Identification of a High-Affinity Receptor for Interleukin 1 α and Interleukin 1 β on Cultured Human Rheumatoid Synovial Cells

Jayne Chin, Elizabeth Rupp, Patricia M. Cameron, Karen L. MacNaul, Paul A. Lotke,* Michael J. Tocci, John A. Schmidt, and Ellen Kahn Bayne

Department of Biochemistry and Molecular Biology, Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey 07065; and

*Department of Orthopedic Surgery, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Abstract

In this report the binding of recombinant human interleukins 1 α and 1 β (rIL-1 α and rIL-1 β) to primary cultures of human rheumatoid synovial cells is measured and compared to the concentrations of these mediators required for stimulation of PGE₂ production by these same cells. The average concentration of IL-1 α required for half-maximal stimulation of PGE₂ was 4.6 $\pm$ 1.5 pM ($\pm$ SEM) (n = 6), whereas for IL-1 β half-maximal stimulation was observed at a concentration of 1.3 $\pm$ 0.24 pM (n = 6). Both direct and competitive binding experiments were performed. In direct binding experiments, IL-1 α bound with a K_d of 66 pM (n = 1), while IL-1 β bound with a K_d of 4 pM (n = 2). In competitive binding experiments, IL-1 α inhibited binding of ¹²⁵I-IL-1 α with a K_i of 33-36 pM (n = 2) and binding of ¹²⁵I-IL-1 β with a K_i of 51-63 pM (n = 2). IL-1 β inhibited binding of ¹²⁵I-IL-1 α with a K_i of 2-3 pM (n = 2) and binding of ¹²⁵I-IL-1 β with a K_i of 7 pM (n = 2). The binding data were best fit by a model specifying a single class of receptors with homogeneous affinity for either IL-1 α or IL-1 β and with an abundance of 3,000-14,000 sites per cell. Autoradiography showed that the vast majority of the synoviocytes within the cultures possessed IL-1 receptors. Comparison of biological response curves with the binding curves indicates that the observed receptors exhibit sufficiently high affinity to mediate the response of human synoviocytes to low picomolar concentrations of IL-1 α and IL-1 β .

Introduction

IL-1 α and IL-1 β are macrophage-derived proteins which, in addition to their effects on lymphoid cells, are now known to be potent activators of connective tissue cells (1, 2). As such IL-1 is thought to play an important role during chronic inflammatory disease and in particular during rheumatoid arthritis. Studies have shown that IL-1 not only can induce bone and cartilage resorption by acting directly on the cells in these tissues (3-7), but that IL-1 also is capable of stimulating the secretion of large quantities of collagenase and prostaglandin E₂ (PGE₂) from synovial lining cells (4, 8-10). Thus much of the joint destruction that characterizes rheumatoid arthritis may be attributed to IL-1. Furthermore, in addition to causing

connective tissue destruction, IL-1 has also been shown to promote the synthesis of fibronectin and collagen by synoviocytes (11). Both of these extracellular matrix proteins are major components of rheumatoid pannus (12-15) and may promote pannus development.

Previously, we compared the specific bioactivities of purified, monocyte-derived IL-1 α and IL-1 β on cultured human rheumatoid synovial cells (16). Our studies demonstrated that both of these molecules were active at very low pM concentrations (i.e., half-maximal activation at concentrations of 0.5-13 pM). This suggested that human synovial cells must possess high-affinity receptors for IL-1. In the present study we have examined the binding of IL-1 α and IL-1 β to synoviocytes and identify such receptors. Autoradiography further showed that the vast majority of the synoviocytes in rheumatoid synovial cell cultures possess IL-1 receptors. Our binding data are consistent with the hypothesis that the bioactivities exerted by both IL-1 α and IL-1 β on synovial cells are mediated through a single class of high-affinity receptor sites that have differential affinity for these mediators.

Methods

Culture of human synovial cells. Human synovial tissue was obtained from patients with rheumatoid arthritis who were undergoing total knee replacement surgery. The tissue was enzymatically dissociated and the cells subsequently cultured (in Dulbecco's modified Eagle's medium with 10% fetal bovine serum; 100 U/ml penicillin, and 100 μ g/ml streptomycin) according to the methods of Baker et al. (17). Cells were cultured for 5-10 d before use and all experiments were performed on primary cultures that had received at least two medium changes. Determinations of cell number were made by counting trypsinized cells in a hemacytometer.

