Interleukin 1 activates jun N-terminal kinases JNK1 and JNK2 but not extracellular regulated MAP kinase (ERK) in human glomerular mesangial cells

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Abstract Interleukin 1 (IL-1) potently activates human glomerular mesangial cells (HMC). In cytosolic extracts of IL-1-stimulated HMC or in anion exchange chromatography fractions we could not find any change in phosphorylation of myelin basic protein (MBP), a good substrate for extracellular regulated kinase (ERK). In contrast, IL-1 stimulated GST-jun kinase activity at least 10-fold. The jun kinase activity could be characterised as JNK1 and JNK2 at the protein and mRNA level. IL-1, TNF, UV light and osmotic stress, but not PMA, LPS, IL-3, IL-4, IL-6, IL-8, IL-10, IL-13, GM-CSF, PDGF, bFGF, TGF-β and interferon-γ were able to stimulate jun kinase activity in HMC, suggesting that jun kinase is selectively mediating signal transduction of the proinflammatory cytokines IL-1 and TNF as well as of cellular stress in HMC.

Key words: Interleukin; Jun N-terminal kinase; Human mesangial cell

1. Introduction

Activated human mesangium cells have been established to play an important role in glomerular inflammatory disease, including glomerulonephritis [1]. IL-1 and, to a lesser extent, TNF-α have emerged as the most potent activators of mesangial cells (MC). IL-1 induces the expression of several genes including growth factors (bFGF, PDGF, GM-CSF, M-CSF), cytokines (IL-1, IL-6), chemokines (IL-8, MCP-1), proteases and matrix components. Therefore, understanding the signal transduction mechanisms induced by IL-1 might be important to generate specific tools to alter mesangial cell function during pathological processes. Little information is available about signal transduction in mesangial cells, mainly through studies on rat cells. It is important to notice that significant differences between human and rat MC exist (reviewed in [2]). For example, IL-1 can induce proliferation of rat mesangial cells [3], but not of human cells [4]. Intracellular protein kinases like the mitogen-activated protein kinases (MAPKs) are key molecules transducing extracellular signals into the nucleus [5]. Depending on the tissue or cell system studied IL-1 has been shown to activate various MAP kinases [6–12]. The aim of the present study was therefore to investigate the regulation of two major MAP kinase pathways, the jun or stress-activated protein kinases and the extracellular regulated kinases, by IL-1 in human mesangial cells.

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Abbreviations: IL-1, interleukin 1; TNF, tumor necrosis factor; JNK, jun N-terminal kinase; SAPK, stress-activated protein kinase; PMA, phorbol 12-myristate 13-acetate; bFGF, basic fibroblast growth factor; PDGF, platelet-derived growth factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; M-CSF, macrophage colony-stimulating factor; LPS, lipopolysaccharide; TGF-β, transforming growth factor β

2. Materials and methods

2.1. Materials

Human recombinant IL-1α was expressed in *E. coli* and purified as described [7]. IL-1β was a gift from Dr. Gallati, Hoffmann LaRoche, Basel, Switzerland; IFN-γ was donated by Dr. B. Otto, Fraunhofer Institute, Hannover, Germany; IL-3, IL-4, IL-13, TNF-α, transforming growth factor β-1 (TGF-β_1) and basic fibroblast growth factor (bFGF) were all from Pharma Biotechnologie, Hannover, Germany; IL-6 was a gift from Dr. W. Fiers, Gent, Belgium; IL-8 was from Dr. I. Lindley, Sandoz, Vienna, Austria; IL-10 was from Laboserv, Giessen, Germany; granulocyte-monocyte colony-stimulating factor (GM-CSF) was generously provided by Dr. K. Welte, Department of Pediatrics, Medical School, Hannover, Germany; platelet-derived growth factor (PDGF) was from Peprotech, London, UK); lipopolysaccharide (LPS) from *E. coli*, E64 (*trans*-epoxysuccinyl-L-leucylamido-(4-guanidino)butane), pepstatin, PMSF (phenylmethanesulphonyl fluoride) and all other chemicals were from Sigma, Deisenhofen, Germany. [γ-^{32}P]ATP was purchased from Hartmann Analytic, Braunschweig, Germany.

The cDNA of the truncated jun protein comprising amino acids 1–135 was a kind gift of Prof. C. Marshall, Institute for Cancer Research, Chester Beatty Laboratories, London, UK. The cDNA for GST p54 MAP kinase β was a kind gift of Dr. J.R. Woodgett (The Ontario Cancer Institute, Toronto, Ont., Canada). GST fusion proteins were expressed and purified from *E. coli* as glutathione S-transferase fusion proteins [8]. The mouse anti-human JNK1 antibody (clone G151-333.8) was from Pharmingen, San Diego, CA, USA. The mouse pan anti-ERK antibody was from Transduction Laboratories, Lexington, KY, USA. The chicken anti-p54 MAP kinase β antibody was produced by immunising chicken with recombinant GST p54 MAP kinase β and purifying the immunoglobulins from egg white.

