DETECTION AND CHARACTERIZATION OF HIGH AFFINITY PLASMA MEMBRANE RECEPTORS FOR HUMAN INTERLEUKIN 1

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Interleukin 1 (IL-1), a polypeptide hormone produced by activated macrophages, has been reported to mediate an apparently diverse range of biological activities (1-3). These include lymphocyte-activating factor (LAF) (4), endogenous or leukocyte pyrogenic activity (5), epidermal cell thymocyte-activating factor (6), bone resorption factor (7, 8), and fibroblast growth factor (9). In general, this set of activities would seem to be consistent with the notion that IL-1 acts as a soluble mediator during inflammatory responses. We have recently purified to homogeneity a polypeptide secreted by human monocytes that produces several IL-1-like effects (10).

The initial step in the action of IL-1 is most likely the binding of this hormone to plasma membrane receptors. The availability of purified IL-1 in our laboratory rendered a search for such receptors possible. For the initial characterization of the plasma membrane (IL-1) receptors, we chose the murine T lymphoma LBRM-33-1A5 as a model cell. This cell line produces interleukin 2 in response to suboptimal doses of phytohemagglutinin (PHA) in an IL-1-dependent fashion, and constitutes a well-defined model system for IL-1 action (11, 12).

Using radiolabeled human IL-1, we show that these cells bind IL-1 specifically and with high affinity. We further show that cell-bound IL-1 is associated with a membrane protein of ~80,000 mol wt. Finally, we have surveyed a broad range of cell types from several species for 125I-IL-1 binding. In general, the data are consistent with the known biological effects of IL-1, in that cell types which bind IL-1 are those previously reported to respond to it. The cell types that showed significant levels of IL-1 binding were either lymphoid in origin or fibroblast/epithelial type cells. The data support the view that, like other polypeptide hormones, IL-1 action is mediated by a specific plasma membrane receptor protein. The characterization of this protein, both at the functional and structural levels, should aid in understanding the diverse biological activities of IL-1.

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Abbreviations used in this paper: BSA, bovine serum albumin; DSP, dithiobis-succinimidyl propionate; DSS, disuccinimidyl suberate; DST, disuccinimidyl tartrate; IL-1, IL-2, interleukins 1 and 2; PBS, phosphate-buffered saline; PHA, phytohemagglutinin; PMSF, phenylmethylsulfonyl fluoride; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.
Materials and Methods

Cell Preparations. Cell lines were propagated in 175-cm² tissue culture flasks at 37°C in 5% CO₂, in RPMI 1640 containing 10% fetal calf serum. Cell lines that grow adherently were harvested by scraping without prior trypsin treatment to avoid the possibility that IL-1 receptors would be lost due to proteolytic degradation. In some cases a brief EDTA treatment was used to aid in detachment.

Murine thymocytes, splenocytes, and lymph node cells were isolated as single-cell suspensions from tissues. Fractionation of thymocytes by peanut lectin (Arachis hypogaea) was done by agglutination (13), followed by separation on fetal bovine serum (14). Human peripheral blood mononuclear cells were isolated from whole human blood by standard procedures (15).

Hormone Preparations. Thyroid-stimulating hormone (TSH), luteinizing hormone (LH), human growth hormone (GH), bovine insulin, and follicle-stimulating hormone (FSH) were purchased from Calbiochem-Behring Corp. (La Jolla, CA). Platelet-derived growth factor (PDGF), epidermal growth factor (EGF), nerve growth factor (NGF), and fibroblast growth factor (FGF), were purchased from Bethesda Research Laboratories, (Gaithersburg, MD). Human recombinant IL-2 was expressed in, and purified from, E. coli and was provided under a collaboration between Hoffmann-La Roche, Inc. (Nutley, NJ) and Immunex Corporation. Colony-stimulating factor 2α (CSF-2α) was purified as described previously (16).

Purification and Radiolabeling of Human IL-1. IL-1 was isolated from supernatants of human mononuclear cells cocultured with heat-killed, formalin-fixed Staphylococcus aureus for 24 h at 37°C. Purification of IL-1 was done by using sequential SP-Sephadex, DEAE-Sephacel, and Procion red agarose column chromatography as described elsewhere (10). This material has a specific activity of ~5 × 10¹⁵ (U/mg) in the LBRM-33-1A5 conversion assay and contains a single 17,500 mol wt protein by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis. Before radioiodination, IL-1 was concentrated to ~10 µg/ml by rechromatography on a 0.5 ml bed volume SP-Sephadex column, and eluted with 0.02 M sodium borate, 0.15 M sodium chloride, pH 8.5. The concentration of the final solution was estimated by silver staining (17) of an aliquot run on SDS-PAGE.