Purification of human IL-1 α and IL-1 β . Nucleic acid sequences encoding residues Leu¹¹⁹-Ser²⁷¹ of IL-1 α and residues Ala¹¹⁷-Ser²⁶⁹ of IL-1 β were expressed in *Escherichia coli* strain JM 105 as previously described for recombinant (r)IL-1 β (18). These residues correspond exactly to those found in native human IL-1 α , pI 5.2 (19) and native human IL-1 β , pI 6.8 (20), respectively. The recombinant proteins were extracted from bacterial lysates and purified as previously described (18, 19).

Each preparation was found to be pure as assessed by reverse-phase HPLC and by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by silver staining (19, 20). Protein concentration was determined by integration of absorbance profiles obtained at 210 nm as the IL-1 species eluted from a reverse-phase HPLC column (19, 20). The integration function of the detection system was calibrated using known amounts of pure ribonuclease. This method was previously validated by amino acid analysis of pure protein (20).

The purified rIL-1 α and rIL-1 β were identical to native human IL-1 α , pI 5.2 (19), and native human IL-1 β , pI 6.8 (20), as shown by a large number of criteria. These included comigration on SDS-PAGE (mol wt 17,500), identical amino terminal sequence analyses, and equal potency in the murine thymocyte proliferation assay and human

Address reprint requests to Dr. Bayne, Merck Sharp & Dohme Research Laboratories, P.O. Box 2000, Rahway, NJ 07065.

Received for publication 20 October 1987 and in revised form 9 February 1988.

J. Clin. Invest.

© The American Society for Clinical Investigation, Inc.

0021-9738/88/08/0420/07 \$2.00

Volume 82, August 1988, 420-426

dermal fibroblast proliferation assay. As previously reported for their native counterparts (16, 19, 20), rIL-1 α and rIL-1 β both gave half-maximal stimulation of murine thymocyte proliferation at a concentration of 24–34 pM and half-maximal stimulation of dermal fibroblast proliferation at a concentration of 1–5 pM. Most relevant to the current study, each recombinant protein and its native counterpart gave superimposable inhibition profiles in competitive receptor binding experiments on MRC-5 human embryonic lung fibroblasts (18 and unpublished observations).

Biological response of synoviocytes to IL-1. As a measure of IL-1 bioactivity, induction of PGE₂ secretion was monitored. Primary cell cultures that had been seeded in 24-well plates were incubated with varying concentrations of pure IL-1 α or IL-1 β in fresh culture medium for 24 h. The culture supernatants were then collected and PGE₂ measured by radioimmunoassay as described by Humes (21).

Preparation of ¹²⁵I-labeled IL-1 α and IL-1 β . Pure rIL-1 α was labeled with Na¹²⁵I using chloramine-T (22), and the radioligand was purified by HPLC gel filtration chromatography as previously described for labeled IL-1 β (23). Pure rIL-1 β was labeled with Bolton Hunter reagent (New England Nuclear, Boston, MA) and the iodinated IL-1 β was then separated from unincorporated label, as well as from unlabeled IL-1 β , as described elsewhere (23). The iodoproteins were purified in the absence of carrier and thus the amount of protein in the iodinated preparations was determined directly, as described above, by integration of the absorbance profiles. The specific radioactivity of various preparations was calculated to be 1,600–2,700 Ci/mmol for ¹²⁵I-IL-1 α and 2,000–2,400 Ci/mmol for ¹²⁵I-IL-1 β . The percentage of labeled IL-1 molecules able to bind to synoviocytes was assessed by successive absorptions as previously described (23). Approximately 75% of the ¹²⁵I-IL-1 α and 50% of the ¹²⁵I-IL-1 β was able to be specifically bound and thus factors of 0.75 and 0.5 were used, respectively, to correct the number of free counts in both the direct and competitive binding experiments (23, 24). Both preparations were > 98% precipitable with cold 10% trichloroacetic acid.

Binding experiments. All binding assays were carried out on primary cultures of synoviocytes that had been seeded into six-well cluster plates and had reached confluence at $\sim 1 \times 10^6$ cells per well. To determine the time required to reach steady state binding, 10 pM radioligand in binding buffer (RPMI medium with 0.5% gelatin and 0.2% sodium azide) was added to replicate cultures and at varying times duplicate wells were washed in phosphate-buffered saline (PBS), solubilized in 2.5 M NaOH, and counted as previously described (23). Nonspecific binding at each time point was assessed by incubating a set of sister cultures with 10 pM radioligand together with a 50-fold molar excess of unlabeled homologous ligand. This second set of cultures was harvested in parallel with the first.

