Specific Bioactivities of Monocyte-derived Interleukin 1α and Interleukin 1β are Similar to Each Other on Cultured Murine Thymocytes and on Cultured Human Connective Tissue Cells

Elizabeth A. Rupp, Patricia M. Cameron, Chitranjan S. Ranawat,* John A. Schmidt, and Ellen Kahn Bayne
*Department of Biochemistry and Molecular Biology, Merck Sharp and Dohme Research Laboratories, Rahway, New Jersey 07065; and *Department of Orthopedic Surgery, Hospital for Special Surgery, Cornell University Medical Center, New York, 10021*

Abstract

In this report we compare the bioactivities of pure, human monocyte-derived interleukin 1 (IL-1) α and β in the standard murine thymocyte proliferation assay, a human dermal fibroblast proliferation assay, and in an assay measuring stimulation of prostaglandin E_2 (PGE_2) release from human rheumatoid synoviocytes. In each case the different species of IL-1 produced saturable stimulation and gave similar dose response curves. Half-maximal stimulation was observed at average IL-1 concentrations of 29 pM in the thymocyte assay, 2 pM in the dermal fibroblast proliferation assay, and 5 pM in the synovial cell assay. Our results show that native, monocyte-derived IL-1α and IL-1β are both potent stimulators of connective tissue cells and that the specific bioactivities of these molecules are similar to each other in tests on human connective tissue cells, as well as on murine lymphoid cells.

Introduction

Although originally described as a thymocyte activation factor, it is now known that interleukin 1 (IL-1)[1] acts on a variety of different target cells and exerts a broad range of biological effects (1, 2). Furthermore, it is clear that human monocytes produce more than one form of IL-1. Several different charged species of human IL-1 have been reported (3, 4) and complementary DNAs (cDNAs) for two distinct IL-1 molecules termed α and β have been isolated (5–7). The relative importance of these molecules during immune responsiveness and inflammation, however, remains to be determined. We have previously purified the isoelectric point (pI) 6.8 species of human IL-1 (IL-1/6.8) and have shown its amino acid sequence to be homologous with that predicted by IL-1β cDNA (8). More recently we have also succeeded in purifying two acidic species of IL-1 having pIs of 5.4 (IL-1/5.4) and 5.2 (IL-1/5.2) (9). Sequence analysis of these molecules indicates that they are homologous with the protein encoded by IL-1α cDNA and that they differ only by the presence of an unidentified NH_2-terminal blocking group in the case of IL-1/5.4 (9). The availability of these purified species of IL-1 has made it possible to directly compare their specific bioactivities on target cells relevant to inflammatory processes. In the present study we have tested native, monocyte-derived IL-1β and the two species of IL-1α for their ability to stimulate fibroblast, as well as thymocyte, proliferation. In addition, the three species of IL-1 have been tested for their ability to stimulate prostaglandin E_2 (PGE_2) release from human synovial fibroblasts, a target cell relevant to the pathogenesis of rheumatoid arthritis. While previous work has suggested that the different species of IL-1 each exhibit the same spectrum of bioactivities (4), without purified protein it was not previously possible to determine the specific activities (units per milligram), and thus the relative potencies, of these molecules. This information is essential, however, in assessing the physiological and pathophysiological roles of the different species of IL-1. In addition, the information provides an important data base for evaluation of recombinant IL-1 preparations, since it will be necessary to determine how such preparations compare with natural, monocyte-derived IL-1.

Methods

Production and purification of monocyte-derived IL-1α and IL-1β. Human IL-1β (pI 6.8) and IL-1α (pI 5.2 and 5.4) were separately isolated from the supernatants of stimulated mononuclear cells as previously described (8, 9). For most of the present work we used the identical pI 6.8, 5.4, and 5.2 IL-1 preparations previously characterized by Cameron et al. (9). Rechromatography of these preparations via reverse-phase high performance liquid chromatography (HPLC) indicated that they were homogeneous, and upon sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and silver staining each of the IL-1 species appeared as a single band with a relative molecular mass (M_r) of 17,500 (9). In addition, amino terminal sequence analysis of purified IL-1/6.8 and IL-1/5.2 has revealed a single NH_2 terminus in each case (8, 9). A second set of IL-1 preparations was also used in some of our experiments. Like the other preparations these were pure as assessed by reverse-phase HPLC and by SDS-PAGE and silver staining. Protein concentrations were determined as previously described (8), by integration of absorbance profiles obtained at 210 nm as the pure IL-1 species eluted from a reverse-phase HPLC column. The integration function of the detection system was calibrated by chromatographing known amounts of pure ribonuclease. This method has been previously validated by obtaining similar concentrations by amino acid analysis of HCl hydrolysates (8).