Purified IL-1 was labeled using diiodo(¹²³I)-Bolton-Hunter reagent (New England Nuclear, Boston, MA) according to the manufacturer’s instructions. Briefly, 200 ng of IL-1 in 20 µl of SP-Sephadex elution buffer was mixed with 5 µl of 0.1 M sodium borate, 0.75 M NaCl, pH 8.5 and added to a vial with 100 µCi of benzene containing 1 mCi (0.23 mmol) of Bolton-Hunter reagent had been evaporated using a gentle stream of dry nitrogen. The reaction was allowed to proceed for 1 h on ice and was terminated by addition of 5 µl of 1 M glycine ethyl ester. 30 µl of 2% gelatin in phosphate (0.05 M)-buffered saline (0.15 M) pH 7.4 (PBS), was added as a carrier, and labeled IL-1 was separated from free Bolton-Hunter reagent by chromatography on a 1 ml bed volume column of Biogel P6 (Bio-Rad Laboratories, Richmond, CA) packed in a Pasteur pipette. The column bed material was blocked with BSA and subsequently washed with 20 ml of PBS to remove unbound bovine serum albumin (BSA) before use. 100-µl fractions were collected and fractions containing protein-bound radioactivity were pooled. Due to the small amounts of IL-1 available to us, direct estimates of the recovery after the radiolabeling were not possible. To estimate recovery, control experiments were done in which IL-1 (200 ng) was mixed with ¹²³I-IL-1 (3 × 10⁴ cpm) and put through the iodination procedure, with the omission of Bolton-Hunter reagent. In five replicate experiments, 54 ± 8% of the counts were recovered after the gel filtration step. In subsequent iodinations, this percentage was taken as the recovery of IL-1 protein. Using this estimate, the specific activity of the radiolabeled IL-1 preparations was 2-5 × 10¹⁵ cpm/mmol, based on a molecular weight of 17,500.

Assay of IL-1 Biological Activity. IL-1 biological activity was monitored using the IL-1 conversion assay as described by Conlon (12). This assay depends on the fact that, in the presence of 0.1% PHA, the LBRM-33-1A5 cell line produces IL-2 only in the presence of exogenous IL-1. The resulting IL-2 produced is assayed, in turn, by the capacity to
maintain proliferation of the murine T cell line CTLL-2. The proliferation of the CTLL-2 cells, measured by \(^{3}H\)thymidine incorporation, is hence dependent on the concentration of IL-1 in which the LBRM-33-1A5 cells are initially incubated. A solution of IL-1 containing 1 U/ml produces 50% of maximal incorporation of \(^{3}H\)thymidine in the assay.

**Binding Assays.** Cells and \(^{125}I\)-IL-1 were incubated in binding medium (RPMI 1640 containing 1% BSA, 0.1% sodium azide, and 20 mM Hepes, pH 7.2) at a concentration of 6.7 \(\times\) 10^7 cells/ml and the appropriate concentration of \(^{125}I\)-IL-1, for a total volume of 150 \(\mu\)l, for a time estimated to allow the system to reach equilibrium (see below). Incubations were carried out at 8°C, on a rocker platform, to ensure continuous mixing of cells and radioactive ligand. Nonspecific binding of \(^{125}I\)-IL-1 was measured by incubations in the presence of at least a 20-fold molar excess of unlabeled IL-1, at a dose of unlabeled ligand predetermined to lead to 100% receptor occupancy. At the end of the incubation, bound and free \(^{125}I\)-IL-1 were separated by removing duplicate 60-\(\mu\)l aliquots from the incubation mixture, layering these on 200 \(\mu\)l of a phthalate oil mixture in 400-\(\mu\)l polyethylene centrifuge tubes (VWR Scientific Div., Univar, San Francisco, CA) and centrifuging for 30 s in a microfuge. The oil mixture, 1.5 parts dibutyl phthalate, 1 part bis(2-ethylhexyl)-phthalate (Eastman Kodak Co., Rochester, NY), allows cells to sediment but is more dense than binding medium. Bound and free ligand were estimated by cutting the tube in half and counting the top and tip as described previously (18, 21). Human gingival fibroblasts (a kind gift of Dr. R. C. Page, University of Washington), were assayed for IL-1 receptors in situ in 24-well Linbro plates after at least 48 h of postpassage culture. For association kinetics experiments, incubation mixtures containing either \(^{125}I\)-IL-1 alone or \(^{125}I\)-IL-1 and excess unlabeled IL-1 were prepared. At the start of the reaction, cells were added. At various subsequent times, duplicate 60-\(\mu\)l aliquots of the mixture were removed; bound and free \(^{125}I\)-IL-1 were separated by the phthalate oil method as described above. The time of measurement was taken as time of initiation of centrifugation. For dissociation kinetics, cells were incubated in the presence of 150 \(\mu\)l of binding medium containing \(^{125}I\)-IL-1 for 4 h. Cells were then sedimented in two separate 75-\(\mu\)l aliquots and resuspended in either 1 ml binding medium or 1 ml binding medium containing 5 \(\times\) 10^-8 M unlabeled IL-1. The time at which the cells were resuspended was taken as the start of the reaction. Samples were taken at various subsequent times, as described for the association kinetics experiments. In general, cell-bound counts per minute in these experiments were 100–5,000; therefore, all samples were counted for 10 min or longer.