For direct binding experiments cultures were incubated with varying concentrations of radioligand, with or without a 50-fold molar excess of unlabeled homologous ligand, in binding buffer for 2 h at 20°C. The cultures were then washed, solubilized, and counted as described above. Specific binding was calculated by subtracting counts bound in the presence of excess unlabeled ligand from the total counts bound in the absence of excess unlabeled ligand.

For competitive binding experiments the cultures were incubated with 4 pM radioligand and increasing concentrations of unlabeled IL-1 α or IL-1 β for 2 h at 20°C, then washed, solubilized, and counted. In addition, experiments were conducted in which the incubations with radioligand were carried out in the presence of excess unlabeled human recombinant tumor necrosis factor- α (TNF α ; kindly provided by Dr. Susan Socher, Merck Sharp & Dohme Research Laboratories, West Point, PA), human recombinant interleukin-2 (rIL-2) (Amgen Corp., Thousand Oaks, CA), or human recombinant γ -interferon (Amgen Corp.).

Data analysis. All binding data were analyzed using the 1986 version of the LIGAND (24) family of programs on an IBM personal computer as previously described (23). One of the features of the program is its ability to assess the goodness of fit provided by models specifying single or multiple receptor sites (24). A single site-two-ligand model

was used to analyze the competitive binding experiments which are tabulated in Table I.

Autoradiography. For autoradiography cultures grown in 35-mm culture plates were incubated for 2 h at 20°C with 200 pM ¹²⁵I-IL-1 β with or without 1 nM unlabeled IL-1 β . After the incubation period the cultures were washed extensively, fixed in 2% glutaraldehyde in PBS, and then washed again, first in PBS and then in water. The cultures were then air dried and coated with undiluted Kodak NTB-2 emulsion (Eastman Kodak Co., Rochester, NY). The exposed emulsion was developed in Kodak D-19 developer at 18°C for 5 min and the resulting autoradiographs were viewed by phase-contrast microscopy.

Results

Bioactivities. We have previously reported that synoviocytes from individual specimens of human rheumatoid pannus respond in a dose-dependent and saturable manner to low picomolar amounts of either IL-1 α or IL-1 β (16). In Fig. 1 the results of six experiments performed on cells from six different patients are averaged. The average concentration of IL-1 α required for half-maximal stimulation of PGE₂ production was 4.6 ± 1.5 pM ($\pm$ SEM), whereas for IL-1 β half-maximal stimulation was obtained at an average of 1.3 ± 0.24 pM. The maximal amount of PGE₂ obtained in different experiments ranged from 1,360 to 2,369 pg/1,000 cells with a mean value ($\pm$ SEM) of $1,840 \pm 178$. The same maximal level of stimulation was observed for both species of IL-1.

Binding experiments. The rate of binding of rIL-1 α and rIL-1 β to cultured human synoviocytes was assessed at 20°C in the presence of azide as described in Methods. Our choice of temperature reflects our previous finding on human lung fibroblasts that IL-1 β binds much more rapidly at 20°C than at 4°C (23). Under these conditions specific binding of radioligand to the synovial cells achieved a steady state within 2 h (data not shown). To determine whether the ligand remained surface-bound or was internalized, cells equilibrated with ¹²⁵I-IL-1 β were washed and exposed to 2% acetic acid (pH 2.5) for 1 min (23). In two separate experiments we found that 80% and 79% of the cell-associated counts were removed by this procedure. Of the counts removed, 95% in the first experiment and 97% in the second experiment were TCA precipitable. Thus most of the label detected upon solubilization of experimental cultures appears to represent nondegraded, surface-bound IL-1. Similarly, the free counts in the supernatant remained 99% TCA precipitable during the course of the 2-h binding assay.

