Similarity between the interleukin 1 receptors on a murine T-lymphoma cell line and on a murine fibroblast cell line

(polypeptide hormone/lymphokines/inflammation)

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ABSTRACT Interleukin 1β (IL-1β), one of two different polypeptide hormones with interleukin 1 (IL-1) biological activity, produced by activated human monocytes, is a 17.5-kDa protein. IL-1β binds specifically to a variety of cells; the cellular distribution of binding is consistent with reported biological responsiveness. In this report we show that two unrelated, but IL-1-responsive, cell lines, LBRM-33-1A5, a T-lymphoma line, and BALB/3T3, a fibroblast line, bind 125I-labeled IL-1β via similar plasma membrane receptor molecules. The T-lymphoma cells possess 238 ± 16 plasma membrane receptors per cell and bind 125I-labeled IL-1β with an affinity of \(3.6 \pm 0.9 \times 10^9\) M\(^{-1}\). The IL-1 receptor has a molecular size of \(\approx 79.5\) kDa, as estimated by affinity cross-linking. The fibroblasts possess 4.8 ± 0.5 \(\times 10^3\) IL-1 receptors per cell and bind 125I-labeled IL-1β with an affinity of \(2.6 \pm 0.5 \times 10^3\) M\(^{-1}\). The molecular size of the receptor molecule on the fibroblasts is \(\approx 78\) kDa. Despite the similarity in the characteristics of the ligand–receptor system on the two different cell types, the biological responses of the two cell types to IL-1β occur at IL-1β concentrations that differ by several orders of magnitude.

Interleukin 1 (IL-1) is a polypeptide hormone that acts as a soluble mediator in inflammatory responses (1–3). A wide range of cell types, including, lymphocytes, synovial cells, epithelial cells, and fibroblasts are involved in inflammation of tissue (1). It is, therefore, not surprising that IL-1 or IL-1-like factors have been reported to elicit biological responses from all of the above cell types (4–7). The diverse range of biological effects of IL-1 raises the issue as to whether this hormone is truly a single molecular species or several different proteins. It has been reported that there are at least two polypeptides in man that by virtue of their source—activated monocytes—and their biological effects can be defined as IL-1 (8). These have been termed IL-1α and IL-1β (8). In addition to producing these IL-1s in recombinant form by using various prokaryotic and eukaryotic expression systems, we have generated relatively large quantities of natural IL-1β in homogenous form, from activated monocytes (9).

The biological actions of polypeptide hormones are mediated by plasma membrane receptors, and we have demonstrated that purified radiolabeled human IL-1β binds specifically to a receptor protein on the surface of the murine T-lymphoma cell line LBRM-33-1A5 (10). This cell line produces IL-2 in response to suboptimal doses of phytohe-magglutinin in an IL-1-dependent fashion and constitutes a well-defined model system for IL-1 action (11).

In this report we compare the binding and biological effects of IL-1β on the murine T-lymphoma cell line LBRM-33-1A5 with those on BALB/3T3 cells, a well-characterized mouse fibroblast line. This 3T3 line constitutes a model for the study of fibroblast biology and has been demonstrated to be responsive to a variety of polypeptide hormones (12, 13). Our data reveal that these two model cell lines, one fibroblast-like and one T cell-like, bind and respond to homogenous IL-1β via similar plasma membrane receptors. This observation is particularly striking since the minimum concentrations of IL-1 necessary to elicit a maximal biological response from the two cell types differ by several orders of magnitude.

MATERIALS AND METHODS

Cells. LBRM-33-1A5 cells (11) were cultured in RPMI 1640 medium with 10% (vol/vol) fetal calf serum (HyClone, Logan, UT) in a humidified 5% CO\(_2\)/95% air atmosphere in 150-cm\(^2\) tissue culture flasks. Cells were harvested by scraping. BALB/3T3 clone A31 cells were purchased from the American Type Tissue Culture Collection (ATCC CCL 163) and maintained in culture as above, except that the cells were propagated in Dulbecco’s modified Eagle’s medium with 10% (vol/vol) calf serum (HyClone, Logan, UT). Prior to binding assays cells were harvested by using a brief 0.5 mM EDTA treatment.