**Affinity Crosslinking and SDS-PAGE.** Crosslinkers disuccinimidyl suberate (DSS), di-thiobis succinimidyl propionate (DSP), and disuccinimidyl tartrate (DST) were purchased from the Pierce Chemical Co. (Rockville, IL). For crosslinking, 10^7 cells were incubated with 5 \(\times\) 10^-10 M \(^{125}I\)-IL-1 in 150 \(\mu\)l of binding medium alone or binding medium containing 8 \(\times\) 10^-8 M unlabeled IL-1 for 2 h at 8°C. Subsequently, the cells were washed twice by centrifugation with 1 ml PBS, pH 7.4 and resuspended in 100 \(\mu\)l PBS. 2 \(\mu\)l of crosslinker (50 mg/ml) in dimethyl sulphoxide were added to give a final concentration of 1 mg/ml, and the mixture was incubated for 1 h at 8°C. The cells were then washed twice with PBS and finally resuspended in 50 \(\mu\)l PBS containing 1% Triton X-100 and 2 mM phenylmethylsulphonyl fluoride (PMSF). The detergent extraction mixture was incubated for 5 min on ice and then centrifuged for 15 min at 8°C in a microfuge to remove nuclei and other cell debris. 10 \(\mu\)l of the supernatant was removed for gamma counting and 40 \(\mu\)l was removed and frozen at -20°C until SDS-PAGE analysis. To each of these samples 2 \(\mu\)l of 10% SDS was added and the sample dried under vacuum. The dried aliquots were resuspended in 60 \(\mu\)l of sample buffer (0.06 M Tris-HCl, pH 6.8, 2% SDS, 10% glycerol) in the presence or absence of 5% 2-mercaptoethanol, boiled for 3 min, and then subjected to electrophoresis in 8% PAGE gels using the stacking gel procedure of Laemmli (19) as described elsewhere (20).

Control experiments were performed in which the time of incubation with crosslinker and the concentration of crosslinker used were varied. Further, a mixture of protease inhibitors (pepsatin, 2 mM final [Sigma Chemical Co., St. Louis, MO], e-phenanthrodine, 2 mM final, and PMSF, 2 mM final) was used in some experiments in an attempt to
Data Analysis. Curve fitting of binding and kinetic data was done using RS/1 (BBN Software Products Corporation, Cambridge, MA), a commercially available scientific data processing package running on a VAX 11/750 under the VMS operating system. Kinetic data were analyzed with functions that are sums of exponential terms as described elsewhere (21, 27); binding data were analyzed with sums of simple Michaelis-Menten terms as described (22); and inhibition data were analyzed with an equation for competitive inhibition between two ligands for one type of site (23).

Results

Radiolabeling of Human IL-1. Fig. 1 shows SDS-PAGE analysis of the 125I-Bolton-Hunter reagent–labeled IL-1 preparation used in the binding studies. The radiolabeled protein in the preparation migrated with an M₀ of ~17,500, characteristic of IL-1. The specific biological activity of this material was only 5% of the activity (biological units per milligram protein) of the material before radiolabeling. However, comparison of the binding of radiolabeled 125I-IL-1 with that of unlabeled IL-1 measured by inhibition revealed little, if any, decrease in binding activity for LBRM-33-1A5 cells (see below).

Several tyrosine-directed methods of iodination were tried, including chloramine T (24), iodogen (25), and lactoperoxidase (26). Of these, only the last gave a product with any detectable binding activity, and this was at least 10-fold lower in affinity than unmodified IL-1. Finally, the source of our material, stimulated peripheral blood mononuclear cells, renders biosynthetic labeling with, for example, [35S]methionine, impractical. We therefore chose to use the Bolton-Hunter method for further studies.

Interaction of IL-1 with Plasma Membrane Receptors on LBRM-33-1A5 Cells. Fig. 2 shows the association kinetics of 125I-IL-1 with LBRM-33-1A5 cells at 8°C; all data are corrected for nonspecific binding. The data in Fig. 2A show that both

![Figure 1](https://example.com/f1.png)

