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Interleukin-1 stimulates de novo synthesis of mitogen-activated protein kinase in glomerular mesangial cells

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Abstract

Interleukin-1 (IL-1) stimulates a time- and concentration-dependent mitogen-activated protein (MAP) kinase activation in rat mesangial cells. A rapid increase in activity (maximal at 10 min) is followed by a second persistent level of activity which steadily increases over 24 h. The second peak of MAP kinase activity is paralleled by a marked de novo synthesis of p42 MAP kinase as measured by immunoprecipitation of [^{35}S]methionine-labelled mesangial cells and by a 60% increase in total p42 MAP kinase protein as detected by Western blot analysis. We propose that IL-1 induced de novo synthesis of p42 MAP kinase is important for the multiplicity of long-term actions of this cytokine in renal mesangial cells.

Key words: Interleukin-1; MAP kinase; Mesangial cell

1. Introduction

Interleukin 1 (IL-1) is the prototype of an inflammatory cytokine that induces the expression of a variety of protein factors that, in turn, trigger acute and chronic inflammatory processes [1]. The local release of growth factors such as IL-1 or tumour necrosis factor α from monocytes is an early event associated with inflammatory reactions and may be an important pathogenetic determinant of structural and functional alterations accompanying immune injury in many organs including the kidney [1].

The mesangium is a highly specialized pericapillary tissue that is involved in most pathological processes of the renal glomerulus. The cross-talk between intrinsic glomerular cells and invading immune cells is of central importance in the pathogenesis of glomerular injury. Three prominent features of intrinsic mesangial cells evolve as a result of the cross-communication with invading monocytes and macrophages: increased mediators production, increased matrix synthesis and increased mesangial cell proliferation [2]. IL-1 has been reported to stimulate the release of a specific type IV collagenase [3], of a group II phospholipase A_2 [4–6], and of prostaglandins [4,7,8] by mesangial cells. IL-1 also triggers the expression of an inducible macrophage-type of nitric oxide synthase [9,10] and a variety of chemokines and cytokines [11] in mesangial cells. Moreover, IL-1 is a co-mitogen in cultured mesangial cells [12,13] and stimulates the rapid phosphorylation of several proteins in the cells [14]. The signalling pathways of IL-1 linking receptor occupancy to cellular responses are not yet known. The IL-1 receptor has no intrinsic tyrosine kinase activity and it has been demonstrated that IL-1 does not activate protein kinase C or protein kinase A in mesangial cells [15]. Nevertheless, one of the earliest cellular responses observed after IL-1 exposure is the phosphorylation of a variety of proteins in mesangial cells and other cell types. Recently, IL-1 has been shown to induce mitogen-activated protein (MAP) kinase activity in fibroblasts and the human epidermoid carcinoma cell line KB [16–18]. MAP kinases are important intermediates in signal transduction pathways stimulated by growth factors, hormones, neurotransmitters and mitogenic agents [19–21]. To date, three distinct MAP kinases have been isolated. p44 MAP kinase and p42 MAP kinase are closely related to each other, whereas the third form encodes a more distantly related kinase that is only 42% homologous to p44 MAP kinase [19–21]. MAP kinases appear to be highly conserved across species which may attest their important functions in signalling processes. Activation of MAP kinases results from phosphorylation of adjacent Tyr and Thr residues within MAP kinase by a dual specificity protein kinase termed MAP kinase kinase [19–21].

Here we show that IL-1 stimulates a biphasic activation of MAP kinase activity in mesangial cells and induces a marked de novo synthesis of p42 MAP kinase. To our knowledge, this is the first report on increased biosynthesis of MAP kinase in response to agonist stimulation.

2. Materials and methods

2.1. Cell culture

Rat renal mesangial cells were cultivated as described previously [4,10]. In a second step, single cells were cloned by limited dilution on 96-microwell plates. Clones with apparent mesangial cell morphology

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were used for further processing. The cells were grown in RPMI 1640 supplemented with 10% (v/v) fetal-calf serum, penicillin (100 units/ml), streptomycin (100 μg/ml) and bovine insulin (0.66 units/ml). For the reported experiments, passages 8–19 of mesangial cells were used.

