Communication

An Interleukin 1β Point Mutant Demonstrates That jun/fos Expression Is Not Sufficient for Fibroblast Metalloproteinase Expression*

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Substitution of glycine for arginine at position 127 of the mature human interleukin 1β protein (IL-1β-proc) generates a mutant IL-1β protein (IL-1β-mut) which binds cellular IL-1 receptors with high affinity but fails to elicit significant proliferation of T-helper cells (Gehrke, L., Jobling, S. A., Paik, L. S. K., McDonald, B., Rensonwasser, L. J., and Auron, P. E. (1990) J. Biol. Chem. 265, 5922-5925). Although both IL-1β and the IL-1β-mut mutein stimulate transcription of fibroblast immediate early (fos and jun) and early (IL-1 β and IL-6) genes, the IL-1 β-mut mutein, in contrast to the wild-type IL-1β protein, induces minimal or no transcription of late genes such as procollagenase and prostromelysin. The effect of the naturally occurring IL-1 receptor antagonist protein (IL-1ra) on fibroblast transcription is distinct from that of the IL-1 β-mut mutein, for the IL-1ra fails to stimulate not only late (procollagenase and prostromelysin) but also immediate early (fos and jun) gene expression. These data suggest that the IL-1 β-mut mutein triggers an incomplete or defective signal transduction cascade and demonstrate that fibroblast fos and jun expression is not necessarily accompanied by increased transcription of genes containing the AP-1 binding site. These data also suggest that at least two events are required for IL-1-mediated late gene induction in fibroblasts.

Interleukin 1 proteins have been demonstrated to be important modulators of cellular responses and involved in the pathogenesis of several types of inflammation, including rheumatoid arthritis (Henderson et al., 1987; Krane et al., 1985). One cell type which has served as a model for such inflammatory states is the fibroblast. Several studies have focused on this cell type and the induction of procollagenase and prostromelysin gene expression as relevant markers for arthritis and tumorigenesis (Angel et al., 1986; Whitham et al., 1986). The induction of these genes has been demonstrated to be dependent upon cell stimulation by IL-1, PMA, or ultraviolet irradiation. In particular, PMA and ultraviolet irradiation have been shown to effect induction of procollagenase transcription via the involvement of the PMA-responsive protein complex AP-1, which is a transcriptional trans-activator consisting of Fos and Jun proteins joined by a leucine zipper dimerization domain (Chiu et al., 1988). Although the mechanism of IL-1 induction of procollagenase has not yet been demonstrated to be mediated exclusively by AP-1, such involvement has been assumed (Brinckerhoff and Auble, 1990; Krane et al., 1990). Since levels of fos and jun mRNA and proteins increase transiently in response to cellular induction (Bartel et al., 1989), it is reasonable to expect that the expression of these genes should precede the activation of the procollagenase gene. The data presented here support a model in which fos and jun transcription is not sufficient for IL-1-mediated induction of metalloproteinase gene expression. We have made use of a mutated IL-1β protein (IL-1 β-mut, mutein) which had previously been shown to bind to the IL-1 receptor without inducing a biological response in T-helper cells. We now demonstrate that this mutein is capable of eliciting normal early transcription signals such as those required for jun and fos transcription but not late signals required for metalloproteinase transcription.

EXPERIMENTAL PROCEDURES

Fibroblast Cell Culture and Induction—Confluent cultures of human foreskin fibroblasts at passage 6-7 were grown at 37°C in humidified 95% air, 5% CO2 and Dulbecco’s modified Eagle’s medium, pH 7.0 (Gibco Laboratories), supplemented with 10% fetal calf serum (Sigma) with changes of media twice per week. Cultures were serum-starved by growth in 0.5% fetal calf serum for 48-52 h before experiments. Recombinant human IL-1β was either expressed in Escherichia coli (a gift from Biogen, Cambridge, MA) or generated by in vitro translation (wt) using a rabbit reticulocyte translation mix (Gehrke et al., 1990). The IL-1β-mut, mutant protein was also synthesized by in vitro translation. The amounts of IL-1β proteins were determined using a high sensitivity IL-1β ELISA kit (Cistron Biotechnology) which reacts quantitatively with both wild-type IL-1β and the IL-1β-mut. The IL-1ra was provided by Synergen Corporation (Boulder, CO).

