Keratinocyte stimulation of matrix metalloproteinase-1 production and proliferation in fibroblasts: regulation through mitogen-activated protein kinase signalling events

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Epithelial tumour cell penetration into the stroma during invasion requires degradation of the basement membrane and the underlying structural collagen. Matrix metalloproteinases (MMPs) with collagenolytic activity are thought to be responsible for much of the connective tissue damage that occurs during invasion (Bauer et al., 1977; Goslen and Bauer, 1986; Muller et al., 1993; Gray et al., 1992; Varani et al., 2000). The tumour cells, themselves, are a source of MMPs (Wright et al., 1994; Putnis et al., 1996), but a variety of evidences suggests that cells of the stromal tissue surrounding the tumour comprise a major source of tissue-destructive MMPs (Kataoka et al., 1993; Heppner et al., 1996; DeClerck, 2000).

We have utilised a human skin organ culture model to study the process of stromal invasion by epithelial cells and the role of MMPs in this process. In this invasion model, human skin is maintained in organ culture for a period of 8–12 days under serum-free, growth factor-free conditions or in the presence of exogenous growth-promoting factors. When the organ culture medium contains no serum or exogenous growth factor, normal histological structure and biochemical function are maintained (Varani et al., 1993, 1994). However, when epithelial growth factor (EGF) is included in the organ culture medium, the epithelial cells undergo proliferation. They grow down into the space occupied by the dermis. Although, for the most part, separated from the stroma by basement membrane, the basement membrane erodes in places and epithelial cells invade the dermis at these sites (Fligiel and Varani, 1993; Zeigler et al., 1996a). Invasion in this model is accompanied by up-regulation of MMP-1 (interstitial collagenase) and MMP-9 (92-kD gelatinase B), and is blocked by the inclusion of tissue inhibitor of metalloproteinases-2 (TIMP-2) in the culture medium along with the exogenous growth factor (Varani et al., 1995; Zeigler et al., 1996b).

Efforts to understand how MMP-9 and MMP-1 are regulated during invasion in this model have shown the following: MMP-9 is elaborated primarily in the epidermis (Varani et al., 1995; Zeigler et al., 1996a); is up-regulated as a direct response of the epidermal cells to the exogenous EGF (Zeigler et al., 1999); and depends on signalling through mitogen activated protein kinase (MAPK) pathways (Zeigler et al., 1999). Specifically, signalling through the extracellular signal-related kinase (ERK) pathway and the jun-N-terminal kinase (JNK) pathway occurs. This leads to formation of active c-fos and c-jun and the combination of these elements to form the AP-1 transcription complex. Interference with ERK signalling using a chemical inhibitor or with JNK signalling...
through use of a dominant-interfering mutant reduces formation of the AP-1 complex and MMP-9 transcription. In contrast, MMP-1 is primarily a dermal fibroblast product. MMP-1 induction reflects a fibroblast response to factors elaborated within the epidermis rather than a response to the exogenously-added EGF. At least two different classes of epidermal-derived factors appear to be important in promoting dermal MMP-1 production – i.e., factors that act through the interleukin-1 (IL-1) receptor, and factors that bind to and activate the EGF receptor (Moon et al., 2001). Little is known about the intracellular signalling events that are induced in fibroblasts by epidermal keratinocytes to bring about MMP-1 elaboration. The present study addresses this issue.

MATERIALS AND METHODS

Reagents

Human recombinant forms of EGF, heparin-binding EGF (HB-EGF), IL-1β and IL-1 receptor antagonist were obtained from R&D Systems (Minneapolis, MN, USA). U0126, a potent inhibitor of ERK1,2 kinase activity (Favata et al., 1998) and SB203580, an inhibitor of p38 kinase activity (Cuenda et al., 1995) were obtained from Calbiochem (San Diego, CA, USA). PD169540, a potent inhibitor of EGF receptor tyrosine kinase activity was a generous gift of Drs W. Leopold and David Fry of Pfizer Global Research and Development, Ann Arbor Laboratories, Ann Arbor, MI, USA. PD169540 is an acrylamide-substituted 4-anilinopyrido[4]pyrimidine designed to irreversibly alkylate Cys-773 within the ATP-binding pocket of c-erbB1 and c-erbB2 (EGF receptor family members) (Smaill et al., 2000).