**Figure 1.** SDS-PAGE of 125I-labeled IL-1. 125I-labeled IL-1 (53,000 cpm) was boiled for 3 min in sample buffer containing 5% 2-mercaptoethanol, and subjected to electrophoresis on a 10-20% polyacrylamide gradient according to the stacking gel procedure of Laemmli (19). The position of various molecular weight markers (Sigma Chemical Co., St. Louis, MO) is indicated at left.
FIGURE 2. Association kinetics of $^{125}$I-IL-1 with LBRM-33-IA5 cells at 8°C. (A) LBRM-33-IA5 cells ($5.3 \times 10^7$ cells/ml) were incubated with (△) $3.62 \times 10^{-10}$ M, (○) $1.16 \times 10^{-10}$ M, or (□) $3.54 \times 10^{-11}$ M $^{125}$I-IL-1 for various periods. Nonspecific binding, measured in the presence of $6 \times 10^{-9}$ M unlabeled IL-1, was $1.15 \times 10^4$ molecules/cell. The continuous curves passing through the data were calculated from the best-fit parameter values using a single exponential term. Infinite time binding: (△) $82.4 \pm 3.4$ molecules/cell, (○) $37.5 \pm 2.0$ molecules/cell, (□) $14.6 \pm 0.9$ molecules/cell; association rate constants: (△) $1.25 \pm 0.21 \times 10^{-1}/(\text{M} \cdot \text{min})$; (○) $0.49 \pm 0.08 \times 10^{-1}/(\text{M} \cdot \text{min})$; (□) $0.35 \pm 0.05 \times 10^{-1}/(\text{M} \cdot \text{min})$. (B) Plot of the value of the association rate constant against the molar concentration of $^{125}$I-IL-1 initially present in the medium.

the final equilibrium amount bound to the cells and the rate of approach to equilibrium are dependent on the concentration of $^{125}$I-IL-1 initially present in the medium. The curves passing through the data in Fig. 2A are best-fit single exponential time dependency curves, consistent with the presence of a single population of receptors on the cells. Fig. 2B shows the dependence of the pseudo first-order forward rate constant (determined by curve fitting the data of Fig. 2A) on the concentration of $^{125}$I-IL-1 in the medium. As described elsewhere (27), for a bimolecular reaction, such data should fit a straight line, the slope being the forward rate constant and the intercept on the ordinate being the reverse rate constant. The data in Fig. 2B do indeed fall on a straight line, and the parameter values were $2.8 \pm 0.3 \times 10^8/(\text{M} \cdot \text{min})$ for the forward rate constant and $2.1 \pm 0.6 \times 10^{-2}/\text{min}$ for the reverse rate constant. The ratio of these numbers gives a range of values for the affinity constant of the radiolabeled IL-1 preparation for its receptor of $0.9-2.0 \times 10^{10}/\text{M}$. These affinity values are on the high end of the range we have found for $^{125}$I-IL-1. Probable reasons for fluctuations in the values of $K_a$ are discussed below.

A striking feature of the data in Fig. 2A is the low number of IL-1 molecules bound to the cells at equilibrium. Since the affinity of IL-1 is high, relative to the concentrations of ligand used in the experiment, this does not reflect low fractional site occupancy but a low level of receptor expression. Such low levels of receptor expression have been a consistent finding in these studies. Finally,
the information obtained from the experiment illustrated in Fig. 2 was used to ensure that, in all subsequent binding experiments, the system had reached equilibrium.

Fig. 3 shows the dissociation of \(^{125}\text{I}-\text{IL-1}\) from LBRM-33-1A5 cells at 8°C. The experimental design followed that originally described by Demeyts et al. (28), and was constructed to test whether the IL-1 receptor exhibits any cooperative properties. A comparison of the dissociation of \(^{125}\text{I}-\text{IL-1}\) from the cells in medium alone, when only a fraction (~20–50%) of the receptors are occupied, with that in the presence of \(5 \times 10^{-9}\) M unlabeled IL-1, when almost all the receptors are occupied, reveals that the rate of dissociation of IL-1 from its receptor is insensitive to occupancy of adjacent receptors. This suggests that IL-1 receptors on LBRM-33-1A5 cells are noncooperative. The dissociation rate constants measured in this experiment were \(2.2 (\pm 0.8) \times 10^{-2}/\text{min}\) in medium alone and \(2.6 (\pm 1.1) \times 10^{-2}/\text{min}\) in the presence of unlabeled IL-1. These values are averages, since neither curve is a simple first-order process. Complex dissociation kinetics appear to be generally observed for cell surface receptor systems (22, 27–30). The cause(s) for such complex kinetics remain unclear, but the complexity does not necessarily result from site heterogeneity. Finally, the data in Fig. 3 show that, during procedures such as affinity crosslinking, it is possible to subject LBRM-33-1A5 cells bound to \(^{125}\text{I}-\text{IL-1}\) to repeated washes and retain a significant fraction of the IL-1 bound to the cell surface.

Fig. 4 shows the binding of \(^{125}\text{I}-\text{IL-1}\) to LBRM-33-1A5 cells at 8°C. The data confirm the results of the association kinetics experiments (Fig. 2), in that the level of receptor expression is low. The background binding, shown by the dashed line (Fig. 4), is a significant fraction of the total. Plotting the specific binding data (total − background) in the Scatchard coordinate system (Fig. 4B) revealed a slight curvilinearity at high concentrations. We therefore attempted to analyze the data using a model containing two classes of sites on the cell surface (22). The analysis showed that the high affinity sites were present at 290
± 70 sites per cell, and that they bound IL-1 with an affinity of 1.96 (±0.8) × 10^9/M. The low-affinity binding was too weak for complete analysis, but the product of the site number and affinity was 8.7 (±1.5) × 10^10 molecules/(cell·M). The curves passing through the specific binding data in both panels of Fig. 4 are calculated from these values. In other experiments the low-affinity binding could not be detected and, when present, may reflect an error in estimating the background rather than a true low-affinity receptor population. In a series of binding experiments with LBRM-33-1A5 cells at 8°C, we found that the level of high affinity IL-1 receptor expression fluctuated considerably; the results are summarized in Table I. In a similar experiment carried out at 37°C, the parameters estimated were 266 ± 50 sites per cell and a 125I-IL-1 binding affinity of 1.8 (±0.5) × 10^9/M. Considering experimental error, the parameters for the high affinity binding were the same at 37°C as at 8°C.

