Journal of Cellular Biochemistry 47:174–183 (1991)

Induction of Interleukin-1β Production in Human Dermal Fibroblasts by Interleukin-1α and Tumor Necrosis Factor-α. Involvement of Protein Kinase-Dependent and Adenylate Cyclase-Dependent Regulatory Pathways

Alain Mauviel, Nathalie Temime, Dominique Charron, Gérard Loyau, and Jean-Pierre Pujol

Laboratoire de Biochimie du Tissu Conjonctif, C.H.U. Côte de Nacre, F-14033 Caen Cédex, France (A.M., G.L., J.-P.P.); Laboratoire d'Immunogénétique Moléculaire, Institut Biomédical des Cordeliers, F-75006 Paris, France (N.T., D.C.)

Abstract It has previously been demonstrated that interleukin-1 (IL-1) is expressed in a variety of fibroblast cell lines. In this study, we investigated the mechanisms involved in the regulation of IL-1β production by cultured human dermal fibroblasts. We have shown that IL-1β is constitutively expressed as a cell-associated form, with no soluble form detectable in control cell or in stimulated cell supernatants. IL-1α and tumor necrosis factor-α (TNF-α) exerted a dose-dependent stimulation on the production of the cell-associated IL-1β, as estimated using a specific enzyme linked immunosorbent assay (ELISA). As expected, this effect was accompanied by a huge release of prostaglandin E_2 (PGE_2) and a transient rise in intracellular cyclic AMP. Furthermore, IL-1β production was elevated to a lesser extent by the addition of increasing concentrations of the protein kinase C activator phorbol myristate acetate or by low concentration (0.001 μg/ml) of PGE_2. In contrast, higher concentrations (0.1 and 1 μg/ml) of PGE_2, as well as exogenous dibutyryl-cyclic AMP, were clearly inhibitory. H7, an inhibitor of protein kinases also reduced the stimulatory effect of IL-1α and TNF-α. Together with the results obtained with phorbol myristate acetate, these data suggest that protein kinase C may play a role in the upregulation of IL-1β expression in normal skin fibroblasts. The addition of indomethacin not only suppressed prostaglandin synthesis, but also dramatically reduced cyclic AMP formation, probably because the PGE_2-induced stimulation of adenylate cyclase was abolished. This resulted in a strong potentiation of the stimulatory effect of IL-1α and TNF-α, supporting the role of both the cyclooxygenase and adenylate cyclase pathways in the endogenous downregulation of IL-1β induction by the two cytokines studied.

Key words: dermal fibroblasts, interleukin-1, tumor necrosis factor-α, protein kinase C, cyclic AMP, prostaglandin E_2

Tumor necrosis factor-α and interleukin-1 are two major secretory products of monocytes/macrophages, both of which are involved in a number of physiological functions performed by these cells. For example, TNF-α and IL-1 have been detected in the synovial fluid of patients with rheumatoid arthritis and have been implicated in the pathogenesis of osteoarticular diseases (Wood et al., 1983; Saxne et al., 1988). Similarly, increased IL-1 secretion by mononuclear cells from systemic scleroderma patients has been reported, suggesting a role for IL-1 in the pathogenesis of this disorder (Alcocer-Varela et al., 1985).

Two distinct, but structurally related, forms of IL-1, known as IL-1α and IL-1β, have been identified (reviewed by Oppenheim et al., 1986). The two molecules exhibit the same biological properties (Rupp et al., 1986) and bind to the same cellular receptor on mouse (Kilian et al.,

Abbreviations used: cAMP, cyclic AMP; db-cAMP, dibutyryl-cAMP; DMEM, Dulbecco's modification of Eagle's medium; ELISA, enzyme-linked immunosorbent assay; FCS, fetal calf serum (heat inactivated); H7, 1-(5-isoquinolinesulfonyl-2-methylpiperazine dihydrochloride); IL-1, interleukin-1; PMA, phorbol myristate acetate; PGE_2, prostaglandin E_2; PKC, protein kinase C; TNF-α, tumor necrosis factor-α.

Received January 2, 1991; accepted June 25, 1991.

Alain Mauviel's present address is Thomas Jefferson University, Jefferson Medical College, Department of Dermatology, 233 South 10th Street, Philadelphia, PA 19107.

Address reprint requests to Dr. Alain Mauviel, Laboratoire de Biochimie du Tissu Conjonctif, C.H.U. Côte de Nacre, F-14033 Caen Cédex, France.

© 1991 Wiley-Liss, Inc.

1986) and human cells (Dower et al., 1986). T-cells, fibroblasts, and epithelial cells express a 80 kDa membrane receptor, whereas the IL-1 receptor on pre-B cells and monocytes is a separate gene product of 68 kDa (Chizzonite et al., 1989). TNF-α, originally described as a factor causing the hemorrhagic lysis of tumors in mice (Carswell et al., 1975), mediates a variety of biological effects common to IL-1, in the areas of immunology, inflammation or tissue repair (reviewed by Le and Vilcek, 1987). For example, these factors can affect extracellular matrix (ECM) metabolism by either stimulating metalloproteases and prostaglandin E_2 production (Dayer et al., 1985, 1986) or modulating the synthesis of ECM components, such as collagen and proteoglycans (Goldring and Krane, 1987; Postlethwaite et al., 1988; Duncan and Berman, 1989; Mauviel et al., 1988a, 1991).

Originally described as a macrophage product, IL-1 is also expressed by a variety of different cell types (reviewed by Oppenheim et al., 1986). Classically described as a soluble protein, IL-1 is expressed in both secreted and cell-associated forms by macrophages, B cells, endothelial cells, or fibroblasts (Kurt-Jones et al., 1985a,b; 1987). Autocrine regulation of IL-1 production has been reported in different experimental models, including synovial and embryonic lung fibroblasts (Dinarello et al., 1987; Warner et al., 1987; Dalton et al., 1989; Yamato et al., 1989). We have recently demonstrated that the two forms of IL-1 and TNF-α upregulate IL-1β gene expression in human dermal fibroblasts (Mauviel et al., 1988b, 1991), whereas no mRNA for IL-1α could be detected (unpublished data). TNF-α also augments cell-associated IL-1 activity at the transcriptional level in fibroblasts (Kurt-Jones et al., 1987; Le et al., 1987).