We next carried out direct binding experiments in which synovial cells were incubated with increasing concentrations of ¹²⁵I-IL-1 α or ¹²⁵I-IL-1 β for 2 h at 20°C in the presence of sodium azide. Results of such experiments are shown in Fig. 2, A and B and tabulated in Table I. Nonspecific binding constituted < 5% of the total counts bound in each case. Specific binding was found to be both dose dependent and saturable for both radioligands. Computer-generated Scatchard analysis of the binding data obtained with ¹²⁵I-IL-1 α (see inset, Fig. 2 A) indicated a single class of receptors with an equilibrium dissociation constant (K_d) of 66 pM ($n = 1$; Table I). Direct binding data for IL-1 β was likewise best fit by a model specifying a single class of high-affinity receptors with a K_d of 4 pM ($n = 2$) (Fig. 2 B; Table I). IL-1 α bound to a maximum of 9,200 receptors per cell while IL-1 β bound to a maximum of 6,100–6,800 receptors per cell (Table I).

Figure 1. PGE₂ secretion by human rheumatoid synoviocytes in response to increasing concentrations of (□) IL-1β or (Δ) IL-1α. The means (±SEM) of duplicate determinations from six different experiments performed on cells obtained from six different patients are shown.

Fig. 2, C and D, shows representative results of competitive binding experiments. In such experiments synovial cells were incubated for 2 h at 20°C with a limiting concentration of either radioligand and increasing concentrations of unlabeled IL-1α or IL-1β. Binding of ¹²⁵I-IL-1α was competed by unlabeled IL-1α with a *K_i* of 33–36 pM and unlabeled IL-1β with a *K_i* of 2–3 pM (*n* = 2; Table I). Binding of ¹²⁵I-IL-1β was competed by unlabeled IL-1α with a *K_i* of 51–63 pM and unlabeled IL-1β with a *K_i* of 7 pM (*n* = 2; Table I). Thus IL-1β competed more efficiently than IL-1α against both radioligands, and the *K_i* values obtained for IL-1α (33–63 pM) or IL-1β (2–7 pM) were similar irrespective of the radioligand employed. Human tumor necrosis factor-α, human IL-2, and human γ-interferon did not inhibit the binding of IL-1α or IL-1β when tested at a concentration of 50 nM (data not shown). As was the case for the direct binding data, a single-site model best fit the homologous competitive binding curves obtained with each species of IL-1. This finding, taken together with the observation that binding of each radioligand is completely inhibited by both species of IL-1 with the same rank order of potency, provided the justification for using a single-site model in the LIGAND program (see Methods) to analyze the competitive binding data shown in Fig. 2, C and D, and Table I. Using this approach the number of receptor sites per cell calculated for IL-1α or IL-1β in the competitive binding experiments was 3,400–13,400 (Table I). The explanation for this degree of interexperimental variation in receptor number is unclear, but may be due to differences in cell cycle, variation from patient to patient, or endogenously produced IL-1.

Autoradiography. Our calculations of receptor number per cell are based on the assumption that all of the cells within the synovial cell preparations bear IL-1 receptors on their surfaces. Because we were dealing with primary cultures that may contain more than one cell type, however, it was important to test this hypothesis. Accordingly, autoradiographic experiments were performed. Cultures incubated with saturating levels of labeled IL-1β (either in the presence or absence of excess unlabeled IL-1β) were fixed, coated with autoradiographic emulsion, and then exposed for varying periods of time (up to 2 mo) before development. An example of the results obtained is shown in Fig. 3. In those cultures incubated in the presence of

Table I. Summary of Results Obtained in Direct and Competitive Binding Experiments on Human Rheumatoid Synovial Cells

Radioligand	K_d	Receptors	
	pM	$sites/cell$	
Direct binding experiments*			
^{125}I -rIL-1 α			
Experiment 1	66	9,200	
^{125}I -rIL-1 β			
Experiment 1	4	6,100	
Experiment 2	4	6,800	
Competitive binding experiments†			
Radioligand	K_i of competing ligand		Receptors
	rIL-1 α	rIL-1 β	
	pM	pM	$sites/cell$ for IL-1 α/β
Competitive binding experiments†			
^{125}I -rIL-1 α			
Experiment 1	36	2	6,700
Experiment 2	33	3	13,400
^{125}I -rIL-1 β			
Experiment 1	63	7	4,100
Experiment 2	51	7	3,400

* Direct binding experiments were performed by incubating varying concentrations of radioligand with or without excess unlabeled homologous ligand as described in Methods. The *K_d* and receptor concentration were calculated by computerized analysis of the binding data (LIGAND [24]) (see Methods).