Human epidermal keratinocytes in monolayer culture

Normal human epidermal keratinocytes were obtained from foreskin tissue (obtained at circumcision) as described previously (Varani et al., 1994). They were maintained in monolayer culture using Keratinocyte Growth Medium (KGM) (Clonetix, Inc., Walkersville, MD, USA) as culture medium. KGM is a low-Ca²⁺ (0.15 mM) modification of MCDB-153 medium. It is supplemented with a mixture of growth factors including 0.1 ng ml⁻¹ EGF, 0.5 μg ml⁻¹ insulin, and 2% bovine pituitary extract. Growth was at 37°C in an atmosphere of 95% air and 5% CO₂. Cells were sub-cultured by exposure to trypsin/EDTA and used intermediately or stored frozen. Normal keratinocytes were obtained using the HaCaT line of immortalised human keratinocytes (Boukamp et al., 1988) and was used in place of normal keratinocytes. HaCaT cells were propagated in exactly the same manner as low-passage keratinocytes, and used interchangeably with keratinocytes.

Culture fluid was prepared from keratinocyte or HaCaT cultures as follows: The cells were plated at 4 x 10⁵ cells per cm² of surface area in 25-cm² or 75-cm² flasks. KGM was used as culture medium. When the cultures were approximately 75% confluent, the cells were washed twice in keratinocyte basal medium (KBM) and incubated for 72 h in KBM supplemented with 1.4 mM Ca²⁺. KBM consists of the same basal formulation as KGM but does not contain exogenous growth factors (EGF, insulin and pituitary extract). At the end of the incubation period, the culture fluid was collected and separated from cells and debris by low-speed centrifugation. The keratinocyte culture fluid obtained in this manner could be used as a source of stimulating factor(s) for dermal fibroblast MMP-1 production.

Human dermal fibroblasts in monolayer culture

Normal human dermal fibroblasts were isolated from neonatal foreskin as described previously (Varani et al., 1994). Fibroblasts were grown in monolayer culture using Dulbecco’s Modified Essential Medium supplemented with non-essential amino acids and 10% foetal bovine serum (DMEM-FBS) as culture medium. Fibroblasts were maintained at 37°C in an atmosphere of 95% air and 5% CO₂. Cells were sub-cultured by exposure to trypsin/EDTA and used at passage 2–3.

For assessment of MMP-1 production and for proliferation, dermal fibroblasts were plated at 2 x 10⁴ cells per cm² of surface area in wells of a 24-well dish using DMEM-FBS as growth medium. After allowing cells to attach, the medium was removed and the cells washed twice in Ca²⁺-supplemented KBM. The cells were then incubated for 48 h in Ca²⁺-supplemented KBM alone or in a 50:50 (v/v) mixture of Ca²⁺-supplemented KBM and keratinocyte-conditioned medium. Additional reagents were added as indicated in Results. At the end of the incubation period, the cells were harvested by trypsinisation and enumerated using an automated particle counter. Culture fluid was clarified by low-speed centrifugation, following which it was assayed for MMP-1 as indicated below.

For assessment of signalling intermediates, cell extracts were prepared by lysis of the fibroblasts in buffer consisting of 20 mM Tris-HCl (pH 7.4), 2 mM sodium vanadate, 2 mM 10 mM phosphate substrate, 100 mM NaCl, 1% NP40 detergent, 0.5% sodium deoxycholate detergent, 25 mg ml⁻¹ each aprotinin, leupeptin and pepstatin and 2 mM each EDTA and EGT. Lysis was performed at 4°C by scraping cells into the detergent-containing buffer and then fragmenting cell debris by several passes through a 26-gauge needle. Cell lysates were cleared by microcentrifugation at 12,000 G followed by incubation at 4°C for 25 min. The supernatant fluid was recovered and protein concentration of the lysate was measured using the BioRad protein assay reagent (BioRad, Hercules, CA, USA).