2.2. Human glomerular mesangial cell culture

HMC, isolated and characterised as described [4], were cultured in RPMI-1640 medium supplemented with nonessential amino acids, L-glutamine (2 mM), sodium pyruvate (2 mM), transferrin (5 μg/ml), insulin (125 U/ml) (all Life Technologies, Eggenstein, Germany) and 10% fetal calf serum (PAA laboratories, Linz, Austria).

2.3. Stimulation and preparation of cellular extracts

After mild trypsinisation, equal cell numbers (4×10000 cells/well) of HMC were seeded in 24-well plates and grown to subconfluence. HMC were activated by adding stimuli to the culture medium and incubating the cells at 37°C, 5% CO_2 for the indicated times. All further steps were carried out at 4°C.

After stimulation the cells were washed in phosphate-buffered saline (PBS) and resuspended by scraping them in lysis buffer (20 mM Tris, pH 7.4, 1 mM EGTA, 1 mM EDTA, 0.2 mM Na_3VO_4, 50 mM NaF, 2 mM dithiothreitol (DTT)) including protease inhibitors (10 μM E64,

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1 mM PMSF, 1 μM pepstatin). For MonoQ chromatography cells were broken mechanically by passaging them three times through a 26-gauge needle. Broken cells were centrifuged at 100 000 × *g* at 4°C and the supernatants taken as cytosolic extract. Alternatively cells were lysed by adding 0.5% NP-40 to the lysis buffer and leaving them on ice for 20–30 min. Detergent lysates were cleared by centrifuging them at 15 000 × *g* for 30 min at 4°C and supernatants collected.

2.4. Protein kinase assays

The assays were carried out in a reaction mixture (15 μl) containing 5 μl of sample, 5 μl of GST-jun or MBP (0.5–1 μg) and 5 μl of 150 mM Tris, pH 7.4, 30 mM $MgCl_2$ and 60 μM ATP (including 1–3 μCi of [γ^{32}P]ATP. Assays were started by adding ATP and incubated at room temperature for 20 min. The phosphorylation reaction was terminated by adding 5 μl of electrophoresis sample buffer (8% SDS, 100 mM Tris, pH 6.8, 4% β-mercaptoethanol, 24% glycerol, 0.02% bromophenol blue). The phosphorylated substrates were separated from the reaction mixture by SDS/PAGE and analysed by autoradiography as described [6]. Autoradiographs were quantitated using a GelDoc 100 System and the Molecular Analyst Program (BioRad, Hercules, CA, USA).

2.5. Western blotting

Proteins were separated on SDS/PAGE and electrophoretically transferred to PVDF membranes (Immobilon® Millipore, Bedford, MA, USA). The membranes were blocked with 5% dried milk in Tris-buffered saline (TBS) for 30 min at 37°C. Membranes were then incubated for 4–24 h with chicken antibody raised against recombinant GST p54 MAP kinase β protein or with the mouse anti-pan ERK antibody. Blots were washed in TBS, and incubated for 2–4 h with the second antibody (rabbit anti-chicken or sheep anti-mouse, coupled to horseradish peroxidase, Sigma, Germany). Proteins were detected using the Amersham enhanced chemiluminescence (ECL) system.

2.6. Immunoprecipitation

A cytosolic extract from one 75-cm^2 flask of stimulated or untreated confluent HMC was incubated with 1 μg mouse IgG1 for 1 h. Then 20 μl of protein G sepharose was added and incubation carried out for another hour. Samples were spun in a microfuge for 10 min and the precleared supernatant incubated with 0.5 μg of mouse α-JNK1 antibody for 2 h. Then 20 μl of protein G Sepharose beads were added for 1 h. Beads were pelleted in a microfuge, washed 5× in PBS and 1× in kinase reaction buffer and resuspended in 10 μl lysis buffer. Immunoprecipitated jun kinase activity was measured by adding 20 μl of kinase buffer including GST jun as described above.

2.7. Anion exchange chromatography

Equal amounts of cytosolic protein from stimulated or unstimulated HMC (six 175-cm^2 flasks each) were loaded on to a MonoQ HR5/5 column run by a FPLC system (Pharmacia). The column was equilibrated in buffer A (20 mM Tris, pH 8.5, 20 mM β-glycerophosphate, 50 mM NaF, 0.1 mM Na_3VO_4, 2 mM DTT, 0.5 mM EGTA, 0.5 mM EDTA). Proteins were eluted with a linear salt gradient (0–0.5 M NaCl). 1-ml fractions were collected and assayed for kinase activity.