In this study, we have examined the role of different effectors on IL-1β production by cultured dermal fibroblasts.

MATERIAL AND METHODS

Cell Cultures

Fibroblasts obtained from explanted infant foreskins were grown in Dulbecco's modification of Eagle's minimum essential Medium (DMEM, Gibco, Paisley, UK) supplemented with antibiotics (100 IU/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml fungizone) and 10% heat inactivated fetal calf serum (FCS, Gibco). The cells were seeded at a density of 20,000 cells/cm^2 in 25 cm^2 flasks for IL-1β ELISA or in 9.6 cm^2 dishes for cAMP determination, and grown to confluency in a 5% CO_2 atmosphere. Experiments were performed between passages 4 and 8.

Experimental Procedure

Upon reaching confluency, the cells were preincubated for 24 h in DMEM supplemented with 2% FCS and 50 μg/ml sodium ascorbate. Fresh medium was then added, containing also β-aminopropionitrile and different concentrations of human recombinant IL-1α (Genzyme Corp., Boston, MA, specific activity: 10^8 U/mg), or TNF-α (kindly provided by Dr. Frickel, Knoll AG, BASF, Federal Republic of Germany). Phorbol myristate acetate (PMA), 1-(5-isoquinolinesulfonyl)-2-methylpiperazine dihydrochloride (H7), prostaglandin E_2, dibutyryl cyclic AMP, and indomethacin were purchased from Sigma Chemical Company, St Louis, MO. After 24 h, the media were collected and the cell layers harvested for IL-1β ELISA assay. Ascorbate and β-aminopropionitrile were added to the cultures with respect to the experimental conditions described in previously published studies on collagen biosynthesis in fibroblasts (Mauviel et al., 1988a, 1991).

Enzyme Linked Immunosorbent Assay (ELISA) for IL-1β

After incubation, cells were recovered by scraping in PBS and were pelleted by centrifugation. Pellets (lysed by 3 cycles of freeze-thawing in 0.15 ml of 10 mM Tris-HCl, pH 7.5) and culture supernatants were assayed by ELISA, using specific antibodies against human IL-1β (Fontaine et al., 1989) (kindly provided by Dr. D. Lando, Roussel Uclaf, Romainville, France). No cross-reactivity was observed with other cytokines such as IL-1α, IL-2, IL-6, IFN-γ, or TNF-α. This assay allows the detection of all forms of IL-1β (active and inactive precursors, and cell-associated and soluble forms). The detection limit is 10–20 pg/ml of IL-1β.

Cyclic AMP Assay

After incubation, the cells were washed three times with cold PBS and stored overnight in 80% ethanol. The cells were then scraped and cell debris was removed by centrifugation. The ethanolic supernatants were evaporated and the

residue was resuspended in 0.05 M Tris–4 mM EDTA, pH 7.5. Cyclic AMP content was estimated using a competitive binding radioimmunoassay kit, according to the manufacturer's instructions (Amersham, U.K.).

Cell Counting

For cell counting, the cells were washed with PBS, detached with trypsin (0.25%) and counted in a Coulter counter (Hycell Inc., Houston, TX).

Statistics

All results are expressed as the mean ± SD unless stated otherwise. The Student's t test was used to evaluate the difference of the means between groups.

RESULTS

Effects of IL-1α and TNF-α on IL-1β Production by Fibroblasts

Human dermal fibroblasts were incubated for 24 h in the presence of IL-1α (1 and 10 U/ml) or TNF-α (0.1, 1, and 10 ng/ml) as described in Materials and Methods. Control cultures exhibited a measurable amount of IL-β in the cell-associated fraction, whereas IL-1β could not be detected in the supernatants (data not shown, values under detection limit). Fibroblasts responded to the cytokines by a dose-dependent increase in the production of cell-associated IL-1β, as estimated by specific ELISA assay (Fig. 1). This effect could not be attributed to enhanced cell proliferation, since cell counting showed no effect of IL-1α on cell number, whereas TNF-α augmented the latter by only 20% at the highest concentration used (data not shown).

Since all forms of IL-1β are detected by the ELISA used in this study, the data demonstrating the induction of IL-1β represent *de novo* production and are not a result of the maturation of previously existing precursor forms of IL-1β stored in the intracellular milieu.

Effect of Exogenous Prostaglandin E_2 and Indomethacin on IL-1β Production by Fibroblasts

As expected, the effects of IL-1α and TNF-α were accompanied by a huge secretion of prostaglandin E_2 (not shown, see similar experiments in Mauviel et al., 1988a, 1991). Since PGE_2 has been suggested to be a potent modulator of IL-1 production in macrophages (Kunkel et al., 1986), we explored the possibility of the involvement of this prostaglandin in the induction of fibroblast IL-1β. As shown in Figure 2, the addition of various doses of exogenous prostaglandin E_2 demonstrates a dual effect of this eicosanoid on IL-1β production; 0.001 μg/ml PGE_2 increased the expression of cell-associated IL-1β, whereas higher concentrations (0.1 and 1 μg/ml) had an inhibitory effect.

In view of these data, we used indomethacin (10^{-5} M) to study the role of prostaglandins in the effect of IL-1α and TNF-α. At this concentration, indomethacin abolishes PGE_2 secretion in our experimental system (Mauviel et al., 1988c). As shown in Figure 3, the response of fibroblasts to IL-1α relative to IL-1β production was almost doubled in the presence of indomethacin. The response to a high dose of TNF-α was also increased in the same proportions, suggesting a downregulation of the production of IL-1β by the endogenous synthesis of PGE_2.

Indomethacin alone reduced the basal levels of cell-associated IL-1β (98.9 ± 17.6 pg/10^6 cells in indomethacin treated-cells vs. 185.8 ± 5.8 in control cultures).

Effect of Addition of Dibutyryl-cAMP on IL-1β Production by Fibroblasts

The release of PGE_2 under IL-1 stimulation was paralleled by a transient rise in intracellular cAMP levels, which peaked at 3–6 h (Fig. 4). To test the role of cAMP in the augmentation of endogenous IL-1β by cytokines, we used dibutyryl cAMP as an exogenous lipophylic analog. As shown in Figure 5, db-cAMP (0.01 to 1 mM) decreased the amount of IL-1β in the cell-associated fraction of fibroblasts in a dose-dependent manner.