Measurement of mRNA and Collagenase Protein Levels—Total cellular RNA was extracted, fractionated on agarose gels, transferred, and probed as previously described (Conca et al., 1989). Collagenase levels were measured by ELISA using a specific polyclonal antiserum

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1 The abbreviations used are: IL, interleukin; PMA, phorbol myristate acetate; TRE, PMA-responsive element; ELISA, enzyme-linked immunosorbent assay; wt, wild type.
prevented human fibroblast collagenase (Cooper et al., 1983; Rifas et al., 1989).

RESULTS

**IL-1β Induces Gene Transcription in Fibroblasts**—Addition of recombinant human IL-1β to human dermal fibroblasts caused expression of procollagenase, prostromelysin, c-fos, cjun, jun-B, IL-1β, and IL-6 genes (Fig. 1). Expression of the immediate early fos and jun genes reached maximal levels by 0.5–1 h but declined rapidly thereafter. The appearance of procollagenase and prostromelysin transcripts was delayed until approximately 3 h after addition of IL-1β (Conca et al., 1989; McCachren et al., 1989), whereas IL-1β and IL-6 mRNAs were detected at 1 h and were maintained at high levels throughout the 6–24-h period (Fig. 1). The *in vitro* transcribed/translated IL-1β proteins used in these studies, IL-1β(wt) and IL-1βR4, possess amino-terminal methionine residues in contrast to the natural mature peptide that is generated by proteolysis of a precursor protein and is devoid of the amino-terminal methionine. In Fig. 1 it is shown that the effects of *in vitro* translated IL-1β(wt) are indistinguishable from those generated by a preparation of recombinant IL-1β expressed in E. coli, IL-1β(E. coli), which was determined to have only 20% amino-terminal methionine (Wingfield et al., 1986).

**IL-1βR4 Does Not Support Late Gene Transcription in Fibroblasts**—The pattern of transcriptional activation observed using the IL-1βR4 mutein is significantly different from that observed using wild-type protein (Fig. 1). In contrast to the results observed using the wild-type IL-1β, the level of procollagenase gene transcription induced by the IL-1βR4 mutein was near background, while prostromelysin transcripts were not detectable (Fig. 1). The IL-1βR4 mutein retained the capacity, however, to induce c-fos, cjun, jun-B, IL-1β, and IL-6 transcription (Fig. 1). The time course of expression of the c-fos, cjun, and jun-B genes in cells stimulated with the IL-1βR4 mutein was similar to that observed with the wild-type protein (Fig. 1), and the levels of mRNA all increased in the presence of 10 μg/ml cycloheximide (data not shown). In contrast the induction of procollagenase requires *de novo* protein synthesis for expression in these cells (Conca et al., 1989). At the 1-h time point transcription of IL-1β and IL-6 genes was induced by the IL-1βR4 mutein to levels similar to those observed using the wild-type IL-1β protein, but with the IL-1βR4 mutein, the levels of these transcripts fell off rapidly at later time points, suggesting that wild-type IL-1 may either stabilize mRNA or induce a longer continuous period of transcription. The relative amounts of collagenase protein (Fig. 2) secreted into the culture medium of cells exposed to wild-type IL-1β or the IL-1βR4 mutein parallel the levels of mRNA observed in Fig. 1. Secreted collagenase is maximally stimulated by the wild-type IL-1β protein, whereas levels of immunoreactive enzyme observed in the presence of IL-1βR4 mutein are indistinguishable from those in control cells. In order to demonstrate that the absence of late gene transcription in the presence of IL-1βR4 mutein is not due to rapid inactivation or degradation of the mutein when incubated with fibroblasts, conditioned media from previously incubated cells were used to elicit the induction of untreated fibroblasts. In Fig. 3 the levels of *c-jun* mRNA in cells exposed to IL-1βR4, mutein which had been previously incubated for 4 hours in culture with fibroblasts is indistinguishable from that in cells exposed to fresh IL-1βR4 (compare lanes G and J) or wild-type IL-1β (lanes C and F). The 4-h time was chosen because it corresponds with the earliest appearance of the metalloproteinase mRNAs.

**IL-1α Induces Neither Early Nor Late Gene Transcription in Fibroblasts**—The transcriptional effects of the recombinant receptor antagonist (IL-1α) (Hannum et al., 1990) or the IL-1 receptor antagonist protein purified from urine (Dayer and Seckinger, 1989; Seckinger et al., 1987) were compared with those of the IL-1βR4 mutein (Fig. 4). The results presented in Figs. 1 and 4 demonstrate that both IL-1β and the IL-1βR4 mutein stimulate IL-6 and c-jun transcription and that
the IL-1βRα-γ, mutein, in contrast to the wild-type protein, had minimal effects on procollagenase expression. The IL-1 receptor antagonist proteins did not stimulate c-jun and IL-6 transcription and did not induce the procollagenase gene (Fig. 4). The capacity of the receptor antagonist proteins to function as inhibitors of IL-1β was confirmed by mixing the recombinant or urine-derived proteins with wild-type IL-1β before addition to fibroblasts and analysis by Northern blotting (Fig. 4). The receptor antagonist proteins block, in a concentration-dependent manner, the IL-1-mediated induction of c-jun, IL-6, and procollagenase transcription.