Fig. 5 shows the inhibition of binding of 125I-IL-1 to LBRM-33-1A5 cells at 8°C by unlabeled IL-1. The experiment was done to determine whether the affinities of labeled and unlabeled IL-1 for the receptor are significantly different. This is crucial in determining whether Bolton-Hunter-labeled IL-1 can be used not only to detect the receptor for the unmodified hormone, but also to characterize those receptors. If the binding constants of the labeled and unlabeled hormone preparations are the same, then affinities, rate constants, and cooperative properties evaluated with the iodinated reagent may be taken as good estimates for the behavior of the unmodified form of the molecule.

Analysis of the data with a single-site competitive inhibition equation (23), yielded an inhibition constant of 2.4 (±0.3) × 10^9/M. The curve passing through the data was calculated with this model (using this value of the inhibition constant). The good fit between theory and experiment is consistent with the
Figure 5. Inhibition of the binding of \(^{125}\text{I}-\text{IL-1}\) by unlabeled IL-1. LBRM-33-1A5 cells (1.1 x 10^8 cells ml) were incubated with 2.73 x 10^{-10} M \(^{125}\text{I}-\text{IL-1}\), in the presence of unlabeled IL-1, at a series of concentrations, for 4 h at 8°C. The continuous curve passing through the data was calculated from a one-site competitive incubation equation using a \(K_d\) value for \(^{125}\text{I}-\text{IL-1}\) of 1.90 x 10^9/M, derived from the data in Fig. 4, and the best-fit \(K_i\) value for unlabeled IL-1 (given in the text). The best-fit value for maximum inhibition was 102 ± 2%. All data are corrected for nonspecific binding measured in the presence of 1.57 x 10^{-7} M unlabeled IL-1; this value was 11 molecules/cell or 4 x 10^{-10} molecules/(cell·M). In the absence of competitor, the cells bound 39 ± 1 molecules/cell of \(^{125}\text{I}-\text{IL-1}\).

Presence of a single class of noncooperative IL-1 receptors on LBRM-33-1A5 cells. In similar experiments with other preparations of IL-1, purified as described by Kronheim et al. (10), we have observed inhibition constants as high as 2 x 10^{-10}/M. The variation in binding activity may be due to variable denaturation or inactivation of the IL-1 during purification, or difficulties in estimating concentrations of protein preparations at the level of 1-10 μg/ml. This may also account for the higher affinity measured by the association kinetics experiment shown in Fig. 2. Nevertheless, a comparison between the affinities of the \(^{125}\text{I}-\)labeled IL-1 used in the experiment shown in Fig. 4 and the unlabeled IL-1 (Fig. 5), both from the same IL-1 preparation, indicates that radiolabeling at the level of 2-5 x 10^{15} cpm/mmol produces little loss in affinity for the receptor.

Fig. 6 illustrates an experiment testing the specificity of the LBRM-33-1A5 IL-1 receptor. Of a set of 12 polypeptide hormones, only IL-1 inhibited the binding of \(^{125}\text{I}-\text{IL-1}\) to LBRM-33-1A5 cells at 8°C. This suggests, at least for murine T cells, that the receptor to which IL-1 binds is specific for this polypeptide hormone.