Thymocyte proliferation assay. The ability of IL-1 to stimulate pro-

Address reprint requests to Dr. Bayne, Merck Research Laboratories, P.O. Box 2000, Rahway, NJ 07065.
Received for publication 30 May 1986.

1. *Abbreviations used in this paper:* cDNA, complementary DNA; HPLC, high performance liquid chromatography; [^{3}H]TdR, tritiated thymidine; IL-1, interleukin 1; PGE_2, prostaglandin E_2; pI, isoelectric point; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

J. Clin. Invest.

0021-9738/86/09/0836/04 $1.00
Volume 78, September 1986, 836–839

liferation of C3H/HeJ thymocytes cultured in the presence of phytohemaglutinin was assessed in the standard murine thymocyte proliferation assay as previously described (10). [^{3}H]thymidine ([^{3}H]TdR) incorporation between 68 and 72 h of assay was measured.

Fibroblast proliferation assay. Normal adult human dermal fibroblasts (CRL 1445) were obtained from the American Type Culture Collection, Rockville, MD at the fourth passage and passaged an additional two to four times in our laboratory before use. IL-1-induced proliferation was then assessed in a [^{3}H]TdR incorporation assay as described by Schmidt et al. (11). Incorporation of [^{3}H]TdR in this assay has been previously shown to correlate with growth as measured by an increase in cell number (11).

Human synoviocyte stimulation. Human synovial tissue samples were obtained from patients with rheumatoid arthritis undergoing total knee replacement surgery and cell cultures were established using the methods of Baker et al. (12). The cells received a medium change ~48 h postplating and 2–3 d later the medium was again removed and test samples in fresh Dulbecco's modified Eagle's medium with 10% fetal calf serum were added. After a 24-h incubation, PGE_2 in the culture supernatants was measured by radioimmunoassay as described by Humes (13).

Results

To evaluate the activities of the different IL-1 preparations, we first tested each in the murine thymocyte proliferation assay, the standard assay that has been used to measure IL-1 bioactivity. Consistent with our previous results (9), the dose response curves obtained for each of the different IL-1 species showed saturability and were nearly superimposable (Fig. 1). Half-maximal stimulation of thymocyte proliferation was observed at IL-1 concentrations averaging 506 pg/ml or 29 pM. The range of IL-1 concentrations required for half-maximal stimulation in four experiments of this kind was 24–34 pM.

Each of the purified species of IL-1 was next tested for its ability to stimulate human dermal fibroblast proliferation. Two different sets of IL-1 preparations were used for these experiments. Fig. 2 shows our results. Each form of IL-1 stimulated [^{3}H]TdR incorporation in a saturable, dose-dependent manner and as was the case in the thymocyte proliferation assay, the dose response curves for the different forms of IL-1 were nearly identical. In the fibroblast assay, however, half-maximal activity was obtained at an average concentration of ~30 pg/ml for each species of IL-1, or 2 pM. Little variability was observed in fibroblast responsiveness from experiment to experiment (Fig. 2). Furthermore, as shown in Fig. 2, two sets of IL-1 preparations

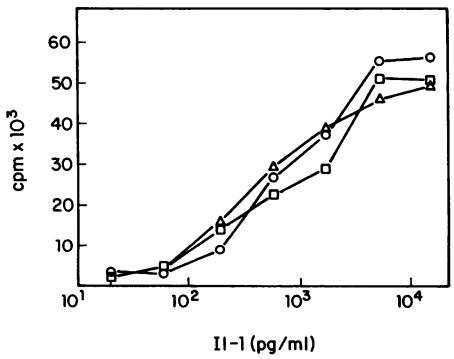

Figure 1. Dose response analysis of human IL-1/6.8 (□), IL-1/5.4 (△), and IL-1/5.2 (○) in the murine thymocyte proliferation assay as determined by [^{3}H]TdR incorporation. Each point represents the mean of triplicate determinations. The SEM was <10% of the mean in each case.