Substrate-embedded enzymography

SDS–PAGE substrate-embedded enzymography (zymography) was used to identify enzymes with collagenase and gelatinase activities. Assays were carried out exactly as described in a previous report (Gibbs et al., 1999). Briefly, denatured but non-reduced culture fluid samples were resolved in 7.5% SDS–PAGE gels prepared with the added incorporation of gelatin (1 mg ml⁻¹) or β-casein (1 mg ml⁻¹) prior to casting. After electrophoresis, gels were washed twice for 15 min in 50 mM Tris buffer containing 1 mM Ca²⁺, 0.5 mM Zn²⁺ and 2.5% Triton X-100. The gels were then incubated overnight in Tris buffer with 1% Triton X-100 and stained the following day with Comassie Brilliant Blue 250-R. Following destaining, zones of enzyme activity were detected as regions of negative staining against the dark background. The zymograms were converted to negative images and digitised. Quantification was accomplished by determining the number of pixels in the negative images. Volumes of 5–35 μl of undiluted culture fluid were normally used for these assays; zones of activity were proportional to the quantity of culture fluid used. Gelatin zymography is useful for detection of MMP-2 (72-kD gelatinase A) in fibroblast cell culture fluids. β-casein zymography is useful for detection of MMP-1, which appears as a doublet in the 54-kD region of the gel. The β-casein zymographic bands co-migrated with purified MMP-1 as detected in Western blotting (Varani et al., 2000). Digestion of native, fibrillar type I collagen was observed in parallel with expression of MMP-1, and blocked in the presence of TIMP-2 or EDTA but not with a battery of serine proteinase inhibitors (Varani et al., 2000).

Western blot analysis of total and phosphorylated forms of ERK and p38

Rabbit IgG antibodies to total and phosphorylated forms of ERK, 1,2 and p38 were obtained from Cell Signaling (Beverly, MA, USA).
Epidermal keratinocyte stimulation of fibroblast MMP-1 elaboration and proliferation: Effects of ERK and p38 inhibitors

Human dermal fibroblasts were exposed to 72-h keratinocyte-conditioned medium and incubated for 2 days. At the end of the incubation period, MMP-1 production and proliferation were assessed. Consistent with our recent findings (Moon et al, 2001), there was a greater than five-fold stimulation of MMP-1 production and an approximately two-fold increase in growth. Effects of two MAPK inhibitors – i.e., U0126 and SB203580 – on MMP-1 elaboration and growth were assessed. In the presence of 10 μM U0126, MMP-1 production was reduced by 88% and fibroblast growth was reduced by 95% (Figures 1A and 2A). The effects of U0126 on both biological responses were dose responsive. Activity (albeit reduced) was observed at a concentration of 1 μM. SB203580 also effectively blocked MMP-1 elaboration induced by keratinocyte-conditioned medium (84% inhibition at 15 μM), but this agent had no effect on the fibroblast proliferative response (Figure 2A). The fibroblast response to SB203580 was also dose responsive, with activity seen at 1.5 μM. In contrast to the results with MMP-1, there was virtually no change in the level of MMP-2 in response to keratinocyte-conditioned medium and no effect with either U0126 or SB203580 (Figure 1, insert).

In a recent study it was demonstrated that ligand(s) which act through the EGF receptor and ligand(s) which act through the IL-1 receptor could together account for virtually all of the MMP-1 – inducing activity in keratinocyte culture fluid while EGF receptor agonists by themselves accounted for proliferation-inducing activity (Moon et al, 2001). We therefore utilised recombinant ligands for these receptors for comparative purposes. As shown in Figures 1B and 2B, EGF (10 ng ml⁻¹) induced both MMP-1 elaboration and proliferation in fibroblasts. In comparison to keratinocyte culture fluid, MMP-1 – inducing activity of EGF was low (2.3-fold increase). Proliferation-inducing activity of purified recombinant EGF was comparable to that of keratinocyte culture fluid. Figures 1B and 2B also demonstrate that in the presence of U0126 (10 μM), EGF-induced MMP-1 production was reduced by 54% while EGF-induced proliferation was reduced by 34%. SB203589 (15 μM) had minimal inhibitory effect on MMP-1 production, and actually enhanced proliferation in response to EGF (Figures 1B and 2B).