Affinity Crosslinking of \(^{125}\text{I}-\text{IL-1}\) to LBRM-33-1A5 Cells. Fig. 7 shows an SDS-PAGE analysis of detergent lysates from LBRM-33-1A5 cells that were incubated with \(^{125}\text{I}-\text{IL-1}\) in the presence or absence of unlabeled IL-1, washed, and exposed to bivalent lysine-directed crosslinking agents. All three of the crosslinkers, DSS, DSP, DST, produced a radiolabeled band at ~100,000 M, when the cell lysates were analyzed under nonreducing conditions (Fig. 7A). The crosslinking of IL-1 to the higher molecular weight species was specific, since unlabeled IL-1 abolished the presence of the bands on the gel (Fig. 7A, lanes 2, 4, 6, and 8). Further, in the absence of crosslinking agents, no cell-associated radiolabeled
FIGURE 6. Specificity of the murine T cell IL-1 receptor. LBRM-33-1A5 cells (3.4 x 10^7 cells/ml) were incubated with 3.50 x 10^-10 M ^125^I-IL-1 alone (A) or with various unlabeled hormone preparations: (B) unlabeled IL-1, 1.94 x 10^-8 M; (C) thyroid-stimulating hormone, 3 \( \mu \)g/ml; (D) platelet-derived growth factor, 3 \( \mu \)g/ml; (E) epidermal growth factor, 3 \( \mu \)g/ml; (F) human luteinising hormone, 3 \( \mu \)g/ml; (G) human growth hormone, 1.67 x 10^-7 M; (H) insulin (bovine), 5.6 x 10^-7 M; (I) nerve growth factor, 3 \( \mu \)g/ml; (J) follicle-stimulating hormone, 3 \( \mu \)g/ml; (K) human IL-2, 2.4 x 10^-7 M; (L) murine CSF-2, 2.7 x 10^-9 M; (M) bovine pituitary fibroblast growth factor, 3 \( \mu \)g/ml. Some hormone preparations were partially pure and their concentrations are given in \( \mu \)g/ml total protein. Concentrations of pure hormones are given in molarity. All incubations were done at 8°C for 2 h.

Five species were detected other than IL-1 (lanes 1 and 2). The uncrosslinked IL-1 runs at the bottom of the 8% gel; however, in other experiments using 12% gels, the \( M_r \) of cell-bound IL-1 was \( \sim 17,500 \), as expected (Fig. 1). Labeled material was also detected at the top of the running gel, suggesting the presence of aggregated protein too large to migrate into the gel. This seemed to be a function of the crosslinker used, since less of the high molecular weight material was observed with DST than with DSS or DSP. There was a faint band in some samples, migrating with an \( M_r \) of 66,000 (e.g., Fig. 7 B, lane 1). We attribute this to a low level of contamination of the ^125^I-IL-1 preparation with ^125^I-BSA, since SDS-PAGE analyses of some of our unlabeled IL-1 preparations revealed the presence of a faint band in this position.

Lysates of cells treated with ^125^I-IL-1 and these crosslinkers were also analyzed under reducing conditions (Fig. 7 B). As expected, no bands other than IL-1 were observed in the reduced samples when the IL-1 had been crosslinked to the cells with the cleavable reagent DSP (Fig. 7 B, lanes 5 and 6). However, use of the crosslinkers DSS and DST resulted in the detection of two bands in the gel: a major band of \( M_r = 97,000 \) and a higher molecular weight band of \( M_r = 133,000 \). The \( M_r = 97,000 \) band was detected under all conditions examined, including when a mixture of protease inhibitors was used during the extraction step. It was also present with the lowest concentration of crosslinker (0.1 mg/ml) and the shortest incubation time (15 min) with crosslinker (data not shown). The appearance of the \( M_r = 133,000 \) band, on the other hand, was variable. Its intensity, relative to the \( M_r = 97,000 \) band, seemed to be a function of the crosslinker, since DSS gave a detectable band at this \( M_r \), while DST yielded a very faint band at
this position. The intensity of the Mr 133,000 band appeared to correlate with the intensity of the material that failed to enter into the nonreducing gel depicted in Fig. 7A. We have focussed on the Mr 97,000 band because it appeared under all crosslinking conditions and in each experiment. It represents $^{125}$I-IL-1 (Mr 17,500) linked to a plasma membrane protein of Mr 79,500.

**Cellular Distribution of IL-1 Receptors.** Table I summarizes the results of a survey of a number of cell types, primary cells, and established cell lines, for the presence of IL-1 receptors. For some cells (Table I, footnote †), complete binding curves were done over a range of $^{125}$I-IL-1 concentrations and receptor numbers per cell were generated by an analysis of the kind used for Fig. 4. In most cases, however, for a preliminary survey, a single concentration of $^{125}$I-IL-1 was used, to determine whether IL-1 receptors were present and their approximate abundance.

With one exception, the level of expression on primary cells was low (Table I). The exception, human fibroblasts, expressed $4.9 \times 10^3$ receptors per cell, which is to some extent reflected in the generally higher levels of receptor expression on the in vitro fibroblast lines in Table I. Among a series of mouse lymphoid tissues tested, only PNA$^+$ thymocytes showed detectable levels of IL-1 receptors; this is striking since these murine lymphocytes are IL-1 responsive (14).
### Table 1

**Cellular Distribution of IL-1 Receptors**

| Species | Cell type | IL-1* | Molecules bound per cell |
|---------|-----------|-------|--------------------------|
| Mouse   | Thymocytes | $4 \times 10^{-10}$ | $<10$ |
| Mouse   | Thymocytes PNA* fraction | $4 \times 10^{-10}$ | $<10$ |
| Mouse   | Thymocytes PNA− fraction | $4 \times 10^{-10}$ | $27 \pm 5$ |
| Mouse   | Spleen cells | $4 \times 10^{-10}$ | $<10$ |
| Mouse   | Lymph node cells | $4 \times 10^{-10}$ | $<10$ |
| Human   | Peripheral blood mononuclear cells | $4 \times 10^{-10}$ | $27 \pm 1$ |
| Human   | T cell line | $4 \times 10^{-10}$ | $100 \pm 0$ |
| Human   | Gingival fibroblasts | — | $4.9 \times 10^{24}$ |

