL-1-induced formation of IL-6 and IL-8. Interestingly, inhibition of the IL-1-induced SAPK/JNK activity correlated in extent with inhibition of IL-6 and IL-8 gene expression, both being more pronounced with antisense RNA overexpression. Furthermore, the relatively weak SAPK/JNK inhibition upon overexpression of the SAPKβ mutant resulted in decreased IL-6 and IL-8 mRNA levels at late time points of IL-1 treatment while being insufficient to suppress the initial mRNA increase. This indicates that full induction of mRNA expression at that time can occur with submaximal SAPK/JNK activation, while maximal activation of the pathway is required for inducing a sustained increase in expression of the IL-6 and IL-8 genes.

While the antisense RNA approach apparently reduces expression of all SAPK/JNK isoforms to similar extents (Fig. 1B), the SAPKβ(K-R) mutant may preferentially suppress activity of the corresponding human (JNK3) isoforms. If so, the results in Fig. 7 suggest that JNK3 isoforms might be important particularly for the prolonged elevation of IL-6 and IL-8 mRNA. Taken together, the results obtained with both the dominant negative mutant and the antisense RNA overexpression, although not enabling us to relate specifically to certain SAPK/JNK isoforms, strongly suggest a crucial role for members of this kinase family in IL-1-induced expression of IL-6 and IL-8.

Although we cannot exclude the possibility that the cell lines generated have genetic defects in other signaling pathways enabling them to survive selection in the presence of reduced SAPK activity, our data suggest that the molecules required for IL-1 signal transduction are present in the cells. This is shown by the fact that IL-6 and IL-8 gene expression could still be induced by IL-1 in all cell lines overexpressing SAPKβ(K-R) or antisense RNA, although to a much lesser extent.

We can only speculate about the molecular mechanism of the IL-6 and IL-8 inhibition. Expression of both genes is controlled at several levels (54–57). In transient transfection studies ev-
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In summary, the results give new insight into the contribution of the SAPK/JNK pathway to IL-1-induced gene regulation in human cells, leading to the conclusion that each of the major signaling pathways activated by IL-1 contributes in a selective manner to the full pattern of gene induction by IL-1.

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