2.8. Polymerase chain reaction

Total cellular RNA from HMC was isolated with RNA clean (AGS, Heidelberg, Germany) followed by a $LiCl_2$ precipitation and determined spectrophotometrically. 1 μg of total RNA was reverse transcribed and amplification with 40 cycles of the resulting cDNA performed in a thermocycler Varius VR (Landgraf, Hannover, Germany) as described [2,11]. 15 μl of 100 μl PCR reaction mixture was restricted with *Pst*I or left untreated and analysed on 1.5% agarose gels. DNA-containing bands were visualised by ethidium bromide staining. Sequences for JNK primer pairs according to published sequences for JNK1 (accession no. L 26318) and JNK2 (accession no. UO 9759):

JNK1, sense (5′–3′):
GCG CGG ATC CTT GCT TGC CAT CAT GAG CAG;

JNK1, antisense (5′–3′):
GCG CGG ATC CCA GAC GAC GAT GAT GAT GGA.

JNK2, sense (5′–3′):
GCG CGG ATC CTG CAT CAT GAG CGA CAG TAA;

JNK2, antisense (5′–3′):
GCG CGG ATC CAA CCT ATC ATC GAC AGC CTT.

3. Results

Initial experiments showed that IL-1 did not induce MBP phosphorylation in HMC (Fig. 1A,B). In cytosolic extracts of cells stimulated with bFGF or PMA increased MBP kinase activity was detected indicating that activation of the ERK group of MAP kinases can be measured under those conditions (Fig. 1C,D).

In contrast IL-1 stimulated jun kinase activity 10–15-fold in comparison to untreated cells (Fig. 1A). IL-1-activated jun kinase activity was detectable after 5 min and peaked between 15 and 30 min of stimulation. After 60 min activity was declining, but still detectable. Immunoprecipitation experiments identified JNK1 as one of the activated JNK isoforms (Fig. 2)

To investigate whether one or more isoforms of JNKs are present and activated in HMC we fractionated cytosolic extracts from stimulated or untreated HMC on anion exchange chromatography (Fig. 3). Most of the jun kinase activity eluted from the column at 200–300 mM NaCl (fractions 5–13) and was strongly regulated by IL-1 (Fig. 3A). This chromatographic behaviour is typical for the stress-activated protein kinases as we have shown during the purification of p54 MAP kinase [7,8]. An anti-p54 MAPK-β antiserum, which cross-reacts with 50- and 55-kDa forms of p54 MAP-α and 45/46-kDa jun kinases in KB epidermal carcinoma and HepG2 hepatoma cells (M. Kracht, unpublished results) recognised two major protein bands with molecular masses of about 46 and 55 kDa on SDS/PAGE in MonoQ fractions 5–13 (Fig. 3B). The proteins comigrated with the jun kinase activity during chromatography. This strongly suggests that IL-1 activates two forms of jun kinase activity in HMC. They most likely represent JNK1 and JNK2 for which a M_r of 46 and 55 kDa has been described.

There was no change in total MBP phosphorylation in fractions 9–17 from extracts of untreated cells or IL-1-stimulated cells (Fig. 3C). The MBP kinase activity coeluted with the 42-kDa MAP kinase ERK2, which was detected by an anti-pan ERK antibody (Fig. 3D).

The expression of JNK1 and JNK2 was further analysed by RT-PCR using specific primer pairs. As shown in Fig. 4, cDNAs of the expected size, 1250 bp for JNK1 and 1300 bp for JNK2, were the only amplified PCR products. The identity of the cDNAs was confirmed by restriction of the PCR products with the endonuclease *Pst*I, which cleaves only the JNK1 cDNA, generating fragments of 527 and 625 bp. The results demonstrate that HMC express both mRNAs for JNK1 and JNK2. It is therefore likely that both kinases are activated by IL-1 in these cells.

HMC respond to a very wide range of stimuli including growth factors, cytokines and chemokines. Thus they represent an excellent system to study the selectivity of jun kinase activation in non-transformed primary cells. Therefore we assayed jun kinase activity upon stimulation with TNF, UV light and osmotic shock (NaCl, sorbitol) as well as cytokines (IL-3, IL-4, IL-6, IL-8, IL-10, IL-13, IFN-γ, TGF-β), tumor promoters (PMA), bacterial cell wall products (LPS) and