Figures 1C and 2C show results from studies in which IL-1β (1 ng ml⁻¹) was used to stimulate fibroblast production of MMP-1 and fibroblast proliferation. In the presence of IL-1β, there was a substantial induction of MMP-1 (5.3-fold), comparable to that seen in the presence of keratinocyte-conditioned medium. In contrast, IL-1β failed completely to stimulate fibroblast proliferation. In the presence of U0126, IL-1β – stimulated MMP-1 production was inhibited by 49%. In the presence of SB203580, 66% inhibition was observed. When proliferation rather than MMP-1 production was assessed, the presence of U0126 had no effect on the (lack of) response to IL-1β. Treatment with SB203580 along with IL-1β, however, actually enhanced proliferation slightly.

ERK 1,2 phosphorylation in fibroblasts stimulated by 72-h keratinocyte-conditioned medium: comparison with EGF- and IL-1β-induced phosphorylation

Figure 3 demonstrates changes in ERK 1,2 phosphorylation at 30 and 60 min in fibroblasts exposed to conditioned medium from 72-h keratinocyte cultures. Increased phosphorylation was observed...
as early as 10 min after stimulation (earliest time-point examined; not shown), reached a maximum at 30 min and decreased to approximately 50% of the maximum value by 60 min. Changes in ERK 1,2 phosphorylation induced by EGF and IL-1β are shown for comparison. In the presence of EGF, ERK 1,2 phosphorylation was increased at the 30-min time-point and remained elevated through 60 min (longest time-point examined) (Figure 3). Although EGF was used as the EGF receptor agonist in most experiments, we also confirmed that a similar profile of ERK 1,2 phosphorylation occurred in the presence of HB-EGF. That is, at 10 ng ml⁻¹, stimulation was seen at 30-min and remained elevated (approximately 50% of maximum) through at least 60 min (data not shown). IL-1β also induced ERK 1,2 phosphorylation. Enhanced phosphorylation was observed at 30 min. However, by 60 min, the level of phosphorylated ERK 1,2 had decreased to below baseline values. Under none of the conditions examined was there a measurable change in steady-state (total) ERK 1,2 protein (Figure 3).

The inhibitor U0126 was used in an effort to block ERK 1,2 phosphorylation. At a concentration of 10 μM, U0126 suppressed ERK 1,2 phosphorylation induced by keratinocyte-conditioned medium to below baseline levels (Table 1). Substantial inhibition was also observed when either EGF or IL-1β was used to induce ERK phosphorylation (Table 1).

**p38 phosphorylation in fibroblasts stimulated by 72-h keratinocyte-conditioned medium: comparison with phosphorylation induced by EGF- and IL-1β**

Figure 4 demonstrates p38 phosphorylation in fibroblasts under the same conditions as assessed with ERK 1,2. In the presence of 72-h keratinocyte culture fluid, p38 phosphorylation was strongly induced at 30 min. However, stimulation was transient. By 60 min, phosphorylation of p38 was decreased by 71% from the value seen at 30 min. When EGF was examined, p38 phosphorylation was not induced at either 30 or 60 min. In contrast, when IL-1β was used as the stimulus, the p38 phosphorylation pattern resembled that induced by keratinocyte culture fluid. Phosphorylation was induced at 30 min but had decreased to almost baseline level by 60 min. Under none of the conditions examined was there a measurable change in steady-state p38 protein (Figure 4).

Additional experiments were conducted in which an IL-1 receptor antagonist and a potent EGF receptor tyrosine kinase antagonist (PD169540) were examined for ability to modulate p38 phosphorylation induced by keratinocyte culture fluid. In the presence of IL-1 receptor antagonist (50 μg ml⁻¹), p38 phosphorylation was inhibited by 80% at 30 min and virtually 100% at 60 min. Not surprising in light of the failure of EGF to induce p38 phosphorylation, PD169540 (1 μM) was completely ineffective in preventing p38 phosphorylation (Figure 5).