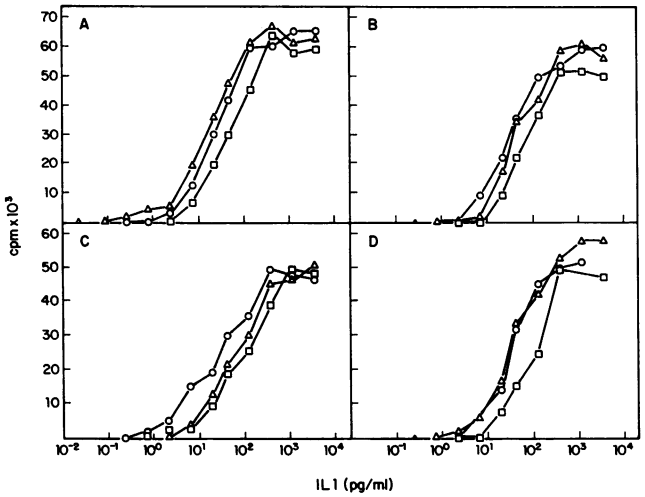

Figure 2. Titrations of human IL-1/6.8 (□), IL-1/5.4 (△), and IL-1/5.2 (○) in human dermal fibroblast proliferation assays as determined by [^{3}H]TdR incorporation. Each panel represents a separate experiment. The assays shown in *A* and *B* were performed with the set of IL-1 preparations used in Fig. 1, while those shown in *C* and *D* were performed with a second, independently purified, preparation of each IL-1 species. Each point represents the mean of quadruplicate determinations.

gave identical results. Half-maximal activities calculated from the different curves shown ranged from 16 to 90 pg/ml (i.e., 1–5 pM).

The abilities of the different species of IL-1 to stimulate synoviocytes isolated from human rheumatoid pannus were also analyzed. Because synoviocytes have been reported to produce large quantities of PGE_2 in response to IL-1 (4, 14), dose response analyses of PGE_2 induction were used to evaluate the specific bioactivities of the different IL-1 species in this system. All experiments were carried out on primary cell cultures and cells derived from seven different patients were tested. The results of two representative experiments performed on cells obtained from different patients are shown in Fig. 3. While in some experiments (Fig. 3 *A*) IL-1/6.8 appeared to be somewhat more active than the acidic species of IL-1, in other cases (Fig. 3 *B*) the same three preparations of the IL-1 species had virtually identical activities. In all experiments, however, we found that the synoviocytes responded to low picomolar concentrations of each of the different forms of IL-1. Half-maximal stimulation was observed at IL-1 concentrations averaging 84 pg/ml or ~5 pM. The IL-1 concentrations required for half-maximal stimulation of PGE_2 release in different experiments ranged from 0.5 to 7 pM for IL-1/6.8, 1 to 11 pM for IL-1/5.4, and 1 to 13 pM for IL-1/5.2.

Discussion

The proteins encoded by IL-1α and IL-1β cDNA have only 26% amino acid homology (6) and the secreted IL-1 molecules corresponding to these cDNAs have little or no antigenic similarity (9, 15). Nonetheless, the present data show that IL-1α and IL-1β have specific activities that are similar to each other in tests on human connective tissue cells as well as on murine lymphoid cells. Although much attention has previously been focused on the bioactivities of IL-1 (for reviews see 1, 2), to our knowledge this is the first study in which the specific activities of native, monocyte-derived IL-1α and IL-1β have been compared, side by side, on different target cell populations. Earlier studies com-