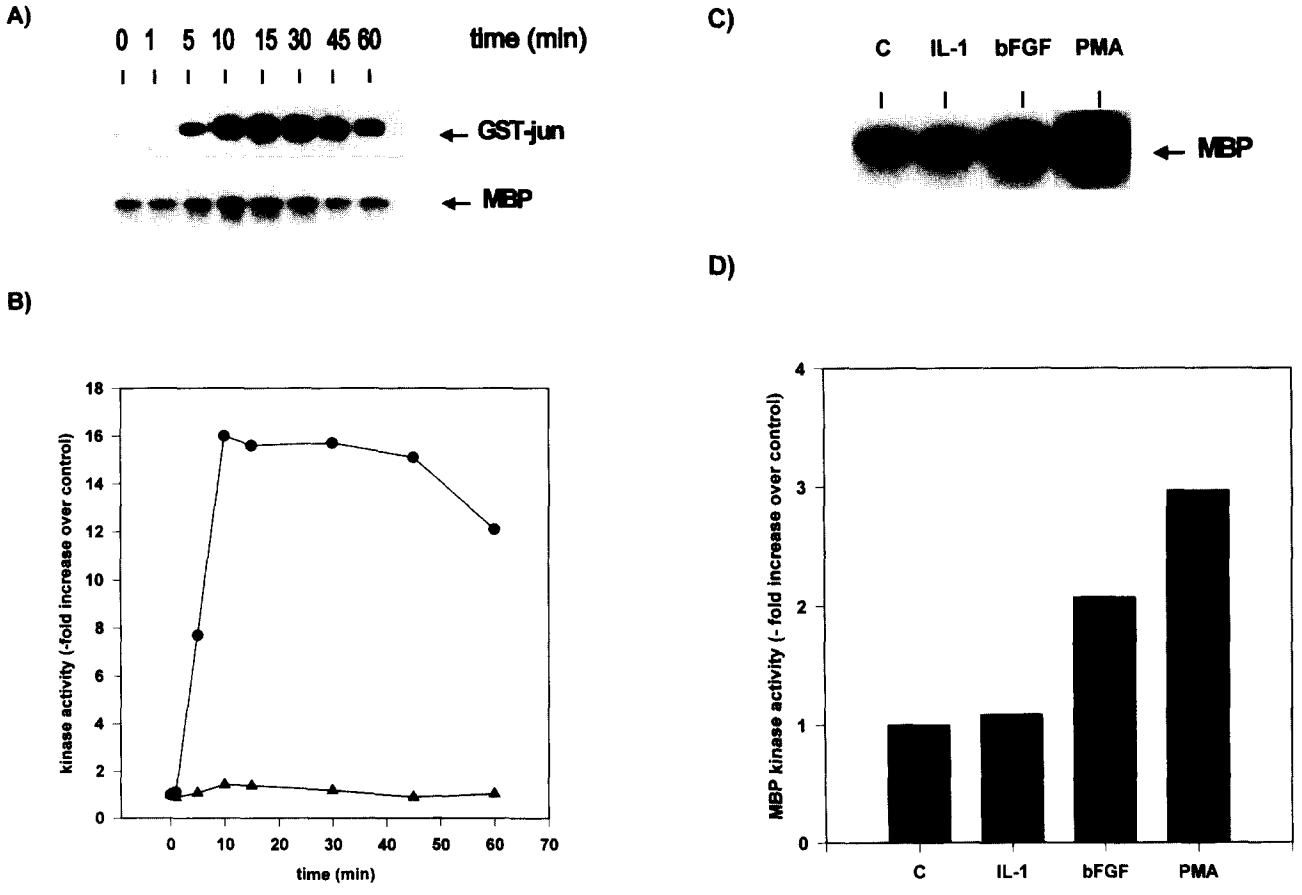

Fig. 1. IL-1 stimulates c-jun but not MBP kinase activity in cytosolic extracts of HMC. HMC were stimulated with IL-1β (20 ng/ml) for the indicated times. Then cytosolic extracts were prepared and jun and MBP kinase activity measured. A region of the autoradiogramm visualising substrate phosphorylation (A) and quantitation of the increase in substrate phosphorylation for jun (●) and MBP (▲) in comparison to untreated cells is shown (B). HMC were also stimulated with IL-1β (20 ng/ml), PMA (100 ng/ml) and bFGF (10 ng/ml) for 20 min and MBP kinase activity in cytosolic extracts measured. The phosphorylated proteins were separated on SDS/PAGE and detected by autoradiography (C) and quantitated (D). See Section 2 for details.

growth factors (GM-CSF, PDGF, bFGF). As shown in Fig. 5, only IL-1 and TNF, and the cell-stressing agents UV light, sorbitol and NaCl were able to stimulate jun kinase activity. The simultaneous activation by IL-1 and growth factors like PDGF or FGF had no additive or synergistic effect on IL-1-induced jun kinase activation, nor did activation by osmotic stress (NaCl or sorbitol) and IL-1 together.

4. Discussion

There is good evidence that IL-1 can activate the p42/p44 ERK group of MAP kinases in rat mesangial cells, based on immunoprecipitation experiments and gel kinase assays with MBP as substrate [13,14]. Activated ERKs are thought to play a role in cell proliferation by using overexpression of dominant negative mutants or antisense RNA [15,16]. In contrast we could not measure increased MBP kinase activity in cytosolic extracts or in MonoQ fractions of IL-1-stimulated human mesangial cells under conditions where the phorbol ester PMA or bFGF did induce MAP kinase activity. The lack of ERK activation by IL-1 is consistent with our observation that IL-1 is not a growth factor for human mesangial cells [4].