**DISCUSSION**

Changes in procollagenase and prostromelysin expression mediated by IL-1 have been associated with the expression and action of transcription factor AP-1 (Conca et al., 1989; Lee et al., 1987; McDonnell et al., 1990; Muegge et al., 1989) which binds to the TRE site located between positions −73 and −42 in the procollagenase gene sequence (Angel et al., 1987a; 1987b). The data presented in this paper demonstrate that expression of immediate early genes c-fos, c-jun, and jun-B following stimulation of fibroblasts with the IL-1βRα-γ, mutein does not lead to expression of genes which contain the TRE. Although wild-type IL-1β increased the accumulation of all mRNAs studied (Fig. 1), the IL-1βRα-γ, mutein induced expression of IL-1β, IL-6, and the immediate early genes without a concomitant increase in the expression of procollagenase and prostromelysin genes (Fig. 1). The stimulation of early but not late gene expression by the IL-1βRα-γ, mutein reveals either a previously unreported element of the signal transduction cascade or failure to stimulate an additional regulatory mechanism associated with IL-1β-dependent induction. Alternatively, the IL-1βRα-γ, mutein may activate expression of a regulatory protein(s) which recognizes adjacent enhancer sequence elements and obstructs the binding of AP-1 to the TRE or obstructs the interaction of bound AP-1 with other elements of the transcriptional initiation complex. The PEA3 (c-ets) transcription factor, whose binding site is immediately upstream of the AP-1 recognition site (Gutman and Wasylyk, 1990; Wasylyk et al., 1990), could be regulated by IL-1 and thereby modulate gene expression. In addition, Jun-B has been reported to act as a negative transcriptional regulatory factor (Chiu et al., 1989). Our observations that jun-B is expressed by cells stimulated with either wild-type IL-1 β or the IL-1βRα-γ, mutein (Fig. 1), however, suggest that the differential transcriptional effects do not result from Jun-B-mediated negative regulation.

Since specific phosphorylation of AP-1 has been proposed as a means of increasing AP-1 activity (Angel et al., 1988; Lee et al., 1987), whereas others (Abate et al., 1990) have reported that the DNA binding properties of Fos and Jun proteins can be regulated by a nuclear protein which modulates oxidation-reduction states, it is possible that posttranslational modification of these proteins may be responsible for the decreased metalloproteinase expression in IL-1βRα-γ-treated cells.

The data presented here also have relevance for understanding the multiple activities of IL-1β by defining a precise region of the IL-1β protein essential for biological activity. Although the IL-1ra is a potent receptor antagonist, the IL-1ra and IL-1β proteins have only 25% sequence identity (Eisenberg et al., 1990; Hannum et al., 1990), which provides little insight into defining the mechanism of the antagonist activity. The profound effects of a single arginine to glycine amino acid substitution on transcriptional activation, however, focus attention on a specific location within the IL-1β molecule. These effects are consistent with our proposal (Gehrke et al., 1990) that the domain surrounding IL-1β Argβ27 is not directly involved in receptor binding but is essential for triggering signal transduction events. Further mutagenesis of the Argβ27 residue strongly suggests that the mechanism of the IL-1βRα-γ, functional activity relates to a change in protein conformation stemming from the absence of a β-carbon at position 127. It therefore appears likely that the IL-1 induction of late genes in fibroblasts requires involvement of either of two distinct regions of the IL-1 receptor which transduce distinct signals or a second receptor which recognizes distinct features on the IL-1β molecule.