2.2. MBP kinase assay

Quiescent mesangial cells in 60 mm-diameter dishes were stimulated with the indicated concentrations of agonists. To stop the reaction, the cells were washed with PBS and scraped into 0.5 ml of kinase buffer (20 mM Tris-HCl, pH 7.5/1 mM EGTA/2 mM $MnCl_2$/0.1 mM sodium orthovanadate/1 mM phenylmethanesulphonyl fluoride/25 μg/ml leupeptin). Cells were then homogenized by passes through a 26-gauge needle, centrifuged for 5 min at 14,000 × *g* and then supernatant was taken for determination of protein concentration. 50 μg of cell extracts were incubated for 15 min at 30°C in the presence of 20 μg of MBP, 10 μM ATP and 2 μCi [γ-^{32}P]ATP. The reaction was terminated by adding 40 μl Laemmli dissociation buffer and subjected to SDS-PAGE (13% acrylamide gel). After fixing in 25% isopropanol, 10% acetic acid, the gels were exposed to Hyperfilm MP for 3–5 h.

2.3. Cell labelling and immunoprecipitation

Confluent mesangial cells in 100 mm diameter dishes were washed with PBS and incubated in methionine-free DMEM in the absence or presence of the stimulators for the indicated time periods. For the last 4 h of incubation [^{35}S]methionine was added (100 μCi/plate). After labeling, cells were washed twice with icecold PBS. Cells were then scraped directly into 1 ml of ice-cold lysis buffer (50 mM Tris-HCl, pH 7.5/150 mM-NaCl/10% glycerol/1% Triton X-100/2 mM EDTA/2 mM EGTA/40 mM β-glycerophosphate/50 mM sodium fluoride/10 mM sodium pyrophosphate/200 μM sodium orthovanadate/10 μg of leupeptin/ml/200 U of aprotinin/ml/1 μM pepstatin A/1 mM phenylmethanesulphonyl fluoride) and homogenized with 10 passes through a 26-gauge needle. The homogenate was centrifuged for 10 min at 14,000 × *g* and the supernatant taken for immunoprecipitation. Samples of 1 ml volume, containing 100 × 10^6 cpm of labelled proteins, 5% fetal calf serum and 1.5 mM iodoacetamide in lysis buffer, were incubated for 4 h at 4°C with a specific p42 MAP kinase antibody [22], diluted as indicated in the figure legends. Then 100 μl of a 50% slurry of protein A-Sepharose 4B-CL in PBS was added and the mixture was incubated for 1.5 h at room temperature under mild shaking. After centrifugation for 5 min at 3000 × *g* immunocomplexes were washed 3 times with 1 ml of a low salt buffer (50 mM Tris-HCl, pH 7.5/150 mM NaCl/0.2% Triton X-100/2 mM EDTA/2 mM EGTA/0.1% SDS), 3 times with 1 ml of a high salt buffer (50 mM Tris-HCl, pH 7.5/500 mM NaCl/0.2% Triton X-100/2 mM EDTA/2 mM EGTA/0.1% SDS) and once with 1 ml of 10 mM Tris. Pellets were boiled for 5 min in 50 μl Laemmli dissociation buffer and subjected to SDS-PAGE (10% acrylamide gel). After fixing in 25% isopropanol/10% acetic acid, the gels were soaked for 30 min in 16% sodium salicylate solution and then dried and exposed to Hyperfilm MP at −70°C.

2.4. Chemicals

Protein A-Sepharose 4B-CL war purchased from Pharmacia Fine Chemicals, Uppsala, Sweden; [^{35}S]methionine (specific activity 55.5 TBq/mmol), [^{32}P]orthophosphate and hyperfilm MP were from Amersham International, Zürich, Switzerland; human recombinant IL-1β was kindly provided by Dr. Klaus Vosbeck, Ciba Ltd., Basel, Switzerland; A antiserum which specifically recognized the p42-isoform was kindly provided by Dr. M.J. Dunn, Cleveland (α1, CP42) [22], all cell culture nutrients were from Gibco BRL, Basel, Switzerland; all other chemicals were from Merck, Darmstadt, Germany or Fluka, Buchs, Switzerland.