In this report we demonstrate for the first time that IL-1 is a potent inducer of jun kinases in HMC. Two protein kinases

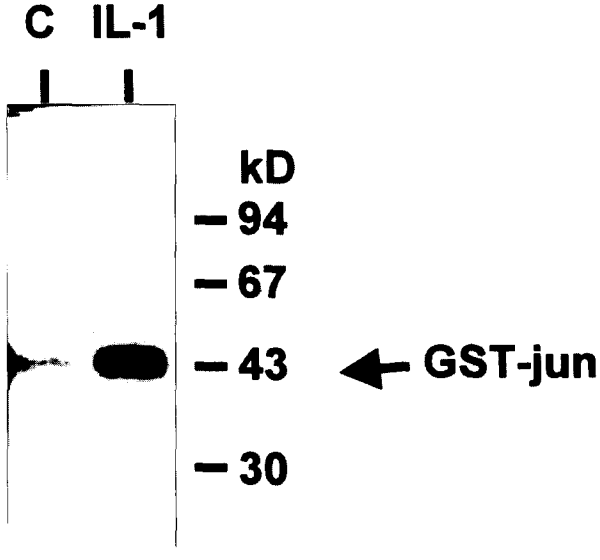

Fig. 2. The IL-1-stimulated jun kinase activity can be immunoprecipitated with an antibody against JNK1. HMC were stimulated for 20 min with IL-1α. Cells were lysed and jun kinase activity was measured in immunoprecipitates as described in Section 2. The phosphorylated jun protein was separated on SDS/PAGE and detected by autoradiography.