**In vitro cell lines**

| Species | Cell type | Designation | IL-1* | Molecules bound per cell |
|---------|-----------|-------------|-------|--------------------------|
| Mouse   | T lymphoma | LBRM-33-1A4 | — | $550 \pm 570^\text{f}$ |
| Mouse   | T lymphoma | LBRM-33-5A4 | $4 \times 10^{-10}$ | $<10$ |
| Mouse   | T lymphoma | FL-4 | $5 \times 10^{-10}$ | $185 \pm 81$ |
| Mouse   | Fibroblast | L929 | $10^{-9}$ | $144 \pm 28$ |
| Mouse   | Fibroblast | SC-1 | $5 \times 10^{-10}$ | $1850 \pm 112$ |
| Human   | T lymphoma | Jurkat-FHCRC | $4 \times 10^{-10}$ | $<10$ |
| Human   | T lymphoma | HSB-2 | $4 \times 10^{-10}$ | $79 \pm 5$ |
| Human   | T leukemia | PEER | $5 \times 10^{-10}$ | $<10$ |
| Human   | Monocyte | U937 | $4 \times 10^{-10}$ | $<10$ |
| Human   | Myelogenous leukemia | KG-1 | $4 \times 10^{-10}$ | $<10$ |
| Human   | Erythroleukemia | K562 | $4 \times 10^{-10}$ | $<10$ |
| Human   | Promyelocyte | HL-60 | $4 \times 10^{-10}$ | $<10$ |
| Human   | Myeloma | ARH77 | $5 \times 10^{-10}$ | $330 \pm 3$ |
| Human   | B lymphoma | BMB | $5 \times 10^{-10}$ | $540 \pm 0$ |
| Human   | Melanoma | A375 | — | $465 \pm 25^\text{f}$ |
| Human   | Hepatoma | SKHEP-2 | $5 \times 10^{-10}$ | $<10$ |
| Rat     | Fibroblast | XC | $5 \times 10^{-10}$ | $450 \pm 22$ |
| Rat     | Hepatoma | HEP-2 | — | $540^\text{f}$ |
| Rat     | Epithelial cell | HTC | — | $510^\text{f}$ |
| Bovine  | Endothelial cell | CPAE | — | $750^\text{f}$ |

* Concentration of $^{125}$-IL-1 used to test for the presence of receptors. All data are corrected for nonspecific binding of radiolabeled ligand measured in the presence of at least $1 \times 10^{-8}$ M unlabeled IL-1.

* Binding was tested with a complete $^{125}$-IL-1 dose response curve.

Among the cell lines tested, in addition to fibroblasts, we found that epithelial, and endothelial cells also showed relatively high levels of IL-1 binding. T cell lines showed variable expression and two B cell lines both had significant levels of IL-1 receptors. The data clearly show that none of the cells have more than a few thousand IL-1 receptors per cell.

**Discussion**

The distribution of IL-1 receptors among the various cell types broadly matches the previously reported pattern of cellular IL-1 responsiveness (7, 9, 11, 12, 14, 32, 34). This is consistent with the hypothesis that the biological effects
of IL-1 are mediated by the plasma membrane receptor(s) identified in these studies.

The level of expression of IL-1-binding sites was low, compared, for example, with receptor levels for IL-2 on T cells (33). Our results suggest that IL-1 can exert its biological effects at levels of 1-100 molecules bound per cell. In the case of thymocytes and LBRM-33-1A5 cells, this may not be surprising, since IL-1 acts by inducing IL-2 production (12, 14), which can presumably provide a large degree of signal amplification. Conversely, where IL-1 is directly mitogenic (for example, on fibroblasts; 9, 34), the levels of receptor expression seem to be somewhat higher. Further, the levels of receptor expression on thymocytes seem to be one to two orders of magnitude less than on LBRM-33-1A5 cells. This correlates with the lower specific activity of purified IL-1 in the thymocyte co-mitogenesis assay (4.4 \times 10^7 \text{ U/mg}) when compared with the LBRM-33-1A5 conversion assay (5.7 \times 10^{10} \text{ U/mg}). This suggests that a minimum absolute level of IL-1/IL-1 receptor complexes may be needed to deliver a signal to a cell, which may be achieved by increasing either IL-1 concentration or receptor number, as has been suggested for IL-2 (35). It may be, however, that only a minority of the PNA^-thymocytes express IL-1 receptors and, hence, produce IL-2 in response to IL-1, while presumably LBRM-33-1A5 all bear receptors since they are of clonal origin.