In the presence of indomethacin, which potentiated the stimulatory effect of both IL-1α and TNF-α on fibroblast IL-1β production, no increase of intracellular cAMP was observed as a result of IL-1α or TNF-α exposure (data not shown). Therefore, the IL-1-induced rise in intracellular cAMP does not result from the direct activation of adenylate cyclase by IL-1, but probably requires PGE_2 production, as suggested in other systems such as synovial cells (Dayer et al., 1986).

From these data, we conclude that cAMP is a potent candidate for the intracellular downregulation of IL-1β production in cytokine-stimulated fibroblasts.

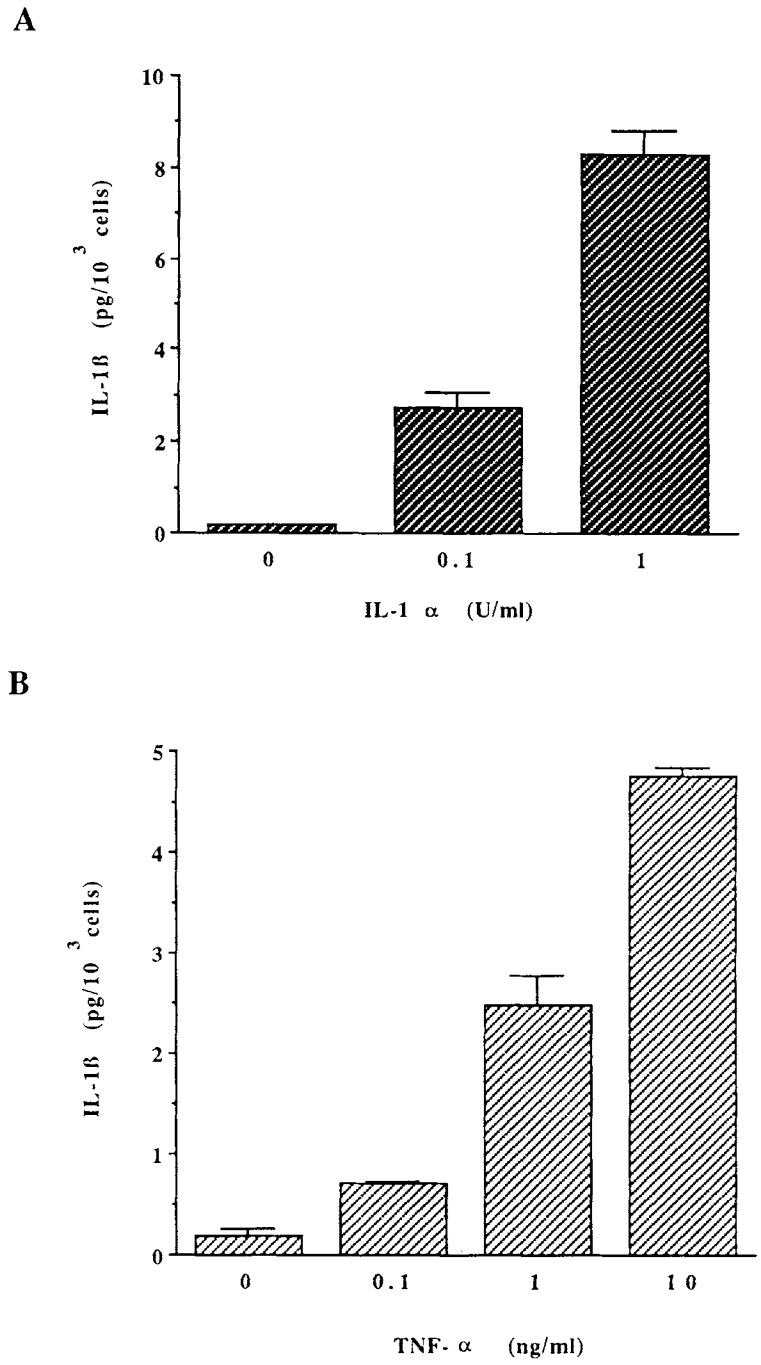

Fig. 1. Effect of human recombinant IL-1α and TNF-α on IL-1β induction in human dermal fibroblasts. Confluent fibroblasts were incubated, as described in Material and Methods, in the presence of varying concentrations of either IL-1α or TNF-α. After 24 h, the IL-1β present in the cell layers and in the supernatants was assayed by specific ELISA. Only results obtained from the cell layers are presented, since no IL-1β could be detected in any of the supernatants. Data expressed as pg/10^3 cells are the mean ± SD of two independent experiments performed on duplicate flasks. All cytokine treatment values plotted are significantly different from untreated control values (Student's t test, $P < 0.01$). **A:** Effect of IL-1α; **B:** Effect of TNF-α.

Effect of Phorbol Myristate Acetate on IL-1β Production

To further investigate the mechanisms whereby IL-1α and TNF-α modulate the production of fibroblast IL-1β, we studied the effect of phorbol myristate acetate as a well-characterized protein kinase C activator. Increasing concentrations of PMA (0.005, 0.5, and 50 ng/ml) induced a dose-dependent increase in the amount

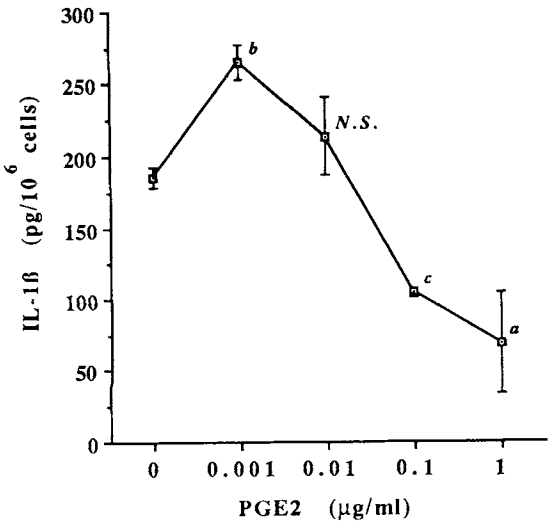

Fig. 2. Effect of exogenous addition of PGE_2 on cell-associated IL-1β amounts in human dermal fibroblasts. Confluent fibroblast cultures were incubated for 24 h as described in Material and Methods with varying concentrations of PGE_2. The amount of cell-associated IL-1β was estimated by ELISA. The data are expressed as pg/10^6 cells and represent the mean ± SD of quadruplicate samples. N.S.: not significant; a: $P < 0.05$; b: $P < 0.02$; c: $P < 0.01$, compared with control.

of cell-associated IL-1β expressed (Fig. 6), suggesting a potential role of PKC in the induction of IL-1β in fibroblasts. However, the extent of stimulation was significantly lower than with IL-1α or TNF-α, even at the highest concentrations of PMA, suggesting that other signaling pathways may be required for the production of a substantial amount of cellular IL-1β under cytokine stimulation.