† Competitive binding experiments were performed by incubating 4 pM radioligand and increasing concentrations of unlabeled IL-1α or IL-1β with synovial cells as described in Methods. *K_i* and receptor concentrations were calculated using LIGAND and a single-site, two-ligand model (see Methods).

excess unlabeled IL-1β (Fig. 3 B) only a very few randomly distributed silver grains were detected and no more than three grains were ever seen associated with a given cell. In all cultures treated with labeled IL-1β alone, however, silver grains could be seen over almost every cell (Fig. 3 A). Cell counts revealed that 97% of the cells in these cultures were labeled with six or more grains. Thus, consistent with the assumptions made above, the vast majority of synoviocytes specifically bound IL-1.

Discussion

The binding of IL-1α and IL-1β to rheumatoid synovial fibroblasts was assessed in direct and competitive binding experiments (Fig. 2, Table I). IL-1α gave a *K_d* of 66 pM in direct binding experiments and in competitive binding experiments gave *K_i*'s of 33–36 pM vs. ¹²⁵I-IL-1α and 51–63 pM vs. ¹²⁵I-IL-1β. These results are in good agreement with each other and a previous study on human embryonic lung fibroblasts where a *K_i* of 50±18 pM (±SEM) for IL-1α vs. ¹²⁵I-IL-1β was obtained (23). IL-1β bound with ~ 10-fold higher affinity than IL-1α

Figure 2. (Upper panels) Direct binding of (A) ^{125}I -IL-1 α and (B) ^{125}I -IL-1 β to human rheumatoid synovial cells. Cultures were incubated with increasing concentrations of (Δ) ^{125}I -IL-1 α or ($\square$) ^{125}I -IL-1 β alone or ($\circ$) in the presence of a 50-fold molar excess of unlabeled IL-1 α or β , respectively. Specific binding of ($\blacktriangle$) ^{125}I -IL-1 α and ($\blacksquare$) ^{125}I -IL-1 β was determined by subtracting counts bound in the presence of excess unlabeled IL-1 from counts bound in the presence of

labeled IL-1 alone. All radioligand concentrations were corrected for bindability. (Insets) The computer-generated Scatchard plots of the data. (Lower panels) Competitive binding experiments. Human rheumatoid synoviocytes were incubated with 4 pM (C) ^{125}I -IL-1 α or (D) ^{125}I -IL-1 β in the presence of increasing concentrations of ($\blacktriangle$) unlabeled IL-1 α or ($\square$) unlabeled IL-1 β .

giving a K_d of 4 pM in two direct binding experiments and K_i 's in competitive binding experiments of 2–3 pM vs. ^{125}I -IL-1 α and 7 pM vs. ^{125}I -IL-1 β . Once again, these results are in good agreement with each other and a previous study on lung fibroblasts where IL-1 β , in direct binding experiments, gave a mean K_d of 8 ± 4 pM and, in competitive binding experiments, gave a mean K_i of 11 ± 3 pM vs. ^{125}I -IL-1 β (23).

The receptors on rheumatoid synovial fibroblasts and lung fibroblasts (23) both exhibit homogeneous affinity for either IL-1 α or IL-1 β . No evidence was found for populations of high and low affinity IL-1 receptors, as has been reported for other receptor systems (e.g., the IL-2 receptor system, by Robb et al. [25]) or for IL-1 receptors on the murine thymoma line, EL4 (26), or porcine synoviocytes (27). This observation, together with the observation that the binding of each radioligand is completely inhibited by both species of IL-1 with the same rank order of potency, argues strongly for a single class of IL-1 receptors on human rheumatoid synovial cells. Complete dis-

placement of IL-1 β by IL-1 α , and vice versa, has been a general property of IL-1 receptors on all cells studied thus far (28, 29).

The number of receptors on rheumatoid synovial fibroblasts (mean of 7,360 from three direct binding experiments and a mean of 6,900 from four competitive binding experiments) is similar to the number of receptors found on human embryonic lung fibroblasts (23), human dermal fibroblasts (22, 27), and porcine synoviocytes (27) but considerably higher than the number found on normal human lymphoid cells (30, and our unpublished observations). In the current study, autoradiography was performed which revealed that most cells in primary cultures from rheumatoid pannus bear IL-1 receptors and that the distribution of receptors among receptor-positive cells appears to be homogeneous. Previous studies (31) and our own unpublished work (Bayne et al., manuscript in preparation) show that the great majority of cells in such cultures lack lymphoid markers and therefore are presumably of connective

Figure 3. Autoradiograph of human rheumatoid synovial cells incubated (A) with 200 pM ^{125}I -IL-1 β alone or (B) with the same concentration of radioligand in the presence of 1 nM unlabeled IL-1 β . Bar, 30 μm .

tissue origin. Whether the small number of mononuclear cells present in such cultures are also receptor positive by this technique must await the results of double labeling studies.