Proteins. Human IL-1β was purified to homogeneity from human monocyte culture supernatants as described (9). Purified IL-1β was radiolabeled with 125I-labeled Bolton–Hunter reagent (New England Nuclear) as described (10). Purity of radiolabeled natural IL-1β was monitored by NaDodSO\(_4\)/polyacrylamide gel electrophoresis. A single 17.5-kDa band was observed. Purified fibroblast growth factor (FGF), isolated from bovine pituitary glands (12), was the kind gift of D. Gospodarowicz. Recombinant (r) IL-1α and IL-1β were expressed in Escherichia coli from appropriately truncated cDNA clones. The rIL-1α was purified by a combination of hydrophobic and ion exchange chromatography (S. Kronheim, personal communication). The rIL-1β was purified with the identical method used to isolate natural IL-1β (9). The purified rIL-1α and rIL-1β were 17 kDa by NaDodSO\(_4\)/polyacrylamide gel electrophoresis. rIL-1α was radiolabeled by a modified chloramine-T method and rIL-1β was radiolabeled by use of 125I-labeled Bolton–Hunter reagent (S.K.D., unpublished observations). NaDodSO\(_4\)/polyacrylamide gel electrophoresis of the radiolabeled recombinant lymphokine preparations showed that they contained a single labeled polypeptide of approximately 17 kDa.

Binding Assays. Binding of radiolabeled IL-1s and inhibition by unlabeled IL-1s and FGF were measured by using a phthalate oil method (14). Briefly, cells and ligands were incubated at 8°C on a rocker platform in a total volume of 150 μl for a time preestablished to be sufficient to reach equilibrium (10). Subsequently, duplicate 60-μl aliquots of cell suspension were transferred to 400-μl polyethylene centri-

Abbreviations: IL, interleukin; FGF, fibroblast growth factor; r, recombinant.
fuge tubes containing 200 μl of a mixture of phthalate oil (1.5 parts dibutyl phthalate to 1 part bis(2-ethylhexylyl) phthalate (vol/vol); Eastman) precooled in an ice/water bath. Tubes were spun for 1 min, and cells and bound ligands (sedimented through the tip) were separated from unbound ligand by cutting the tip off with a razor blade. Since the amount of bound 125I-labeled ligand gave cpm in the range 100–10,000, all samples were counted for 10 min or longer.

**Assays of IL-1β Biological Activity.** IL-1-dependent IL-2 production by LBRM-33-1A5 cells was assayed as described by Conlon (15). Briefly, IL-2, produced in response to IL-1 and 0.1% phytohemagglutinin, is assayed for its growth stimulation of the murine T-cell line CTLL-2 (16) by using [3H]thymidine incorporation. One unit of IL-1 activity is defined as the amount producing 50% of maximal IL-2-dependent CTLL-2 [3H]thymidine incorporation.

IL-1-dependent growth effects on BALB/3T3 cells were measured as described for human dermal fibroblasts (7, 17). Briefly, 3T3 cells were harvested from subconfluent cultures in 175-cm² flasks by a brief trypsin/EDTA treatment and plated at 5 × 10⁴ cells per well in 100 μl of growth medium in 96-well flat-bottom microtiter plates. After 24 hr the medium was replaced by serum-free medium. After a further 24 hr the serum-free medium was replaced by medium containing the test substances, and incubation was continued for 90 hr. Subsequently 0.5 μCi of [3H]thymidine per well (1 Ci = 37 GBq; New England Nuclear) was added, and incubation was continued for 8 hr, at which time the medium was removed and replaced with trypsin solution (0.5 mg/ml of 0.15 M NaCl); after 15 min cells were harvested with an automated harvester, deposited onto glass fiber filters, and counted.

**Affinity Crosslinking.** Affinity crosslinking of 125I-labeled IL-1β to cells was done as described (10). Briefly, cells were incubated with 125I-labeled IL-1β for 1 hr at 8°C, then washed three times with phosphate-buffered saline (0.05 M sodium phosphate, pH 7.2, 0.15 M NaCl) to remove unbound IL-1β. As described (10) most of the surface-bound IL-1β is retained during this procedure. The cells were then resuspended in 100 ml of phosphate-buffered saline, and 2 μl of 50 mg of dithiobis(succinimidylpropionate)/ml (Pierce) in dimethyl sulfoxide were added. Incubation was continued for 1 hr at 8°C. The cells were washed twice, as above, and resuspended in 50 μl of phosphate-buffered saline containing 1% Triton X-100 and 2 mM phenylmethylsulfonyl fluoride. The extraction mixture was incubated on ice for 5 min then centrifuged for 15 min at 8°C in a microfuge, to remove nuclei and other debris. The extract was analyzed by polyacrylamide gel electrophoresis on 8% slab gels as described (18).