Effects of H7 on IL-1α- and TNF-α-Induced IL-1β Production

To further characterize the role of protein kinase C in cytokine-induced upregulation of fibroblast IL-1β production, we used the isoquinolinesulfonamide derivative H7, a well-known inhibitor of protein kinase C (inhibition constant Ki = 6.0 µM) and cyclic nucleotide-dependent protein kinases (cGMP-dependent protein kinase Ki = 5.8 µM; cAMP-dependent protein kinase Ki = 3.0 µM) (Hidaka et al., 1984), concommitantly with the exposure of fibroblasts to various concentrations of both IL-1α and TNF-α. H7 alone decreased the amounts of cell-associated IL-1β (−15% at 10^{-6} M, −70% at 10^{-4} M, data not shown). As shown in Figure 7, H7 (10^{-4} M) reduced IL-1β induction by IL-1α and TNF-α, but did not abolish the stimulatory effect of the two cytokines studied. Together with the data

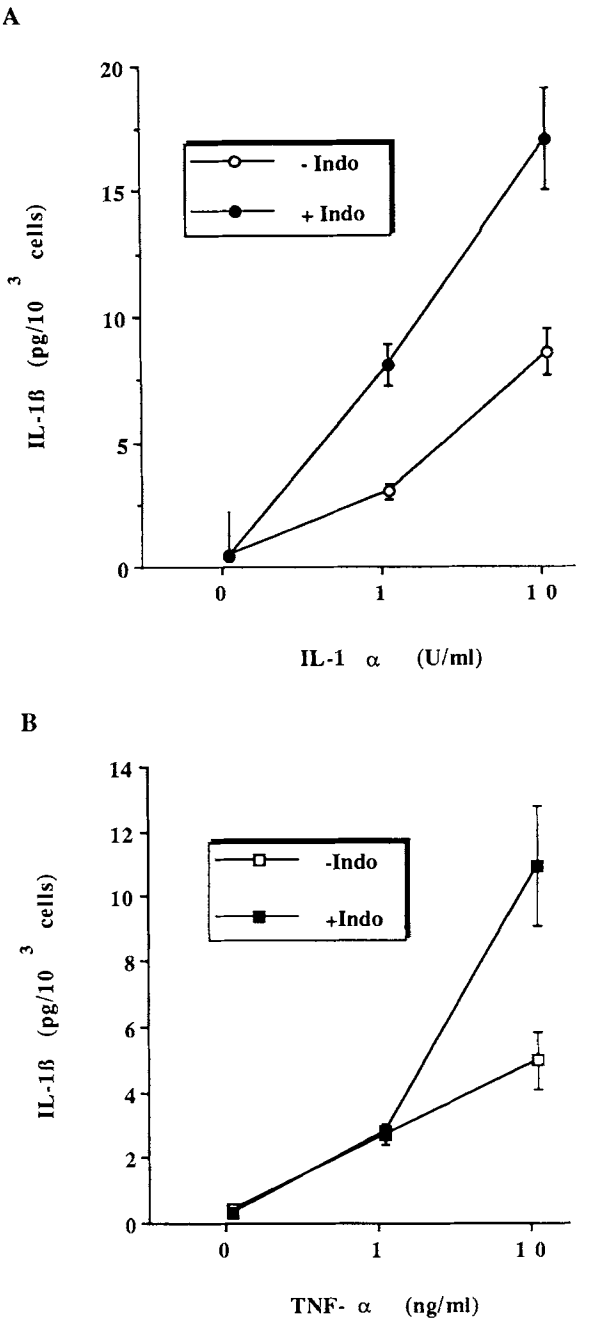

Fig. 3. Effect of indomethacin 10 µM on the induction of cell-associated IL-1β by IL-1α and TNF-α in human dermal fibroblasts. Cells were incubated as described in Material and Methods with varying concentrations of IL-1α or TNF-α, in the presence or absence of 10 µM indomethacin. The amount of cell-associated IL-1β was estimated by ELISA. Data are expressed as pg/10^3 cells and represent the mean ± SD of two independent experiments performed on duplicate flasks. All indomethacin treatment values plotted were significantly different from untreated values (Student's t test, $P < 0.01$) except for TNF-α at 1 ng/ml. **A:** Effect of IL-1α in presence of indomethacin; **B:** Effect of TNF-α in presence of indomethacin.

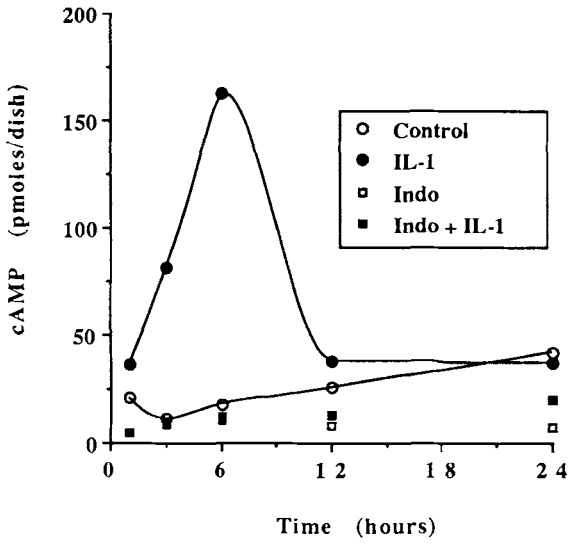

Fig. 4. Effect of IL-1α on intracellular cAMP levels in human dermal fibroblasts. Confluent cultures in 9.6 cm^2 dishes were incubated, as described in Material and Methods, with 1 U/ml IL-1α in the presence or absence of 10 μM indomethacin, and extracted with 80% ethanol at different time points for cAMP determination. The results were expressed as pmoles/dish and represent the mean of triplicate dishes.

obtained with PMA, these experiments indicate that the modulation of PKC and/or cyclic nucleotide-dependent protein kinase activity participate in the regulation of IL-1β induction in fibroblasts.