In the next set of experiments, effects of SB203580 on p38 phosphorylation induced by keratinocyte culture fluid was assessed. Consistent with the findings presented in Figure 4, p38 phosphorylation was increased at 30 min under control conditions (i.e., in the presence of keratinocyte-conditioned medium) and then decreased almost to baseline by 60 min. In the presence of both the keratinocyte culture fluid and the inhibitor, the level of phosphorylation seen at 30 min was increased slightly over that seen with culture fluid alone. More impressively, the decrease seen at 60 min in the presence of culture fluid was substantially inhibited in the presence of SB203580 (Figure 6). Similar results were observed when IL-1β was used as the stimulus in place of keratinocyte culture fluid (Figure 6).

### Table 1

| Treatment group                 | ERK 1,2 phosphorylation (Fold-Induction) |
|---------------------------------|------------------------------------------|
|                                 | 30 min                                   | 60 min                                   |
| KC-conditioned medium           | 3.1 ± 0.1                                 | 3.5 ± 0.5                                |
| +U0126                          | 0.6 ± 0.4                                 | 0.5 ± 0.4                                |
| EGF                             | 2.2                                      | 3.0                                      |
| +U0126                          | 0.5                                      | 0.4                                      |
| IL-1β                           | 3.4                                      | 0.3                                      |
| +U0126                          | 0.7                                      | 0.2                                      |

Table I Effects of U0126 on ERK 1,2 phosphorylation induced by keratinocyte-conditioned medium, EGF and IL-1β

Fibroblasts were exposed for 30 or 60 min to culture medium alone or to a 50:50 mixture of culture medium and keratinocyte-conditioned medium with or without 10 μM U0126. EGF (10 ng ml⁻¹) and IL-1β (1 ng ml⁻¹) served as controls. At the end of the incubation period, extracts were prepared and assayed for phospho-ERK 1,2 expression as described in the Materials and Methods section. Values shown are expressed as fold-induction relative to the level detected in cells exposed to culture medium alone. Values for keratinocyte-conditioned medium (KC-conditioned medium) are presented as averages of two experiments ± the differences between individual values and averages.

**Figure 2** Fibroblast proliferation in response to stimulation by (A) keratinocyte-conditioned medium, (B) EGF and (C) IL-1β. Fibroblasts were exposed to culture medium alone or to a 50:50 mixture of culture medium and keratinocyte-conditioned medium. U0126 (10 μM) or SB203580 (15 μM) was included as indicated. At the end of the 2-day incubation period, cell number was assessed as described in the Materials and Methods section. Values shown are means and standard deviations based on four separate experiments, each with duplicate or triplicate samples.
Finally, keratinocyte culture fluid was examined for ability to stimulate c-Jun phosphorylation in the absence and presence of SB203580. In the absence of the inhibitor, there was an approximately 3.6-fold increase in phosphorylation at 30 min, which decreased to 1.5-fold at 60 min. The presence of SB203580 had virtually no effect at 30 min, but completely inhibited the decrease at 60 min (Figure 7). The level of c-jun steady-state protein remained constant (Figure 7).

DISCUSSION

MMPs are thought to play a major role in tissue destruction associated with tumour invasion (reviewed in: Stetler-Stevenson et al, 1993; Stamenkovic, 2000; Herouy, 2001). Utilising an organ culture model of epithelial cell invasion in skin, we have demonstrated that stromal invasion in this model was accompanied by the up-regulation of MMP-9 and MMP-1 and inhibited in the presence of exogenous TIMP-2 (Varani et al, 1995; Zeigler et al, 1996b). MMP-9 was elaborated in the epidermis as a direct response to the exogenous growth factors used to stimulate invasion. In contrast, MMP-1 was produced largely in the stroma. Induction of this enzyme occurred as a response to factors elaborated by the epidermis. At least two distinct epithelial cell factors—one, a ligand for the EGF receptor, and the other an agonist for the IL-1 receptor—appeared to be responsible for enzyme induction (Moon et al, 2001).