**Data Analysis.** Binding data were analyzed using RS-1 (Bolt, Beranek, and Newman, Boston, MA) a scientific data processing package running on a VAX 11/750 computer under the VMS operating system. Binding data were analyzed with an equation for bimolecular binding as described (19). Inhibition data were analyzed with an equation for competitive inhibition between two types of ligand for one type of site (20).

**RESULTS**

**Comparison of the Binding of 125I-labeled IL-1β to LBRM-33-1A5 Cells and BALB/3T3 Cells.** Fig. 1 shows the binding of 125I-labeled Bolton–Hunter reagent labeled-IL-1β to LBRM-33-1A5 and BALB/3T3 cells at 8°C. We have described (10) the determination of the forward and reverse rate constants for interaction of IL-1β with the receptor on the murine T-lymphoma line LBRM-33-1A5. These values can be used to estimate the minimum time needed for binding of the radiolabeled ligand to the cells to reach equilibrium. By this estimate the system had reached equilibrium under the conditions used in the experiment illustrated in Fig. 1.

The data show that the fibroblast line possesses approximately 20 times more IL-1 receptors per cell than does the T-lymphoma cell line. We have indicated (10) that a preliminary survey of a variety of cell types showed that, in general, fibroblasts and epithelial-type cells possess more IL-1 binding sites than do cells of lymphoid origin. A possible explanation for this difference is that on fibroblasts IL-1 binds to the receptor for some other polypeptide hormone. Since IL-1 has been described as an FGF (7), we tested the possibility that the IL-1 receptor on fibroblasts also binds FGF. The results of this experiment are shown in Fig. 2. The binding of 125I-labeled IL-1β to both LBRM-33-1A5 cells and BALB/3T3 cells.

**Fig. 1.** Comparison of the binding of 125I-labeled IL-1β to LBRM-33-1A5 cells and BALB/3T3 cells. LBRM-33-1A5 cells (○, ○) (7.3 × 10⁵ cells per ml) or BALB/3T3 cells (●, ●) (8.7 × 10⁵ cells per ml) were incubated with various concentrations of 125I-labeled IL-1β for 2 hr at 8°C. For both cell types, viability was >90% by trypan blue exclusion at the end of the assay. Nonspecific binding, measured in the presence of 19 nM unlabeled IL-1, was 1.7 × 10⁶ molecules per cell per M for the LBRM-33-1A5 cells and 2.7 × 10⁶ molecules per cell per M for the BALB/3T3 cells. All data shown have been corrected for these background values, by subtraction. The continuous curves passing through the data were generated from a bimolecular binding model with the best-fit parameter values given in Table 1. The error bars on the data points, given where error values exceed the symbol size, are calculated from duplicate measurements.

**Fig. 2.** Specificity of IL-1β binding to LBRM-33-1A5 and BALB/3T3 cells. LBRM-33-1A5 cells (○, ○) (7.3 × 10⁵ cells per ml) and BALB/3T3 cells (●, ●) (8.7 × 10⁵ cells per ml) were incubated at 8°C in the presence of 0.21 nM 125I-labeled IL-1β and various concentrations of either unlabeled IL-1β (△, △) or FGF (○, ○) for 2 hr. Data were analyzed by non-linear least-squares fitting with a competitive inhibition equation. This equation was used to calculate the continuous curves from the best-fit parameter values given in Table 1. Maximal binding for the 3T3 cell was 2050 molecules per cell; background was 564 molecules per cell; for the 1A5 cells maximal binding was 146 molecules per cell and the background was 35 molecules per cell.
the very top of the stacking gel and at the interface between the stacking gel and the running gel. These are, therefore, presumably due to extensive crosslinking of a variety of cell surface components.

Close comparison of the position of the high molecular size band generated with LBRM-33-1A5 with that obtained from the BALB/3T3 cells reveals that the latter migrates slightly lower (93–96 kDa) than the former. Whether this represents a true difference in the polypeptide size or different glycosylation of the two proteins cannot be resolved from these data. The similarity in the crosslinking patterns taken in conjunction with the similar affinity with which IL-1 binds to the two cell types (Table 1) is, however, consistent with the hypothesis that the receptor molecules are closely related.