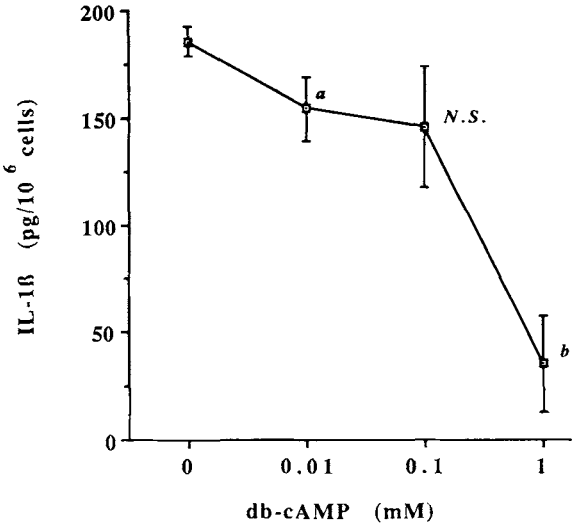

Fig. 5. Effect of dibutyryl-cyclic AMP on cell-associated IL-1β induction in human dermal fibroblasts. Cells were incubated as described in Material and Methods with varying concentrations of db-cAMP. Cell-associated IL-1β amounts were estimated by ELISA. The data, expressed as pg/10^6 cells, are the mean ± SD of quadruplicate samples. N.S.: not significant; a: $P < 0.05$; b: $P < 0.01$, compared with control.

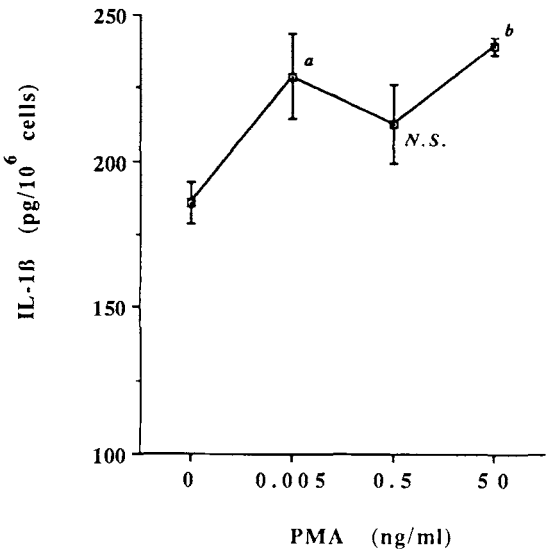

Fig. 6. Effect of phorbol myristate acetate on cell-associated IL-1β amounts in human dermal fibroblasts. Cells were incubated as described in Material and Methods with varying concentrations of PMA. The amount of cell-associated IL-1β was estimated by ELISA. These data, expressed as pg/10^6 cells, represent the mean ± SD of quadruplicate samples. N.S.: not significant; a: $P < 0.05$; b: $P < 0.01$, compared with control.

DISCUSSION

Misregulation of connective tissue organization in a number of skin disorders, such as dermal fibrosis, keloids, or scleroderma, is not fully understood, although cytokines such as IL-1 and TNF-α have been implicated in several immunoinflammatory responses (reviewed by Larrick and Kunkel, 1988). Since IL-1 is present in increased amounts in the affected sites and connective tissue cells are known to express and produce IL-1, the purpose of this study was to determine the signaling pathways which are responsible for IL-1β production in cultured human dermal fibroblasts.

In contrast to monocytes which require activation, dermal fibroblasts produce cell-associated proIL-1β constitutively, with no detectable mature, soluble form. We had previously demonstrated the constitutive presence of the mRNA for IL-1β in non-stimulated human dermal fibroblasts (Mauviel et al., 1988b). When primed by IL-1α or TNF-α, an increased production of cellular IL-1β was observed in dermal fibroblasts. This cellular form of fibroblast IL-1β, shown to be the 33 kDa precursor with no biological activity, is localized in the cytosol and does not transfer into intracellular organelles, in contrast to other secreted proteins such as tissue plasminogen activator (Kurt-Jones et al., 1985b; Elias et