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**REFERENCES**

Abate, C., Patel, L., Rauscher, F. J., and Curran, T. (1990) Science **249**, 1157–1161

Angel, P., Pötting, A., Mallick U., Rahmsdorf, H. J., Schorpp, M., and Herrlich, P. (1986) Mol. Cell. Biol. **6**, 1760–1766

Angel, P., Baumann, L., Stein, B., Delius, H., Rahmsdorf, H. J., and Herrlich, P. (1987a) Mol. Cell. Biol. **7**, 2256–2266

Angel, P., Imagawa, M., Chiu, R., Stein, B., Imbra, R. J., Rahmsdorf, H. J., Jonat, C., Herrlich, P., and Karin, M. (1987b) Cell **49**, 729–739

Angel, P., Hattori, K., Smeal, T., and Karin, M. (1988) Cell **55**, 875–885

Bartel, D. P., Sheng, M., Lai, L. F., and Greenberg, M. E. (1989) Genes & Dev. **3**, 304–313

Brickerhoff, C. E., and Auble, D. T. (1990) Ann. N. Y. Acad. Sci. **580**, 355–374

Chiu, R., Boyle, W. J., Meek, J., Smeal, T., Hunter, T., and Karin, M. (1988) Cell **54**, 541–552

Chiu, R., Angel, P., and Karin, M. (1989) Cell **59**, 979–986

Conca, W., Kaplan, P. B., and Krane, S. M. (1988) J. Clin. Invest. **83**, 1753–1757

Cooper, T. W., Bauer, E. A., and Eisen, A. Z. (1983) Collagen Relat. Res. **3**, 205–216

Dayer, J. M., and Seckinger, P. (1989) in Interleukin-1, Inflammation & Disease (Bomford, R. H. R., and Henderson, B., eds) pp. 283–302, Elsevier, New York

Eisenberg, S. P., Evans, R. J., Arend, W. P., Verderber, E., Brewer, M. T., Hannum, C. H., and Thompson, R. C. (1990) Nature **345**, 341–346

Gehrke, L., Jobling, S. A., Paik, L. S. K., McDonald, B., Rosenwater, 2 Unpublished observations, in which arginine at position 127 was selectively substituted to several other amino acids, have demonstrated that only the glycine substitution at this position is capable of reducing biological activity. These observations and a careful analysis of the crystal structure data deposited in the Brookhaven crystallographic database (identifier codes 111B, 211B, and 411B) suggest that the β-carbon of the position 127 side chain stabilizes the conformation at Glnβ27.

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L. J., and Auron, P. E. (1990) *J. Biol. Chem.* **265**, 5922–5925
Gutman, A., and Wasylyk, B. (1990) *EMBO J.* **9**, 2241–2246
Hannum, C. H., Wilcox, C. J., Arend, W. P., Joslin, F. G., Dripps, D. J., Heimdal, P. L., Arnes, L. G., Sommer, A., Eisenberg, S. P., and Thompson, R. C. (1990) *Nature* **343**, 336–340
Henderson, B., Pettipher, E. R., and Higgs, G. A. (1987) *Br. Med. Bull.* **43**, 415–428
Krane, S. M., Dayer, J.-M., Simon, L. S., and Byrne, M. S. (1986) *Collagen Relat. Res.* **5**, 98–118
Krane, S. M., Conca, W., Stephenson, M. L., Amento, E. P., and Goldring, M. B. (1990) *Annu. N. Y. Acad. Sci.* **580**, 340–354
Lee, W., Mitchell, P., and Tjian, R. (1987) *Cell* **49**, 741–752
McCachren, S. S., Greer, P. K., and Niedel, J. E. (1989) *Arthritis Rheum.* **32**, 1539–1545
McDonnell, S. E., Kerr, L. D., and Matrisian, L. M. (1990) *Mol. Cell. Biol.* **10**, 4284–4293
Muegge, K., Williams, T. M., Kant, J., Karin, M., Chiu, R., Schmidt, A., Siebenlist, U., Young, H. A., and Durum, S. K. (1989) *Science* **246**, 249–251
Rifas, L., Halstead, L. R., Peck, W. A., Avioli, L. V., and Welgus, H. G. (1989) *J. Clin. Invest.* **84**, 686–694
Seekinger, P., Williamson, K., Balavoine, J.-F., Mach, B., Mazzei, G., Shaw, A., and Dayer, J.-M. (1987) *J. Immunol.* **139**, 1541–1545
Wasylyk, B., Wasylyk, C., Flores, P., Begue, A., Leprince, D., and Stehelin, D. (1990) *Nature* **346**, 191–193
Whitham, S. E., Murphy, G., Angel, P., Rahmsdorf, H. J., Smith, B. J., Lyons, A., Harris, T. J. R., Reynolds, J. J., Herrlich, P., and Docherty, A. J. P. (1986) *Biochem. J.* **240**, 913–916
Wingfield, P., Payton, M., Tavernier, J., Barnes, M., Shaw, A., Rose, K., Simons, M. G., Demczuk, S., Williamson, K., and Dayer, J.-M. (1986) *Eur. J. Biochem.* **160**, 491–497