**Comparison Between the Biological Effects of IL-1β on LBRM-33-1A5 and BALB/3T3 Cells.** Fig. 4 shows the binding and biological effects of IL-1β on both T cells (Fig. 4A) and fibroblasts (Fig. 4B). For the T cells, the proliferation of CTLL-2 cells is stimulated by IL-2 that is secreted by LBRM-33-1A5 cells in response to IL-1β + 0.1% phytohemagglutinin, as described (11, 15). For the fibroblasts, the cells proliferate in response to IL-1β, presumably in combination with other serum factors such as insulin, epidermal growth factor, and platelet-derived growth factor.

For the LBRM-33-1A5 cell line, the data in Fig. 4A are typical of numerous experiments and consistent with the reported (9) specific activity of purified IL-1β in the conversion assay (5.7 ± 10^-10 units/mg of protein). For the fibroblast line, BALB/3T3 cells, the data are representative of several experiments in our laboratory (unpublished data). In an extensive series of experiments with BALB/3T3 fibroblasts, the response of the cells to IL-1β was quite variable, both with regard to maximal stimulation over background and the serum concentration at which the largest differences between experimental and control wells were observed. Nevertheless, the IL-1β concentration range in which proliferation was factor dependent was reproducible. Hence, the data shown in Fig. 4B are typical with respect to the concentration of IL-1β needed to produce half-maximal stimulation of proliferation. For comparison we show in Fig. 4 the binding of unlabelled IL-1β to the two cell types, measured by inhibition of 125I-labeled IL-1β binding. This is shown, rather than the binding of 125I-labeled IL-1β, since unlabelled IL-1β was used in the biological response assays.

Clearly, while the receptor binding and biological response are highly congruent for the fibroblast line, there is a large apparent discrepancy between the receptor occupancy and degree of biological effect for the T-cell line. Indeed the binding data suggest that maximal biological response is elicited from the T cells at only 1–10 molecules of IL-1β bound per cell. Whatever the mechanism of IL-1β action on the T cells may be, it is striking that these cells respond to the hormone at much lower concentrations than the fibroblasts, despite the fact that the latter cell line possesses 20–fold more IL-1 receptors (Table 1).

**Cross Competition of IL-1α and IL-1β for the Murine T-Cell IL-1 Receptor.** Fig. 5 shows the effects of unlabelled natural

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**Table 1. Comparison of receptor binding and biological action of IL-1β on LBRM-33-1A5 cells and BALB/3T3 cells**

<table>
<thead>
<tr>
<th></th>
<th>LBRM-33-1A5</th>
<th>BALB/3T3</th>
</tr>
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<tbody>
<tr>
<td>IL-1 receptors per cell, no.</td>
<td>238 ± 16</td>
<td>4800 ± 500</td>
</tr>
<tr>
<td>Kₐ for 125I-labeled IL-1β binding, M⁻¹</td>
<td>3.6 ± 0.4 × 10⁰</td>
<td>2.1 ± 0.6 × 10⁰</td>
</tr>
<tr>
<td>Kᵣ for unlabelled IL-1β binding, M⁻¹</td>
<td>7 ± 1 × 10⁻⁶ (99 ± 3)</td>
<td>2.6 ± 0.5 × 10⁻⁶ (98 ± 4)</td>
</tr>
<tr>
<td>Concentration of IL-1β at half-maximal biological activity, M</td>
<td>5 × 10⁻¹⁴</td>
<td>5 × 10⁻¹¹</td>
</tr>
<tr>
<td>Mᵣ of IL-1 receptor</td>
<td>79,500</td>
<td>78,000</td>
</tr>
</tbody>
</table>
IL-1, rIL-1-β, and rIL-1α on the binding of radiolabeled natural IL-1-β, rIL-1-β, and rIL-1α to LBRM-33-1A5 cells at 8°C in the presence of sodium azide. In each case the inhibition of binding of radiolabeled hormone in the presence of a large excess of unlabeled autologous hormone was set to 100%. The data show that for all three 125I-labeled proteins, there were no sites on 1A5 cells that did not also bind the other two unlabeled lymphokines. While this is not surprising for natural and rIL-1-β, it is striking that rIL-1α also binds to the same receptor population. Finally, the data suggest that the natural IL-1-β preparation is not contaminated with any polypeptide hormone capable of binding specifically to 1A5 cells, since rIL-1-β blocks the binding of natural 125I-labeled IL-1-β as completely as natural IL-1-β. If there were contaminating labeled hormones present in the natural material, these would not be present in the recombinant protein and, hence, their binding would not be inhibited by it.