3. Results

IL-1β is a potent stimulator of MAP kinase activity in mesangial cells and induces a biphasic activation of the enzyme as shown in Fig. 1. A first peak of activity is observed as early as 10 min after exposure of cells to IL-1β (1 nM) and is followed by a second increase in activity which steadily increases over 24 h (Fig. 1). The degree of activation of MAP kinase, measured after 10 min exposure of mesangial cells to IL-1β is concentration-dependent (Fig. 2) and is paralleled by an increased phosphorylation of p42 MAP kinase as determined by immunoprecipitation from $^{32}P_i$-labelled mesangial cells (Fig. 3). To study whether IL-1β has an effect on de novo synthesis of MAP kinase, mesangial cells were stimulated with IL-1β (1 nM) for different time periods and

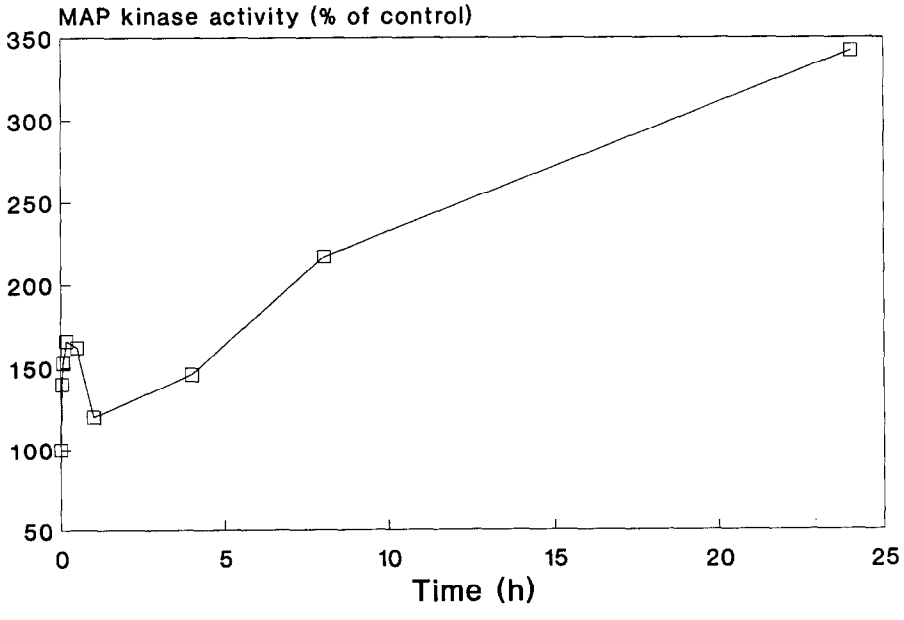

Fig. 1. Kinetics of MAP kinase activation of IL-1β. Confluent mesangial cells were stimulated with IL-1β (1 nM) for the indicated time periods and MAP kinase-stimulated MBP phosphorylation was measured as described in section 2.

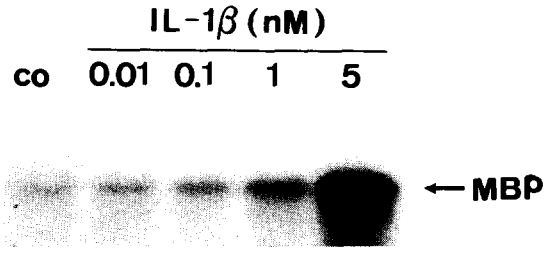

Fig. 2. Concentration-dependency of IL-1β stimulated MAP kinase activation. Confluent mesangial cells were stimulated for 2 min with the indicated concentrations of IL-1β and MAP kinase-stimulated MBP phosphorylation was measured as described in section 2.

[^{35}S]methionine was added for the last 4 h of the stimulation period. p42 MAP kinase was immunoprecipitated with a specific polyclonal antibody and subjected to SDS-PAGE. Whereas under basal conditions only a faint band of p42 MAP kinase labelling is detectable (Fig. 4), addition of IL-1β dramatically increases biosynthesis of the enzyme after 4 and 8 h of stimulation (Fig. 4). This increased de novo synthesis of p42 MAP kinase is also detectable by Western blot analysis as after 24 h stimulation of mesangial cells with IL-1β (1 nM) there is an approximately 60% increase in p42 MAP kinase protein as evaluated by densitometry.