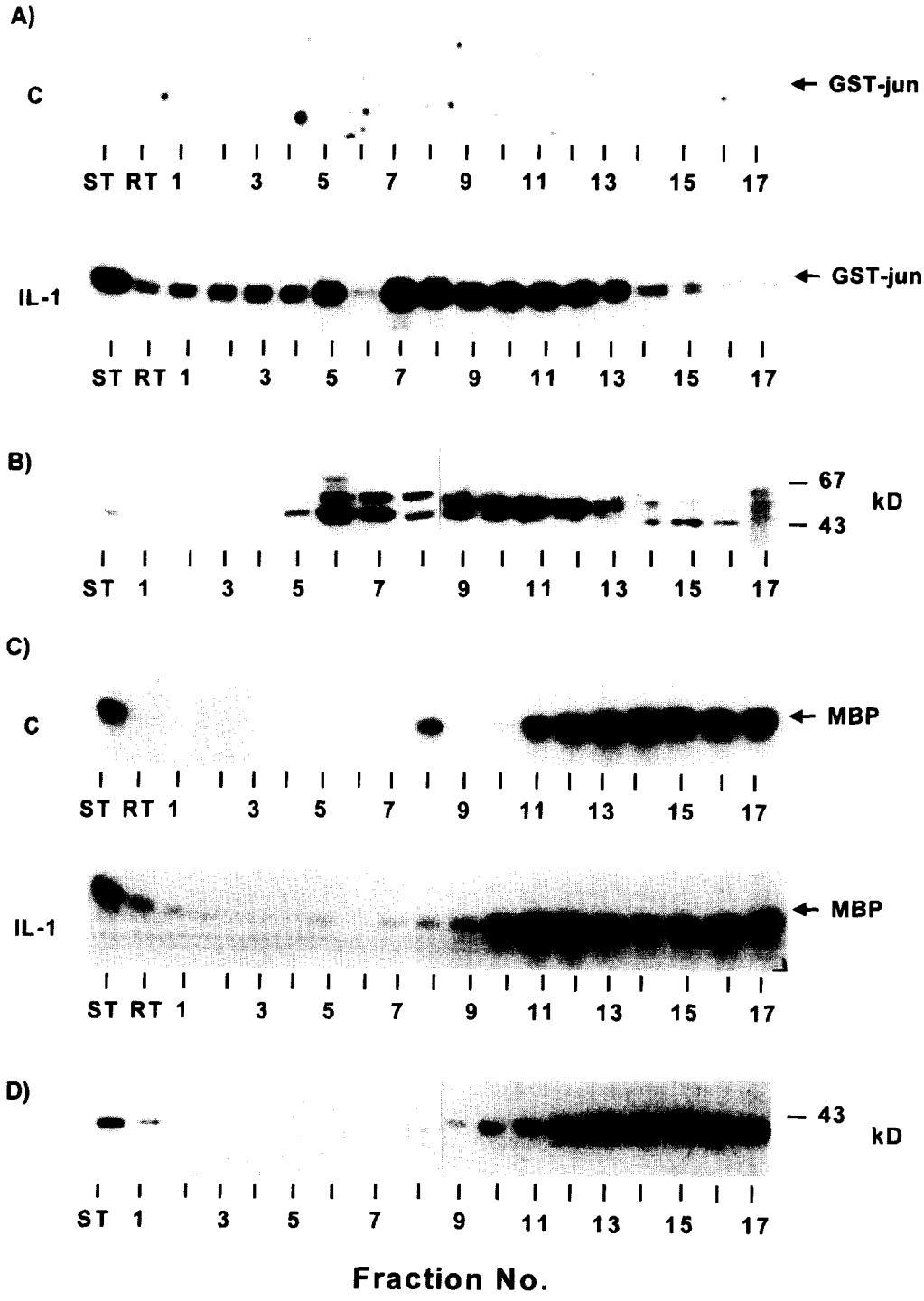

Fig. 3. Identification of jun kinases and extracellular regulated kinases in anion exchange chromatography fractions from resting or stimulated HMC. HMC were stimulated with IL-1α for 20 min or left untreated. A cytosolic extract was prepared. Equal amounts of protein were than loaded on to a MonoQ column and eluted with a linear salt gradient (0–0.5 M NaCl) in 20 fractions of 1 ml. A: Jun kinase activity was measured in aliquots of fractions. B: Proteins from 50 μl of each fraction were separated on SDS/PAGE, transferred to membranes and probed with an antibody against GST p54 MAPK β. C: MBP kinase activity in aliquots of the fractions. D: Proteins from 50 μl of each fraction were separated on SDS/PAGE, transferred to membranes and probed with an antibody against ERKs. Chromatography, protein kinase assays, separation of phosphorylated proteins on SDS/PAGE, detection by autoradiography and Western blotting were performed as described in Section 2. ST = material loaded on to the column, RT = material that did not bind to the column.

with a M_r of 46 and 55 kDa were identified by specific antibodies. They are very likely to represent the two human isoforms of stress-activated protein kinases, JNK1 and JNK2, since we could also detect the mRNA of both these kinases. Identifying as precisely as possible JNK or SAPK isoforms expressed and activated within a cell or tissue is important, since it is unclear whether isoforms can display functional differences. Several isoforms of JNKs or SAPKs have been cloned or purified so far. In the rat three genes, encoding p54 MAP kinase α, β, and γ, and alternatively spliced variants

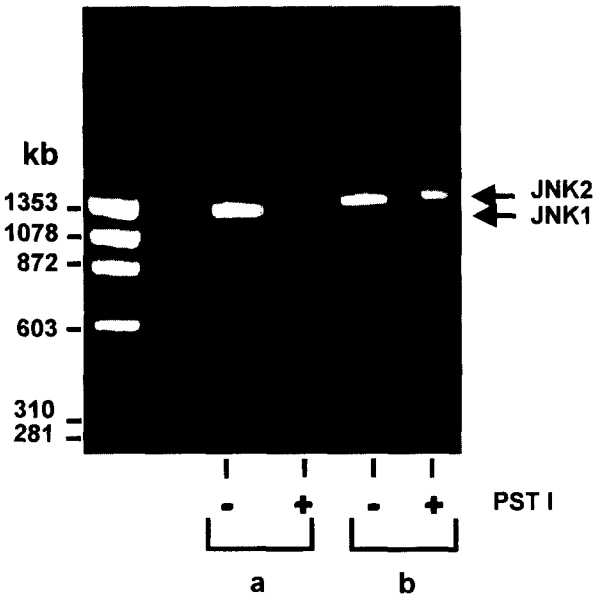

Fig. 4. HMC express mRNA for JNK1 and JNK2. RNA from HMC was isolated and RT-PCR with primer pairs specific for JNK1 (a) and JNK2 (b) performed as described in Section 2. The resulting PCR products were left untreated (−) or digested with *Pst*I (+) and separated on a 1.5% agarose gel. A photography of the ethidium bromide-stained gel is shown, JNK1 and JNK2 cDNAs are indicated by arrows. DNA size markers (ϕ174 DNA digested with *Hae*I) are shown in the left lane.

have been cloned [17]. Up to now two human cDNAs encoding JNK1 and JNK2 have been cloned [18,19]. All genes are highly homologous and encode proteins with a molecular mass between 46 kDa (JNK1, p54 MAP kinase γ) and 5a5 kD (JNK2, p54 MAP kinase α and β) when expressed in COS cells. Recently we purified from rat liver two 50- and 55-kDa forms of IL-1-activated p54 MAP kinase α [8] and an IL-1-activated 46-kDa SAP kinase from the human epidermal cell line KB [7].

In agreement with our data one recent report describes the activation of two jun kinases by IL-1 and protein synthesis inhibitors in rat MC [20]. The kinases were detected in gel kinase assays but not further characterised, and their relation to the cloned rat enzymes (see above) remains unclear.