The specific activity of IL-1 when assayed on LBRM-33-1A5 cells suggests that \( \sim 10^{-14} \text{ M} \) IL-1 is sufficient to induce 50% maximal response from LBRM-33-1A5 cells at 37°C over 24 h. The binding studies show that the affinity of unmodified IL-1 for LBRM-33-1A5 cells is \( 10^9 \) to \( 10^{10} \text{ M} \); hence the concentration of IL-1 required to produce 50% maximal receptor occupancy is \( 10^{-9} \) to \( 10^{-10} \text{ M} \). These concentrations are approximately four orders of magnitude higher than those required to produce the biological effects. The kinetic data show that the equilibrium binding studies closely reflect the true receptor affinity, since the measured reverse rate constant of \( 2 \times 10^{-2} \text{ /min} \) gives a maximum halftime of \( \sim 30-50 \text{ min} \) for binding of IL-1 to LBRM-33-1A5 cells, and the assays were done for 4 h. However, we observed a slowly exchanging component in the dissociation kinetics experiments, and it may be that this reflects a subpopulation of receptors capable of effectively and irreversibly binding IL-1. This interaction may be primarily responsible for the capacity of IL-1 to produce biological effects at such low concentrations on cells with low levels of receptor expression. We have only studied in detail the binding of IL-1 to the LBRM-33-1A5 cell line; whether these observations can be generalized to other IL-1-responsive cells remains to be investigated.

The crosslinking experiments provided some preliminary structural information on the murine T cell IL-1 receptor protein. The most likely candidate for the IL-1-binding protein is the one with an \( M_r \) of 79,500. This protein, when crosslinked to \(^{125}\text{I}-\text{IL-1} \), resulted in an \( M_r \) 97,000 band on polyacrylamide gels under reducing conditions. It migrated on nonreducing gels at a similar molecular weight, suggesting that the binding protein is not covalently linked to other subunits as part of a receptor complex. The \( M_r \), 97,000 protein was detected under all conditions. It appeared within minutes of the addition of crosslinker to cells, and was present as the only detectable band when cells were lysed in the
presence of a mixture of protease inhibitors. It is therefore unlikely to be related to the M, 133,000 band as a result of either proteolysis or aggregate formation of the M, 97,000 protein due to extensive crosslinking. The M, 133,000 band was most intense in samples from cells that had been crosslinked with DSS, a more hydrophobic reagent than the crosslinker DST. DSS may partition more effectively into the plasma membrane, and crosslink the $^{125}$I-IL-1/IL-1 receptor complex to a second, closely associated membrane protein of M, 36,000. Alternatively, $^{125}$I-IL-1, in the presence of DSS, could be crosslinked to an M, 115,500 protein closely associated with the M, 79,500 protein on the plasma membrane. These suggestions are speculative. The data do not allow a definite choice between the models.

The receptor protein we have identified and characterized in these studies was detected using IL-1 purified to homogeneity from activated human monocytes (10). We and others (36, 37) have recently cloned the gene encoding this protein (IL-1β). A second form of human IL-1 (IL-1α) has been identified in our laboratory using a recombinant DNA approach (36). Preliminary data suggest that IL-1α and IL-1β both bind to the M, 79,500 receptor protein on LBRM-33-1A5 cells.

Finally, the availability of an assay for the presence of IL-1 receptors makes it possible to screen for monoclonal antibodies directed against this protein, and to search for cell lines expressing high levels of receptor. These will be important steps towards isolating and characterizing the receptor protein(s) and the gene(s) that encode it.

Summary

Interleukin 1 (IL-1) is a polypeptide hormone that acts as a central mediator of inflammation. Since IL-1 action is presumably mediated by specific cell surface receptor(s), we have characterized the binding of this hormone to cells. Purified human IL-1 was labeled to high specific activity with $^{125}$I, using Bolton-Hunter reagent. The labeled protein binds specifically to LBRM-33-1A5 (a murine T lymphoma line previously shown to produce IL-2 in response to phytohemagglutinin and IL-1) with an affinity of \( \sim 0.2-2 \times 10^{10} / M \) and, at saturation, to \( \sim 500 \) receptors per cell, on intact cells at 8°C in the presence of sodium azide. The affinity of unmodified IL-1 for the murine plasma membrane receptor is \( 0.9-2 \times 10^{10} / M \), as measured by the inhibition of $^{125}$I-IL-1 binding. The murine receptor specificity has been confirmed by demonstrating that, among a series of 12 polypeptide hormones, only IL-1 inhibits $^{125}$I-IL-1 binding to LBRM-33-1A5 cells. Treatment of surface-bound $^{125}$I-IL-1 with bivalent water-soluble crosslinkers identified a membrane polypeptide of M, 79,500 to which IL-1 is crosslinked.

A variety of cell types have been surveyed for the capacity to bind $^{125}$I-IL-1 specifically. The presence of specific binding correlates with the capacity of the cells tested to respond to IL-1. Our results indicate that the biological effects of the polypeptide hormone IL-1 are mediated by high affinity plasma membrane receptors. The identification of these receptors should provide valuable insight into the apparently diverse biological activities of IL-1.
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