If one averages the K_d and K_i values obtained for IL-1 α or IL-1 β (Table I), one obtains mean equilibrium binding constants of 49.8 ± 6.8 ($\pm$ SEM) pM for IL-1 α and 4.5 ± 0.86 pM for IL-1 β . These values represent the mean concentrations of ligand necessary for occupation of half of the available receptors. Comparison of these values with the lower concentrations of these mediators required for half-maximal stimulation of PGE $_2$ (IL-1 α , 4.6 pM; IL-1 β , 1.3 pM; Fig. 1), shows that occupation of a small, but finite, percentage of the available receptors at any one time results in a proportionately larger biological response. Using the mass action equation, occupation of ~ 550 receptors by IL-1 β or ~ 480 receptors by IL-1 α would occur at the mean concentrations of mediator required for half-maximal PGE $_2$ secretion. Signal amplification has been reported for other receptor systems (32) and appears to be a property of IL-1 receptor systems on most cells examined to date (23, 28, 29, 33).

The receptor affinities obtained in the current study on primary cultures of human rheumatoid synovial cells differ from many of the values obtained by other groups on various types of connective tissue cells. Dower et al. (22) reported that human IL-1 α and human IL-1 β bound to human dermal fibroblasts with K_d 's of 625 and 555 pM, respectively, and to murine 3T3 cells with K_d 's of 333 and 476 pM, respectively.

More recently, Mizel et al. (34) reported a K_d of 40–50 pM for recombinant human IL-1 α on murine 3T3 cells. Bird and Saklatvala (27) reported that porcine IL-1 α ("IL-1/5") and porcine IL-1 β ("IL-1/8") bound to porcine synoviocytes with K_d 's of 170 and 150 pM, respectively. Thus all but one of these studies have reported considerably lower affinities for IL-1 α binding to connective tissue cells and all have reported that IL-1 β binds with at least 30–100-fold lower affinity than found in the current study. The lower affinities reported by others appear to be inconsistent with the exceedingly low concentrations of IL-1 α and IL-1 β required for rheumatoid synovial cell activation (16, and current study). Direct comparison of our results with those of others is difficult, however, because the other studies measure the binding of human IL-1 to murine 3T3 cells (22, 23), or porcine IL-1 to human or porcine connective tissue cells (27). Some of these cell types, particularly 3T3 cells (33), appear to be considerably less sensitive to human IL-1 activation than rheumatoid synovial cells. There is only one previous study (22), aside from our own (23), in which binding of human IL-1 to human connective tissue cells was measured. In that study, the biological responsiveness of the target cells to IL-1 was not determined. The current binding studies on rheumatoid synovial cells, and our previous study on human embryonic lung fibroblasts (23), are the only reports directly comparing the biological and binding activities of human IL-1 molecules on human connective tissue cells.

IL-1 is a potent inflammatory mediator which, in view of

its demonstrated activities on the connective tissue cells of the joint (35), is likely to be responsible for much of the destruction that occurs in rheumatoid arthritis as well as in other inflammatory joint diseases. The present work demonstrates that synovial cells from patients with rheumatoid arthritis possess specific receptors for both IL-1 α and IL-1 β which have sufficiently high affinity to mediate the biological properties of these mediators. The evidence shows that both species of IL-1 compete for a common class of receptors on these cells. The results obtained on rheumatoid synovial cells are in good agreement with those obtained on normal human embryonic lung fibroblasts (23) thus minimizing the possibility that abnormal IL-1 receptors play an etiologic or pathophysiologic role in rheumatoid arthritis. More detailed studies of IL-1 receptors found on more readily available human connective tissue cells can now be performed with the reasonable assurance that the results will be applicable to rheumatoid synovial cells as well.

Acknowledgments

The authors thank Mrs. Marcella Paoline and Ms. Lynne Purdy for careful preparation of the manuscript.

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