4. Discussions

IL-1 is a major product of activated monocytes and is also released by many cell types, including mesangial cells, when exposed to an inflammatory environment. IL-1 exerts a wide range of activities and contributes to the pathogenesis of haemodynamic shock, as well as to many inflammatory and degenerative diseases [23]. These biological activities of IL-1 are initiated by binding of the cytokine to two types of IL-1 receptors designated as IL-1R type I and type II. Although the cytoplasmic portions of both IL-1 receptors do not contain kinase domains or motifs homologous to any other known signalling system, rapid intracellular protein phosphorylation occurs in response to IL-1 stimulation [16]. The nature of the primary signal triggered by IL-1 receptor activation remains poorly understood and controversial [1], but it is clear that IL-1 does stimulate protein kinase activity in a wide variety of different cell types. Recently, IL-1 and tumour necrosis factor have been reported to induce rapid MAP kinase activation in fibroblasts and KB cells [16–18]. We extend these studies and report that IL-1β triggers a biphasic activation of the MAP kinase signalling pathway with a first rapid peak at 10 min and a second sustained activation lasting for more than 24 h. In addition, IL-1β stimulates de novo synthesis of p42 MAP kinase and thus provides mesangial cells with an increased capacity to transmit signals via this key signalling enzyme that has been shown to regulate diverse protein kinases, protein phosphatases, nuclear transcription factors and phospholipase A_2 [19–21]. The IL-1-stimulated biosynthesis and activation of MAP kinase may contribute to the proliferative response [12,13] and the activation of phospholipase A_2 [4–6] that has been observed in mesangial cells in response to this cytokine.

A coordinate, biphasic activation of MAP kinase has also been demonstrated in hamster fibroblasts exposed to thrombin and is paralleled by a biphasic increase in ribosomal S6 kinase activity [24]. The authors, however, did not check whether the second peak of MAP kinase activity is due to increased de novo synthesis of the enzyme as shown here for mesangial cells. Obviously, a maintained signal flow along the MAP kinase cascade is required to drive a cell into mitosis [12,13] and to initiate other long- term cellular responses such as phospholipase A_2 activation [4–6]. In mesangial cells MAP kinase activation has been reported for endothelin, epidermal growth factor, thrombin [22] and for platelet-derived growth factor and extracellular nucleotides (Huwiler and Pfeilschifter, manuscript submitted) which all are potent mitogens. The mechanisms by which IL-1 stimulates MAP kinase activation and biosynthesis are unknown and tracing this signalling cascade more upstream may help to unravel the primary signalling event triggered by IL-1. Recently, Saklatvala and colleagues [25] reported that IL-1 and tumour necrosis factor activate MAP kinase kinase in fibroblasts and KB cells. Whether the cytokines do so by activating c-Raf kinase or MAP kinase kinase kinase remains to be evaluated. Additional studies are also required to determine the pathophysiological significance of IL-1β-stimulated de novo synthesis of MAP kinase in mesangial cells and its possible role in inflammatory kidney diseases.

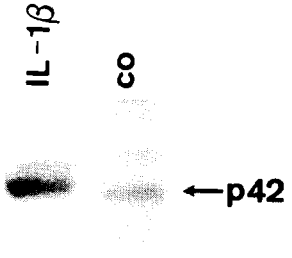

Fig. 3. IL-1β stimulates p42 MAP kinase phosphorylation. Mesangial cells labelled with [^{32}P]orthophosphate were stimulated with vehicle (co) or IL-1β (1 nM) for 10 min. Cells were then lysed and p42 MAP kinase was immunoprecipitated with a specific antiserum at a dilution of 1:200. The immunoprecipitates were separated on SDS-PAGE (10% acrylamide gel) and exposed to Hyperfilm MP.

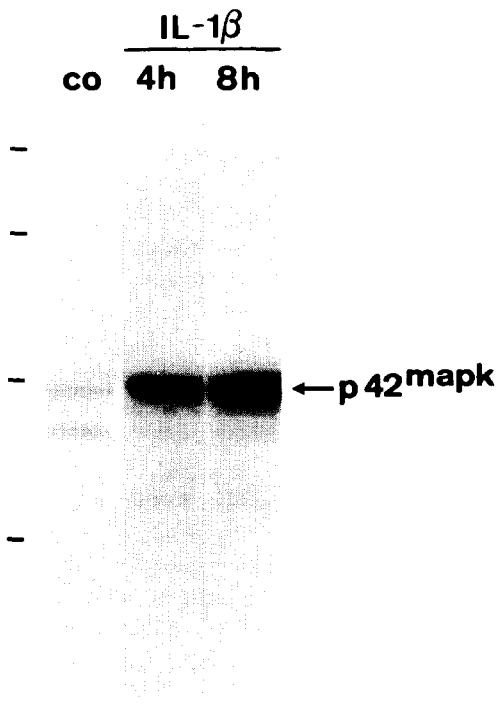

Fig. 4. Kinetics of IL-1β-stimulated MAP kinase de novo synthesis. Confluent mesangial cells were stimulated with vehicle (co) for 8 h or IL-1β (1 nM) for the indicated time periods. During the last 4 h [^{35}S]methionine was added. Then p42 MAP kinase was immunoprecipitated as measured as described in section 2.

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