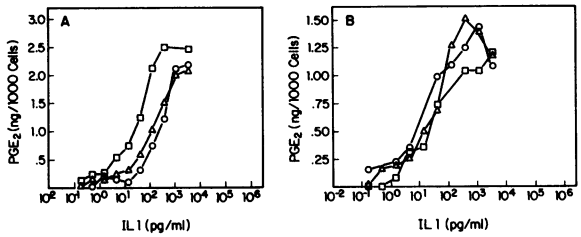

Figure 3. Secretion of PGE_2 by human rheumatoid synoviocytes in response to IL-1. Various concentrations of IL-1/6.8 (□), IL-1/5.4 (△), and IL-1/5.2 (○) were incubated with adherent synovial cells for 24 h and the PGE_2 in the conditioned medium was then measured by radioimmunoassay. Points are the means of duplicate determinations. *A* and *B* show experiments on cells from two different patients.

paring the bioactivities of different charged species of IL-1 secreted by monocytes into their culture supernatants, were limited in that only partially purified IL-1 preparations were available. Moreover, while the work indicated that acidic and neutral forms of human IL-1 exhibited similar arrays of bioactivities (4), because it was not possible to assess the concentrations of IL-1 in the different fractions that were tested, no information on the relative potencies of the different IL-1s could be obtained. Likewise, the absolute concentrations of IL-1 required to stimulate the biological responses of various cell types remained unclear.

From the present study it is clear that monocyte-derived IL-1α and IL-1β are both potent activators of connective tissue cells. Half-maximal stimulation of human dermal fibroblast proliferation occurred at IL-1 concentrations of 2 pM and PGE_2 release from human rheumatoid synovial cells was induced at similar low picomolar concentrations of each of the forms of IL-1. These concentrations are ~200-fold lower than those reported by Dower et al. (16) for half-maximal stimulation of BALB/3T3 fibroblast proliferation by recombinant human IL-1β. These authors, however, did not report the activity of their preparation on human connective cells. The values that we obtain are also lower than those reported by Gubler et al. (17) in experiments where they test recombinant human IL-1α on fibroblasts. These authors utilized the same human fibroblast strain that we use in the present study and observed half-maximal stimulation of proliferation at 160 pM. Half-maximal stimulation of PGE_2 release from the same cells was obtained at 286 pM. In a note added in proof, however, the authors state that they observe at least a 20-fold increase in specific activity in the murine thymocyte proliferation assay when they use recombinant IL-1α that has been purified from *Escherichia coli* without the use of chaotropic agents. Were a similar increase in bioactivity to be observed in the fibroblast assays, the specific bioactivity of the recombinant IL-1α molecule would then approach that which we currently report for monocyte-derived IL-1.

In the present study, we observe half-maximal stimulation of fibroblast proliferation at concentrations of IL-1 fivefold to 34-fold lower than those required for half-maximal stimulation of murine thymocyte proliferation. This may mean that the murine IL-1 receptor has lower affinity than the receptors found on human fibroblasts for human IL-1 or that more receptor binding sites need to be occupied in the case of murine cells to obtain full activation. Receptor binding studies on these different target cells with labeled human IL-1 need to be performed to understand the difference in sensitivity of these different cells to human IL-1 molecules.

Given the nearly identical specific bioactivities observed for each of the human IL-1 species on all three target cells studied, the data are consistent with the hypothesis that the different IL-1 molecules activate target cells via a single class of receptor sites. Cross-competition between IL-1α and IL-1β has been reported in receptor binding assays on a murine T-lymphoid line (16). However, the receptors identified exhibited low affinity (equilibrium dissociation constant [K_d] ~ 1 nM) and thus are unlikely to mediate the biological effects observed at low picomolar concentrations of IL-1. Competitive receptor binding studies performed on human connective tissue cells should clarify whether one or two types of receptors mediate the biological responses of these highly sensitive cells to IL-1.

The role of IL-1 in chronic inflammatory disease is likely to be a complex one. Connective tissue cells can respond to IL-1 in a number of ways. In addition to stimulating release of PGE_2 (4, 14) and increased cellular proliferation (11, 18), IL-1 has also been reported to stimulate production of collagenase and proteoglycanase (19–22), to stimulate cartilage and bone resorption (21, 23–26), and to stimulate synthesis of a tissue inhibitor of metalloproteinases (20). Increased collagen and fibronectin synthesis has also been reported (27). The present results demonstrate that human dermal fibroblasts and human synoviocytes are exquisitely sensitive to both the acidic and neutral species of human IL-1 and thus suggest that IL-1α and IL-1β are similar in their ability to modulate the functions of connective tissue cells.