**DISCUSSION**

IL-1 has been reported to have a diverse range of biological activities centered around its role as a soluble mediator of inflammation (1–3). Clearly the evidence that human macrophages can produce two different polypeptides with similar overall structures, sharing some of the classical IL-1 activities, raises the possibility that the major secreted protein IL-1-β may not be responsible for all IL-1 activities (8). The data presented in this report, nevertheless, suggest that IL-1-β can bind to and stimulate two different types of cells, fibroblasts and T cells. We show that two murine cell lines, LBRM-33-1A5 and BALB/3T3, bind human IL-1-β through similar receptors, as judged by the affinity for IL-1-β and the molecular size of the receptors. It is striking that much higher concentrations of IL-1-β are required to elicit a response from the 3T3 cells than from the 1A5 cells. The concentration dependence of the biological response of the 3T3 cells is not surprising in light of the relatively high number of IL-1 receptors (ca. 5 × 10⁶ per cell) and the affinity of IL-1-β binding. However, in the case of the 1A5 cells, a biological response can be elicited at very low levels of cell-bound IL-1-β. One reason for this may be that the assay system for the 1A5 cells is an indirect one, involving the IL-1 triggered IL-2 secretion. This is analogous to enzyme-mediated systems such as the complement pathway (21) that uses a “cascade” mechanism to produce a large amplification of a small signal. There are precedents for such responses; both chemotaxis, and OKT-3-driven T-cell proliferation seem to operate at very low levels of receptor occupancy (22, 23), and it has been argued that other cell surface receptor systems require only fractional receptor occupancy to produce maximal biological effect (24, 25). Cuatrecasas and Hollenberg (20) have discussed the great diversity in different hormone-receptor systems with respect to the relationship between receptor occupancy and biological response. Specifically, these authors describe the effects of insulin in two systems: stimulation of glucose oxidation by rat epididymal fat cells (half-maximal response at 30 pM) and stimulation of proliferation of cultured human fibroblasts (half-maximal response at 1 nM), a situation analogous to that reported for IL-1 in this paper. Epidermal growth factor-stimulated proliferation of fibroblasts is another example that reaches maximal levels at only 25% epidermal growth factor-receptor occupancy (20).

While there can probably be no general mechanistic explanation for nonstoichiometric relationships between receptor occupancy and biological effect, one reasonable hypothesis is that levels of intracellular targets may differ from one cell type to another (20). In the IL-1-dependent IL-2 production by T cells and fibroblast proliferation, it is reasonable to suppose that quite different intracellular sys-
tems are affected by hormone–receptor binding. In the 1A5 cells it is possible that the transcription of only the IL-2 gene is affected; while in 3T3 cells a more complex system may be required to accelerate cellular proliferation. Regardless of the mechanism of IL-1β action on either cell type, the data suggest that either the signals in the two cell types are different or the cell lines respond differently to a similar signal.

The data suggest an analogy between the in vivo and in vitro effects of IL-1β. If IL-1β is released by macrophages at sites of tissue disruption, the concentrations of the hormone in tissue should increase with the duration and magnitude of the response. T-cell activation is a relatively early event in the process, whereas connective tissue proliferation and wound healing are late events (1). Thus the model system data is consistent with the in vitro IL-1β response of fibroblasts that requires a higher concentration of the hormone and the in vitro response of T cells that requires a lower concentration of hormone. Confirmation of this proposal requires information on the in vivo half-life of IL-1β and its local retention in tissue.

Despite these reservations, the binding and biological activities of IL-1β in the two model systems, we have investigated, correlate well with known events in inflammatory responses and are consistent with the view that IL-1β is centrally involved in these events as a soluble mediator.

Finally, our data show that IL-1α, like IL-1β, probably exerts its effects on cells via the ~80-kDa receptor protein. This situation, where two related polypeptide hormones bind to identical or closely related receptor structures, has been observed with insulin and the insulin-like growth factors. It is curious that two different polypeptide hormones produced by the same cells should act on target cells via the same receptor. Examination of the biological half-lives, of the two factors and the receptor binding properties in a variety systems, should help to clarify this issue.

We thank our colleagues at Immunex who have been involved in this work, in particular Drs. Carl March and Shirley Kronheim for providing us with purified natural IL-1α, rIL-1α, and rIL-1β, and Dr. Paul Conlon and Ms. T. Washkewicz for performing the IL-1 conversion assays. These studies are part of a collaborative project between the Immunex Corporation and the Syntex Corporation.