In the present study we have examined MAPK signalling events that underlie MMP-1 induction in fibroblasts following exposure to conditioned medium from keratinocyte cultures. To summarise, activation of both ERK and p38 pathways occurs in fibroblasts exposed to keratinocyte-conditioned medium, and both pathways contribute to enzyme induction. Several important issues can be addressed relative to these findings.

One is the relationship between MAPK signalling events triggered by keratinocyte-conditioned medium and MMP-1 induction by the same factors. Our data indicate that activation of ERK 1,2 by keratinocyte-conditioned medium reflects the summation of action of ligands for both EGF receptor and IL-1 receptor, while p38 activation is mediated primarily (if not exclusively) by IL-1 receptor agonists. This is based on the similarity of phosphorylation patterns seen at 30 and 60 min after exposure of the cells to either keratinocyte-conditioned medium or to prototypelike ligands for the two receptors (i.e., EGF and IL-1β) as indicated in Figures 3 and 4. Consistent with this, the ERK pathway inhibitor U0126 strongly inhibited MMP-1 production in fibroblasts exposed to keratinocyte culture medium, and concomitantly reduced MMP-1 production in fibroblasts exposed to either EGF or IL-1β. The p38 pathway inhibitor SB203580 also inhibited MMP-1 induction by keratinocyte-conditioned medium. This inhibitor blocked MMP-1 production in response to IL-1β, but in contrast to U0126, had no effect of MMP-1 production following EGF stimulation. Taken together, these data indicate that EGF-like ligands induce MMP-1 production in fibroblasts by acting through the ERK pathway (independent of p38). In contrast, the IL-1 receptor agonist(s) appear to stimulate MMP-1 production by concomitantly signalling through both ERK and p38 pathways.

It should be noted that while the actual keratinocyte-derived ligands for the EGF and IL-1 receptors were not identified in this

**Figure 3** ERK 1,2 phosphorylation in fibroblasts in response to stimulation by keratinocyte-conditioned medium, EGF and IL-1β. Fibroblasts were exposed for 30 or 60 min to culture medium alone or to a 50:50 mixture of culture medium and keratinocyte-conditioned medium. At the end of the incubation period, extracts were prepared and assayed for phospho-ERK expression as described in the Materials and Methods section. Values shown are expressed as fold-induction relative to the level detected in cells exposed to culture medium alone. Values are means and standard deviations based on n=6 separate experiments for keratinocyte-conditioned medium and n=5 for EGF and IL-1β. The insert demonstrates a Western blot from one experiment. Top: ERK 1,2 phosphorylation in an extract from cells exposed to culture medium alone at time-zero; Ctrl: ERK 1,2 phosphorylation in extracts from cells exposed to control medium alone at each time-point. ERK-P=phosphorylated ERK 1,2, ERK-SS=total ERK 1,2 protein.

**Figure 4** p38 phosphorylation in fibroblasts in response to stimulation by keratinocyte-conditioned medium, EGF and IL-1β. Fibroblasts were exposed for 30 or 60 min to culture medium alone or to a 50:50 mixture of culture medium and keratinocyte-conditioned medium. At the end of the incubation period, extracts were prepared and assayed for phospho-p38 expression as described in the Materials and Methods section. Values shown are expressed as fold-induction relative to the level detected in cells exposed to culture medium alone. Values are means and standard deviations based on n=6 separate experiments for keratinocyte-conditioned medium and n=5 for EGF and IL-1β. The insert demonstrates a Western blot from one experiment. Top: p38 phosphorylation in an extract from cells exposed to culture medium alone at time-zero; Ctrl: p38 phosphorylation in extracts from fibroblast exposed to culture medium alone at each time-point. p38-P=phosphorylated p38. p38-SS=total p38 protein.
Experimental Therapeutics

Szabowski et al could be inhibited with an antibody to HB-EGF (Varani in a recent study that epidermal hyperplasia in organ-cultured skin (Stoll et al, 2001). IL-1 agonists, but of these, only HB-EGF is strongly up-regulated in the IL-1 receptor. Keratinocytes produce several EGF receptor agonists, and among these, IL-1 is the major keratinocyte ligand for the IL-1 receptor. Keratinocytes produce several EGF receptor agonists, but of these, only HB-EGF is strongly up-regulated in organ-cultured skin (Stoll et al, 1999; Maas-Szabowski et al, 2000).