References

1. Dinarello, C. A. 1984. Interleukin 1. *Rev. Infect. Dis.* 6:51–95.

2. Durum, S. K., J. A. Schmidt, and J. J. Oppenheim. 1985. Interleukin 1: an immunological perspective. *Annu. Rev. Immunol.* 3:263–287.

3. Lachman, L. B., M. P. Hacker, and R. E. Handschumacher. 1977. Partial purification of human lymphocyte activating factor (LAF) by ultrafiltration and electrophoretic techniques. *J. Immunol.* 119:2019–2023.

4. Wood, D. D., E. K. Bayne, M. B. Goldring, M. Gowen, D. Hamerman, J. L. Humes, E. J. Ihrie, P. E. Lipsky, and M.-J. Staruch. 1985. The four biochemically distinct species of human interleukin-1 all exhibit similar biologic activities. *J. Immunol.* 134:895–903.

5. Auron, P. E., A. C. Webb, L. J. Rosenwasser, S. F. Mucci, A. Rich, S. M. Wolff, and C. A. Dinarello. 1984. Nucleotide sequence of human monocyte interleukin 1 precursor cDNA. *Proc. Natl. Acad. Sci. USA.* 81:7907–7911.

6. March, C. J., B. Mosley, A. Larsen, D. P. Cerretti, G. Braedt, V. Price, S. Gillis, C. S. Henney, S. R. Kronheim, K. Grabstein, P. J. Conlon, T. P. Hopp, and D. Cosman. 1985. Cloning, sequence and expression of two distinct human interleukin-1 complementary DNAs. *Nature (Lond.).* 315:641–647.

7. Furutani, Y., M. Notake, M. Yamayoshi, J. Yamagishi, H. Nomura, M. Ohue, R. Furuta, T. Fukui, M. Yamada, and S. Nakamura. 1985. Cloning and characterization of the cDNA's for human and rabbit interleukin-1 precursor. *Nucleic Acids Res.* 13:5869–5882.

8. Cameron, P., G. Limjuco, J. Rodkey, C. Bennett, and J. A. Schmidt. 1985. Amino acid sequence analysis of human interleukin 1 (IL-1). Evidence for biochemically distinct forms of IL-1. *J. Exp. Med.* 162:790–801.

9. Cameron, P. M., G. A. Limjuco, J. Chin, L. Silberstein, and J. A. Schmidt. 1986. Purification to homogeneity and amino acid sequence analysis of two anionic species of human interleukin 1. *J. Exp. Med.* 164:237–250.

10. Schmidt, J. A. 1984. Purification and partial biochemical characterization of normal human interleukin 1. *J. Exp. Med.* 160:772–787.

11. Schmidt, J. A., S. B. Mizel, D. Cohen, and I. Green. 1982. Interleukin 1, a potential regulator of fibroblast proliferation. *J. Immunol.* 128:2177–2182.

12. Baker, D. G., J.-M. Dayer, M. Roelke, H. R. Schumacher, and S. M. Krane. 1983. Rheumatoid synovial cell morphologic changes induced by a mononuclear cell factor in culture. *Arthritis Rheum.* 26:8–14.

13. Humes, J. L. 1981. Prostaglandins. *In* Methods for Studying Mononuclear Phagocytes. D. H. Adams, H. Kuren, and P. Edelson, editors. Academic Press, Inc., New York. 641–654.

14. Mizel, S. B., J.-M. Dayer, S. M. Krane, and S. E. Mergenhagen. 1981. Stimulation of rheumatoid synovial cell collagenase and prostaglandin production by partially purified lymphocyte-activating factor (interleukin-1). *Proc. Natl. Acad. Sci. USA.* 78:2474–2477.