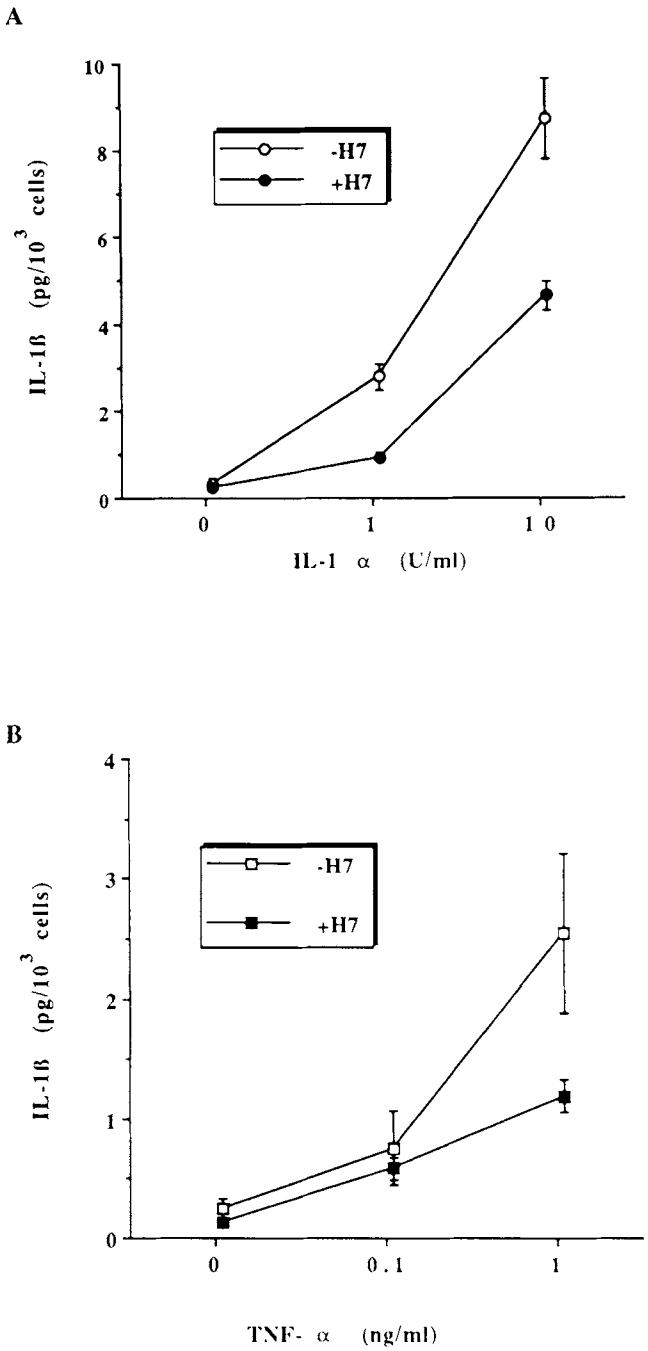

Fig. 7. Effect of H7 on the induction of cell-associated IL-1β induction by IL-1α and TNF-α. Cells were incubated as described in Material and Methods, with varying concentrations of IL-1α or TNF-α, in the presence or absence of H7 (10^{-4} M). The amount of cell-associated IL-1β was estimated by ELISA. These data, expressed as pg/10^3 cells, represent the mean ± SD of two independent experiments performed on duplicate flasks. All H7 treatment values plotted are significantly different from values obtained from corresponding samples treated with cytokines but without H7 (Student's t test, $P < 0.01$). **A:** Effect of IL-1α; **B:** Effect of TNF-α. A: Effect of IL-1α in presence of H7; B: effect of TNF-α in presence of H7.

al., 1989). No active soluble form was released, thus clearly differing from monocytic cells (Dinarello et al., 1987) and endothelial cells (Warner et al., 1987), although in the latter case IL-6 or other soluble factor(s) could account for the released thymocyte stimulating activity, as evidence by Elias et al. (1989).

The response of dermal fibroblasts to cytokines is different from that of lung fibroblasts, which accumulate cellular IL-1β only when stim-

ulated by a combination of both IL-1 and TNF-α, whereas IL-1 and TNF-α taken individually failed to stimulate lung fibroblasts to produce detectable amounts of cellular IL-1β, despite the accumulation of the corresponding mRNA (Elias et al., 1989). This observation supports the concept of tissue and/or cell-specificity of the regulation of IL-1 production. Interestingly, despite their different responses to cytokines, fibroblasts from either lung or skin were unable to secrete significant amounts of IL-1β protein. This observation may reflect a defect in fibroblasts in either the secretion of IL-1β or the proteolytic cleavage (maturation) of pro-IL-1β.

We used phorbol myristate acetate and H7 to study the possible involvement of protein kinases in IL-1β induction. From the data obtained using PMA, it appears that the stimulation of PKC cannot explain by itself the increased production of cellular IL-1β in fibroblasts under IL-1α or TNF-α treatment, since these cytokines are approximately ten times as potent as PMA at the highest concentration used. However, since H7 reduces the extent of stimulation exerted by the two cytokines, it is conceivable that protein kinases (PKC, cGMP- or cAMP-dependent protein kinases) participate in this phenomenon. These data support the study by Yamato et al. in 1989, obtained on the human embryonal lung fibroblast cell line, WI-38. These authors also demonstrated that WI-38 cells exhibit a low but detectable IL-1 activity in their supernatants, which can be potentiated by TNF-α, and further by PMA. However, the eventual interference by IL-6 with the thymocyte proliferation test used by these authors could not be excluded. Phorbol esters also induce IL-1β mRNA in human histiocytic lymphoma cells (Nishida et al., 1988) and in THP-1 human monocytic leukemia cells (Fenton et al., 1988). Taken together, these data suggest that PKC activation may be an essential process in the induction of IL-1β production in many cell types, of either monocytic or mesenchymal origin.

Neither low concentrations of PGE_2 nor PMA could stimulate IL-1β induction to the levels reached by IL-1α or TNF-α. This observation suggests the existence of additional signaling pathways. For example, Ca^{++} ionophores have been shown to enhance IL-1 secretion and processing by macrophages (Suttles et al., 1990). Ouabain, an inhibitor of the cellular Na^+/K^+ antiport, known to increase intracellular levels of Ca^{++}, has been shown to increase IL-1β mRNA in the WI-38 fibroblastic cell line (Yamato et al., 1989). In contrast, calmodulin kinase inhibitors such as W7, trifluoroperazine, or calmidazolum, inhibit IL-1 production (Kovacs et al., 1988). Thus, it cannot be excluded that calmodulin kinase dependent pathways may be involved in the induction of IL-1β in fibroblasts by cytokines.

Using indomethacin to block PGE_2 secretion, we strongly potentiated the IL-1β production induced by IL-1α and TNF-α. These data are in good agreement with the theory that IL-1 can autoregulate its production through a self-induced inhibitor, PGE_2 (Kunkel et al., 1986). However, low concentrations of exogenous PGE_2 exert a stimulatory effect on IL-1β production. A similar dual effect of the prostaglandin was also reported for the release of TNF-α from macrophages (Renz et al., 1988). Thus, PGE_2 cannot be solely considered as an endogenous inhibitory product for IL-1 or TNF-α production. The biphasic response to PGE_2 may be explained through the induction of intracellular cAMP which may occur only in the presence of high concentrations of PGE_2, since indomethacin suppresses the IL-1-induced rise in intracellular cAMP and we have shown that the cAMP analog dibutyryl-cAMP inhibits the production of cell-associated IL-1β. Thus, the rise in cAMP in response to PGE_2 secretion may represent an intracellular suppressive signal for IL-1β production (Knudsen et al., 1986). A potential limitation of this hypothesisis the observation that the addition of db-cAMP to cells leads to a sustained increase in intracellular levels of this cyclic nucleotide, which does not mimic the transient rise in natural intracellular cAMP induced by hormonal stimulation of adenylate cyclase.