While ligands for both EGF and IL-1 receptors participate in the induction of MMP-1 elaboration, the data suggest that ligands acting through the IL-1 receptor provide the predominant stimulus. In the presence of EGF, a 2.3-fold induction of MMP-1 was seen while in the presence of IL-1β, induction was 3.5-fold. Both EGF (as well as HB-EGF) and IL-1β were examined over a wide range of concentrations and these were the maximal values obtained. More importantly, it was demonstrated in a recent study that a potent EGF receptor tyrosine kinase inhibitor completely blocked MMP-1 induction by EGF but was only modestly effective in blocking stimulation by keratinocyte-conditioned medium. In contrast, IL-1 receptor antagonist was highly-effective in blocking MMP-1 induction by either IL-1β or keratinocyte culture fluid (Moon et al, 2001).

A corollary issue is the relative contribution of each of the two signalling pathways to MMP-1 induction and the possible interaction between the two. Greatly reduced MMP-1 production in the presence of either the ERK pathway inhibitor or the p38 pathway inhibitor strongly suggests that signalling through both MAPK pathways is required for induction of MMP-1 by keratinocyte-conditioned medium. The contribution of EGF (acting through the ERK pathway) appears to be independent of p38, based on the lack of effect of the p38 inhibitor in preventing EGF-induced MMP-1 up-regulation. The fact that c-Jun is still phosphorylated (and presumably activated) in the presence of SB203580, provides a p38-independent path to the formation of the AP-1 transcription complex (Zeigler et al, 1999). This does not appear to be the case with the component of stimulation due to IL-1β. Stimulation of MMP-1 production via IL-1 receptor activation can be blocked by inhibiting either ERK or p38 signalling. Since almost identical results were achieved when either keratinocyte-conditioned medium or recombinant IL-1β was used as agonist, the presumption is IL-1 receptor activation is the major pathway leading to MMP-1 up-regulation and that signalling through both ERK and p38 pathways is required for maximal stimulation via this receptor.

The findings presented here are of interest in regard to what has been seen in other cell types or in fibroblasts stimulated with other agonists. Recent studies have shown that in human dermal fibroblasts, MMP-1 – inducing agents as diverse as okadaic acid, 

Figure 6 Effects of an IL-1 receptor antagonist and an EGF receptor tyrosine kinase inhibitor PD169540 on p38 phosphorylation induced by keratinocyte-conditioned medium or IL-1β. Fibroblasts were exposed for 30 or 60 min to culture medium alone or to a 50:50 mixture of culture medium and keratinocyte-conditioned medium with or without 15 μM SB203580. At the end of the incubation period, extracts were prepared and assayed for phospho-p38 expression as described in the Materials and Methods section. Values shown are expressed as fold-induction relative to the level detected in cells exposed to culture medium alone. The insert demonstrates a Western blot from one experiment. p38-P=phosphorylated p38. p38-SS=total p38 protein.
ceramide and EMMPRIN (extracellular matrix proteinase inducer) are susceptible to inhibition of either ERK or p38 signalling (Lim et al., 1998; Reunanen et al., 1998; Westermark et al., 1998). In contrast, MMP-1 induced by basic fibroblast growth factor is susceptible to inhibition of either ERK or p38 signalling (Lim et al., 1998). Additional experiments will be needed to fully address this and other possibilities. In summary, while no in vitro culture system can fully mimic what occurs in vivo, these data allow one to suggest that keratinocyte-fibroblast interactions are mediated by multiple stimulating agents acting on specific receptors to induce signalling through different MAPK pathways in order to jointly alter expression of key biological functions.

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