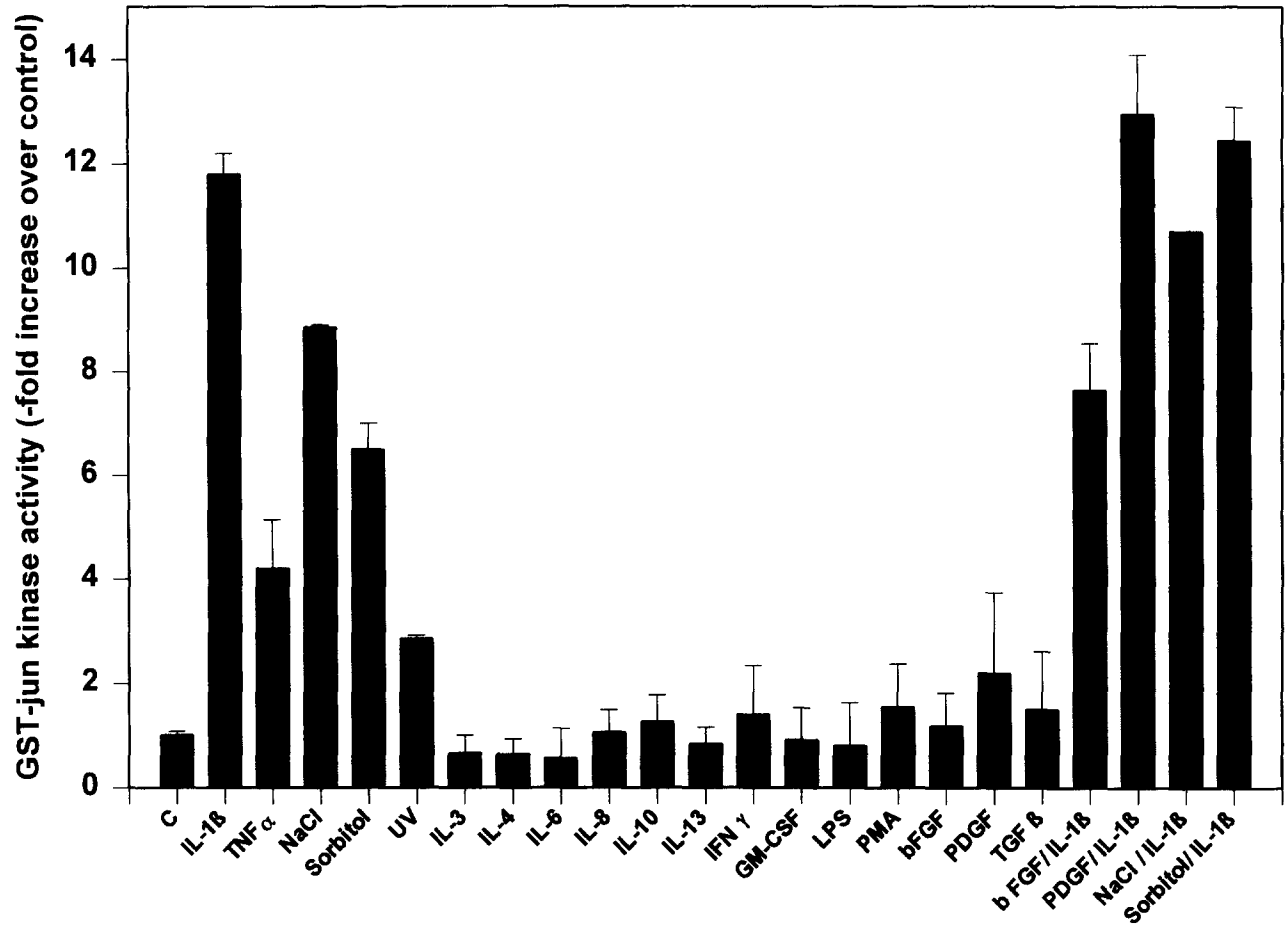

Fig. 5. Selective activation of jun kinase activity by IL-1, TNF and stress in HMC. Confluent HMC were left untreated (C) or stimulated for 15 min with the following stimuli: IL-1β (20 ng/ml), TNF-α (1000 U/ml), NaCl (400 mM), sorbitol (500 mM), UV light (312 nm), IL-3 (50 ng/ml), IL-4 (10 ng/ml), IL-6 (1 ng/ml), IL-8 (50 ng/ml), IL-10 (50 ng/ml), IL-13 (10 ng/ml), interferon-γ (IFN-γ, 500 U/ml), GM-CSF (50 ng/ml), LPS (10 μg/ml), PMA (100 ng/ml), bFGF (10 ng/ml), PDGF (10 ng/ml), TGF-β (50 ng/ml) and the combinations of IL-1β with bFGF, PDGF; NaCl or sorbitol. After stimulation cells were lysed with NP-40 and jun kinase activity measured. Phosphorylated jun protein was separated on SDS/PAGE. ^{32}P incorporated into jun substrate was visualised by autoradiography and quantitated as described in Section 2. Shown is the increase in jun phosphorylation induced by the different stimuli compared to untreated cells from three independent experiments.

In an extension of our study we were also interested in the effects of other extracellular stimuli which can act on HMC and for which the expression of receptors has been shown. Very interestingly TNF, osmotic stress and UV light were the only other stimuli that were effective in activating jun kinase. To our knowledge this is the only study where such a wide range of cytokines has been tested for the ability to activate jun kinases.