Moreover, we cannot rule out the possibility of cross-talk between protein kinase C and the prostaglandin-adenylate cyclase system, as described in different experimental models (Yoshimasa et al., 1987; Izuka et al., 1989; Bell and Brunton, 1986; Parker et al., 1987). Such interactions between the different cellular signaling systems may explain the differences in the extent of responses observed between cytokine-stimulated cells and cells receiving an exogenous effector specific for one transduction system.

In conclusion, the regulation of fibroblast IL-1β production appears to be dependent on the interconnection of many different signaling mechanisms, including the regulation of protein kinases activity, as well as the synthesis of intra-

cellular cyclic nucleotides. The functional significance of the accumulation of cellular IL-1β, which corresponds almost exclusively to the inactive precursor form (Kurt-Jones et al., 1985a; Elias et al., 1989), without any detectable release of active IL-1β, is not understood as yet. However, during inflammatory situations, fibroblasts are likely to be exposed to both IL-1 and TNF-α produced by inflammatory cells and to contain large amounts of pro-IL-1β. This precursor may have intracellular regulatory effects or may be released from the cells upon cell death or transient cell membrane disruption (Young et al., 1988), and could subsequently be activated by locally accumulated proteases (Mosley et al., 1987). Moreover, fibroblasts may process the precursor form and secrete active IL-1β when primed by other unknown stimuli. The result of any of these hypotheses would lead to the production of high amounts of active IL-1β which may participate in an autocrine loop controlling both local IL-1β production and the synthesis of ECM components associated with tissue remodeling.

ACKNOWLEDGMENTS

We wish to thank Dr. D. Lando (Roussel-Uclaf, Romainville, France) for the kind gift of anti-IL-1β. We also wish to thank Dr. A. Christiano (Jefferson Medical College, Philadelphia, PA) for careful reading of the manuscript. N. Temime is the recipient of an IFSBM-ARC fellowship.

This work was presented in abstract form at the Keystone Symposium "Cytokines and Their Receptors, from Clonal to Clinical Investigations," held in Keystone, CO, April 1–7, 1991. (J Cell Biochem (Suppl) 15F, p 135, abstract P538).

REFERENCES

Alcocer-Varela J, Martinez-Cordero E, Alargon-Segovia D (1985): Spontaneous production of, and defective response to, interleukin-1 by peripheral blood mononuclear cells from patients with scleroderma. Clin Exp Immunol 59:666–672.

Bell JD, Brunton LL (1986): Enhancement of adenylate cyclase activity in S49 lymphoma cells by phorbol esters. Withdrawal of GTP-dependent inhibition. J Biol Chem 261:12036–12041.

Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B (1975): An endotoxin-induced serum factor that causes the necrosis of tumors. Proc Natl Acad Sci USA 72:3666–3670.

Chizzonite R, Truitt T, Kilian PL, Stern AS, Nunes P, Parker KP, Kaffka KL, Chua AO, Lugg DK, Gubler U (1989): Two high-affinity interleukin-1 receptors represent separate gene products. Proc Natl Acad Sci USA 86:8029–8033.

Dalton BJ, Connor JR, Johnson WJ (1989): Interleukin-1 induces interleukin-1α and interleukin-1β gene expression in synovial fibroblasts and peripheral blood monocytes. Arthritis Rheum 32:279–287.

Dayer J-M, Beutler BM, Cerami AC (1985): Cachectin/tumor necrosis factor stimulates collagenase and prostaglandin E_2 production by human synovial cells and dermal fibroblasts. J Exp Med 162:2163–2168.

Dayer J-M, deRochemonteix B, Burrus B, Demczuk S, Dinarello CA (1986): Human recombinant interleukin-1 stimulates collagenase and prostaglandin E_2 production by human synovial cells. J Clin Invest 77:645–648.

Dinarello CA, Ikejima T, Warner SJC, Orencole SF, Lonneman G, Cannon JG, Libby P (1987): Interleukin 1 induces interleukin 1. I. Induction of circulating interleukin 1 in rabbits in vivo and in human mononuclear cells *in vitro*. J Immunol 139:1902–1910.

Dower SK, Kronheim SR, Hopp TP, Cantrell M, Deeley M, Gillis S, Henney CS, Urdal DL (1986): The cell-surface receptors for interleukin-1α and interleukin-1β are identical. Nature (London) 324:266–268.

Duncan MR, Berman B (1989): Differential regulation of collagen, glycosaminoglycan, fibronectin, and collagenase activity production in cultured human adult dermal fibroblasts by interleukin-1 alpha and beta and tumor necrosis factor alpha and beta. J Invest Dermatol 92:699–706.

Elias JA, Reynolds MM, Kotloff RM, Kern JA (1989): Fibroblast interleukin-1: Synergistic stimulation by recombinant interleukin-1 and tumor necrosis factor and posttranscriptional regulation. Proc Natl Acad Sci USA 86:6171–6175.

Fenton MJ, Vermeulen MW, Clark BD, Webb AC, Auron PE (1988): Human proIL-1β gene expression in monocytic cells is regulated by two distinct pathways. J Immunol 140:2267–2273.

Fontaine S, Damais C, Lando D, Cousin MA (1989): Monoclonal antibodies to human interleukin-1β and their use in a sensitive two-sites enzyme linked immunosorbent assay. Lymphokine Res 8:129–139.

Goldring MB, Krane SM (1987): Modulation by recombinant interleukin 1 of synthesis of types I and III collagens and associated procollagen mRNA levels in cultured human cells. J Biol Chem 262:16724–16729.

Hidaka H, Inagaki M, Kawamoto S, Sasaki Y (1984): Isoquinoline sulfonamides, novel and potent inhibitors of cyclic nucleotide dependent protein kinase and protein kinase C. Biochemistry 23:5036–5041.

Izuka H, Sakai H, Tamura T (1989): Effects of the tumor promoter, phorbol 12-myristate, 13-acetate, on the epidermal adenylate cyclase system: Evidence for adenylate cyclase-regulation by protein kinase C. J Invest Dermatol 93:387–391.