15. Simon, P. L., C. L. Green, and J. C. Lee. 1985. Preparation and biological activities of a goat IgG antibody to human pI 6.9 interleukin-1. *In* The Physiologic, Metabolic, and Immunologic Actions of Interleukin 1. M. J. Kluger, J. J. Oppenheim, and M. C. Powanda, editors. Alan R. Liss, Inc., New York. 481–489.

16. Dower, S. K., S. M. Call, S. Gillis, and D. L. Urdal. 1986. Similarity between the interleukin 1 receptors on a murine T-lymphoma cell line and on a murine fibroblast cell line. *Proc. Natl. Acad. Sci. USA.* 83:1060–1064.

17. Gubler, U., A. O. Chua, A. S. Stern, C. P. Hellmann, M. P. Vitek, T. M. Dechiara, W. R. Benjamin, K. J. Collier, M. Dukovich, P. C. Familletti, C. Fiedler-Nagy, J. Jenson, K. Kaffka, P. L. Kilian, D. Stremlo, B. H. Wittreich, D. Woehle, S. B. Mizel, and P. T. Lomedico. 1986. Recombinant human interleukin 1: purification and biological characterization. *J. Immunol.* 136:2492–2497.

18. Schmidt, J. A., C. N. Oliver, J. L. Lepe-Zuniga, I. Green, and I. Gery. 1984. Silica-stimulated monocytes release fibroblast proliferation factors identical to interleukin 1. *J. Clin. Invest.* 73:1462–1472.

19. McCroskery, P. A., S. Arai, E. P. Amento, and S. M. Krane. 1985. Stimulation of procollagenase synthesis in human rheumatoid synovial fibroblasts by mononuclear cell factor/interleukin-1. *FEBS (Fed. Eur. Biochem. Soc.) Let.* 191:7–12.

20. Murphy, G., J. J. Reynolds, and Z. Werb. 1985. Biosynthesis of tissue inhibitor of metalloproteinases by human fibroblasts in culture. Stimulation by 12-O-tetradecanoylphorbol 13-acetate and interleukin 1 in parallel with collagenase. *J. Biol. Chem.* 260:3079–3083.

21. Saklatvala, J., L. M. C. Pilsworth, S. J. Sarsfield, J. Gavrilovic, and J. K. Heath. 1984. Pig catabolin is a form of interleukin 1. Cartilage and bone resorb, fibroblasts make prostaglandin and collagenase, and thymocyte proliferation is augmented in response to one protein. *Biochem. J.* 224:461–466.

22. Postlethwaite, A. E., L. B. Lachman, C. L. Mainardi, and A. H. Kang. 1983. Interleukin 1 stimulation of collagenase production by cultured fibroblasts. *J. Exp. Med.* 157:801–806.

23. McGuire-Goldring, M. B., J. E. Meats, D. D. Wood, E. J. Ihrie, N. M. Ebsworth, and R. G. G. Russell. 1984. In vitro activation of human chondrocytes and synoviocytes by a human interleukin-1-like factor. *Arthritis Rheum.* 27:654–662.

24. Gowen, M., D. D. Wood, E. J. Ihrie, M. K. B. McGuire, and R. G. G. Russell. 1983. An interleukin 1-like factor stimulates bone resorption in vitro. *Nature (Lond.).* 306:378–380.

25. Dewhirst, F. E., P. P. Stashenko, J. E. Mole, and T. Tsurumachi. 1985. Purification and partial sequence of human osteoclast-activating factor: identity with interleukin 1β. *J. Immunol.* 135:2562–2568.

26. Gowen, M., and G. R. Mundy. 1986. Action of recombinant interleukin 1, interleukin 2, and interferon-α on bone resorption in vitro. *J. Immunol.* 136:2478–2482.

27. Krane, S. M., J.-M. Dayer, L. S. Simon, and M. S. Byrne. 1985. Mononuclear cell conditioned medium containing mononuclear cell factor (MCF), homologous with interleukin 1, stimulates collagen and fibronectin synthesis by adherent rheumatoid synovial cells: effects of prostaglandin E_2 and indomethacin. *Collagen Relat. Res.* 5:99–117.