The combination of IL-1 and bFGF synergistically induces PGE2 synthesis and secretion of chemokines like RANTES, MCP-1 and IL-8 (P. Uciechowski, M. Schwarz, V. Kaever, K. Resch and H.H. Radeke, manuscript in preparation). However, we did not observe any effect of bFGF or PDGF on IL-1-induced jun kinase activation. Both growth factors activate the classical MAP kinase cascade in rat MC, as has been shown by activation of raf, MAP kinase and ERK2 [21]. The synergistic action of IL-1 and for example bFGF on gene expression in human MC could therefore be a result of the convergence of different MAP kinase pathways, rather than enhanced activation of the same pathway.

In summary, we present evidence that a novel MAP kinase pathway is activated in HMC by IL-1, involving JNK1 and JNK2. We also show that IL-1 does not activate the ERK group of MAP kinases in these cells. The striking selectivity of JNK activation by IL-1 and TNF potentially makes this protein kinase pathway a target for pharmacological intervention, at least in those inflammatory or stress-dependent pathological processes of the kidney where glomerular mesangial cells are involved.

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References

[1] Sedor, J.R., Konieczkowski, M., Huang, S., Gronich, J.H., Nakazato, Y., Gordon, G. and King, C.H. (1993) Kidney Int. 43, 65–70.
[2] Davies, M. (1994) Kidney Int. 45, 320–327.
[3] Lovett, D.H., Ryan, J.L. and Sterzel, R.B. (1983) J. Immunol. 131, 2830–2836.
[4] Francki, A., Uciechowski, P., Floege, J., von der Ohe, J., Resch, K. and Radeke, H.H. (1995) Am. J. Pathol. 147, 1372.
[5] Cano, E. and Mahadevan, L.C. (1995) Trends Biochem. Sci. 20, 117–122.
[6] Guesdon, F., Freshney, N., Waller, R.J., Rawlinson, L. and Saklatvala, J. (1993) J. Biol. Chem. 268, 14343–14352.
[7] Kracht, M., Shiroo, M., Marshall, C.J., Hsuan, J.J. and Saklatvala, J. (1994) Biochem. J. 302, 897–905.
[8] Kracht, M., Truong, O., Totty, N.F., Shiroo, M. and Saklatvala, J. (1994) J. Exp. Med. 180, 2017–2025.
[9] Bird, T.A., Kyriakis, J.M., Tyshler, L., Gayle, M., Milne, A. and Virca, G.D. (1994) J. Biol. Chem. 269, 31836–31844.
[10] Raingeaud, J., Gupta, S. Rogers, J.S., Dickens, M., Han, J., Ulevitch, R.J. and Davis, R.J. (1995) J. Biol. Chem. 270, 7420–7426.
[11] Saklatvala, J., Rawlinson, L.M., Marshall, C.J. and Kracht, M. (1993) FEBS Lett. 334, 189–192.
[12] Freshney, N., Rawlinson, L.M., Guesdon, F., Jones, E., Cowley, S., Hsuan, J. and Saklatvala, J. (1994) Cell 78, 1039–1049.
[13] Huwiler, A. and Pfeilschifter, J.(1994) FEBS Lett. 350, 135–138.
[14] Sugiura, T., Wada, A., Itoh, T., Tojo, H., Okamoto, M., Imai, E., Kamada, T. and Ueda, N. (1995) FEBS Lett. 370, 141–145.
[15] Pages, G., Lenormand, P., L'Allemain, G., Chambard, J.-C., Meloche, S. and Pouyssegur, J. (1993) Proc Natl. Acad. Sci. USA 90, 8319–8323.
[16] Seger, R., Seger, D., Reszka, A.A., Munar, E.S., Eldar-Finkelmann, H., Dobrowolska, G., Jensen, A.M., Campbell, J.S., Fischer, E.H. and Krebs, E.G. (1994) J. Biol. Chem. 269, 25699–25709.
[17] Kyriakis, J.M., Banerjee, P., Nikolakaki, E., Dai, T., Rubie, E.A., Ahmad, M.F., Avruch, J. and Woodgett, J.R. (1994) Nature 369, 156–160.
[18] Kallunki, T., Su, B., Tsigelny, I., Sluss, H.K., Derijard, B., Moore, G., Davis, R.J. and Karin, M. (1994) Genes Dev. 8, 2996–3007.
[19] Derijard, B., Hibi, M., Wu, I.-H., Barrett, T., Su, B., Deng, T., Karin, M. and Davis, R.J. (1994) Cell 76, 1025–1037.
[20] Guan, Z., Tetsuka, T., Baier, L.D. and Morrison, A.R. (1996) Am. J. Physiol. 270, F634–641.
[21] Huwiler, A., Stabel, S., Fabbro, D. and Pfeilschifter, J. (1995) Biochem. J. 305, 777–784.