Kilian PL, Kaffka KL, Stern AS, Woehle D, Benjamin WR, Decharia TM, Gubler U, Farrar JJ, Mizel SB, Lomedico PT (1986): Interleukin 1α and interleukin 1β bind to the same receptor on T cells. J Immunol 136:4509–4514.

Knudsen PJ, Dinarello CA, Strom TB (1986): Prostaglandins posttranscriptionally inhibit monocyte expression of interleukin 1 activity by increasing intracellular cyclic adenosine monophosphate. J Immunol 137:3189–3194.

Kovacs EJ, Radzioch D, Young HA, Varesio L (1988): Differential inhibition of IL-1 and TNF-α mRNA expression by agents which block secon mesenger pathways in murine macrophages. J Immunol 141:3101–3105.

Kunkel SL, Chensue SW, Phan SH (1986): Prostaglandins as endogenous mediators of Interleukin 1 production. J Immunol 136:186–192.

Kurt-Jones EA, Beller DI, Mizel SB, Unanue ER (1985a): Identification of a membrane associated interleukin 1 in macrophages. Proc Natl Acad Sci USA 82:1204–1208.

Kurt-Jones EA, Kiely JM, Unanue ER (1985b): Conditions required for expression of membrane IL-1 on B cells. J Immunol 136:1548–1540.

Kurt-Jones EA, Fiers W, Pober JS (1987): Membrane interleukin 1 induction on human endothelial cells and dermal fibroblasts. J Immunol 139:2317–2324.

Larrick JW, Kunkel SL (1988): The role of tumor necrosis factor and interleukin-1 in the immunoinflammatory response. Pharm Res 5:129–139.

Le J, Vilcek J (1987): Biology of disease. Tumor necrosis factor and interleukin-1: Cytokines with multiple overlapping biological activities. Lab Invest 56:234–248.

Le J, Weinstein D, Gubler U, Vilcek J (1987): Induction of membrane-associated interleukin 1 by tumor necrosis factor in human fibroblasts. J Immunol 138:2137–2142.

Mauviel A, Daireaux M, Rédini F, Galéra P, Loyau G, Pujol J-P (1988a): Tumor necrosis factor inhibits collagen and fibronectin synthesis in human dermal fibroblasts. FEBS Lett 236:47–52.

Mauviel A, Temime N, Charron D, Loyau G, Pujol J-P (1988b): Interleukin-1 α and β stimulate interleukin-1β gene expression in human dermal fibroblasts. Biochem. Biophys Res Commun 156:1209–1214.

Mauviel A, Kahari V-M, Heino J, Daireaux M, Hartmann DJ, Loyau G, Pujol J-P (1988c): Gene expression of fibroblast matrix proteins is altered by indomethacin. FEBS Lett 231:125–129.

Mauviel A, Heino J, Kähäri V-M, Hartmann DJ, Loyau G, Pujol J-P, Vuorio E (1991): Comparative effects of interleukin-1 and tumor necrosis factor-α on collagen production and corresponding mRNA levels in human dermal fibroblasts. J Invest Dermatol 243:243–249.

Mosley B, Urdal DL, Prickett KS, Larsen A, Cosman D, Conlon PJ, Gillis S, Dower SJ (1987): The interleukin-1 receptor binds the human interleukin-1α precursor but not the interleukin-1β precursor. Biol Chem 262:2941–2944.

Nishida T, Tanako M, Kawakami T, Nishino N, Nakai S, Hirai Y (1988): The transcription of the interleukin 1β gene is induced with PMA and inhibited by dexamethasone in U937 cells. Biochem Biophys Res Commun 156: 269–274.

Oppenheim JJ, Kovacs EJ, Matsushima K, Durum SK (1986): There is more than one interleukin 1. Immunol Today 7:45–56.

Parker J, Daniel LW, Waite M (1987): Evidence of protein kinase C involvement in phorboldiester-stimulated arachidonic acid release and prostaglandin synthesis. J Biol Chem 262:5385–5393.

Postlethwaithe AE, Raghow R, Stricklin GP, Poppleton H, Seyer JM, Kang AH (1988): Modulation of fibroblast functions by interleukin 1: Increased steady-state accumulation of type I procollagen messenger RNAs and stimulation of other functions but not chemotaxis by human recombinant interleukin 1 α and β. J Cell Biol 106:311–318.

Renz H, Gong J-H, Schmidt A, Nain M, Gemsa D (1988): Release of tumor necrosis factor-α from macrophages. Enhancement and suppression are dose-dependently regulated by prostaglandin E_2 and cyclic nucleotides. J Immunol 141:2388–2393.

Rupp EA, Cameron PM, Ranawat CS, Schmidt JA, Bayne EK (1986): Specific bioactivities of monocyte-derived interleukin 1-alpha and interleukin 1-beta are similar to each other on cultured murine thymocytes and on cultured human connective tissue cells. J Clin Invest 78:836–839.

Saxne T, Palladino MA Jr, Heinegard D, Talal N, Wollheim FA (1988): Detection of tumor necrosis factor α but not tumor necrosis factor β in rheumatoid arthritis synovial fluid and serum. Arthritis Rheum 31:1041–1045.

Suttles J, Giri JG, Mizel SB (1990): IL-1 secretion by macrophages. Enhancement of IL-1 secretion and processing by calcium ionophores. J Immunol 144:175–182.

Warner SJC, Auger KR, Libby P (1987): Interleukin 1 induces interleukin 1. II. Recombinant human interleukin 1 induces interleukin 1 production by adult human vascular endothelial cells. J Immunol 139:1911–1917.

Wood DD, Ihrie EJ, Dinarello CA, Cohen PL (1983): Isolation of an interleukin 1-like factor from human joint effusion. Arthritis Rheum 26:975–983.

Yamato K, El-Hajjaoui Z, Koeffler HP (1989): Regulation of levels of IL-1 mRNA in human fibroblasts. J Cell Physiol 139:610–616.

Yoshimasa T, Sibley DR, Bouvier M, Lefkowitz RJ (1987): Cross-talk between cellular signaling pathways suggested by phorbol ester-induced adenylate cyclase phosphorylation. Nature 327:67–70.

Young PR, Hazuda DJ, Simon PL (1988): Human interleukin-1β is not secreted from hamster fibroblasts when expressed constitutively from a transfected cDNA. J Cell Biol 107:447–456.