Interleukin-1 Signal Transduction

INCREASED GTP BINDING AND HYDROLYSIS IN MEMBRANES OF A MURINE THYMOMA LINE (EL4)*


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The post-receptor events which follow the binding of interleukin 1 (IL1) to cells are unclear. The present studies provide evidence for the activation of a guanine nucleotide binding protein (G protein) by IL1 in the membranes of an IL1 receptor-rich strain (NOB-1) of the EL4 murine thymoma line. IL1α and β increased the binding of the GTP analogue [35S]guanosine 5'-[γ-thio]triphosphate (GTPγS) to membranes prepared from these cells. By 1 min after addition of IL1 there was a 2-fold enhancement in binding which was dose dependent in the range 0.1–100 ng/ml. A qualitatively similar result was obtained with IL1β although it was 10 times less potent. Specific neutralizing antisera to IL1α and IL1β abolished the response. Experiments in which the concentration of [35S]GTPγS was varied revealed that IL1 increased the affinity of the binding sites for [35S]GTPγS and not their number. IL1α was shown to stimulate GTPase activity in the membranes, the time and concentration dependence of this was similar to that observed for increased [35S]GTPγS binding. Half-maximal enhancement of [35S]GTPγS binding by IL1α, measured after 4 min, occurred at 5% IL1 receptor occupancy. Maximal stimulation was achieved when 30% of receptors were occupied. Experiments with pertussis and cholera toxins revealed that pretreating membranes with pertussis toxin (100 ng/ml) inhibited by 50% the IL1-induced [35S]GTPγS binding and [γ-32P]GTP hydrolysis. Cholera toxin (100 ng/ml) was without effect. However, both pertussis and cholera toxins at concentrations of 100 ng/ml inhibited IL1-induced IL2 secretion in EL4 NOB-1 cells. These results show that the IL1 receptor of a responsive thymoma line activates, and may be coupled to, a G protein(s). This is a possible mechanism of IL1 signal transduction.

Interleukin 1 is a proinflammatory cytokine made by activated mononuclear phagocytes and other cells. It occurs as two homologous forms, α and β, which mediate many features of inflammation such as fever, the acute phase response, leucocyte accumulation, and tissue destruction by acting on a range of cell types (1, 2). The cellular actions of IL1 have been intensively studied since it was originally defined on the basis of its ability to activate T lymphocytes by augmenting their synthesis of IL2 (3). It stimulates production of other cytokines (4), proteinases, and prostaglandins (5) by connective tissue cells. Similar changes occur in vascular endothelial cells, and in addition their expression of leukocyte adhesion molecules is increased (6). IL1 directly induces a pattern of acute phase protein synthesis in hepatocytes (7), it is a cofactor for early growth of stem cells in bone marrow (8), and in the brain it can induce slow wave sleep (1).

IL1α and β share a common high affinity receptor on the cell surface which is thought to mediate their effects. On several cell types this has been identified as an 80-kDa glycoprotein (9–11), and the murine form has been cloned (12). Neither the mechanism by which the occupied receptor generates signals, nor the nature of the signals are understood. There have been conflicting reports of changes in second messengers (13–20), which are discussed later, and there is evidence from early changes in protein phosphorylation (21–23) that IL1 alters intracellular protein kinase activity.

Many ligand-receptor complexes interact with heterotriomic guanine nucleotide binding proteins (G proteins) (24). These proteins transduce signals from occupied receptors to effector enzymes such as adenyl cyclase or phospholipase C (24), which in turn generate intracellular messengers. Activation of G proteins by ligand-receptor complexes is associated with enhancement of their binding of GTP, which is generally demonstrated by the use of a non-hydrolysable analogue, and increased GTPase activity. Recent studies using such criteria have shown that several polypeptide growth factors activate G proteins. These include tumor necrosis factor α (25) which shares many of the biological properties of IL1 (13), colony stimulating factor 1 (26), interleukin 2 (27), and bombesin (25). We have therefore examined the effect of IL1 upon GTP binding and hydrolysis by membranes made from a murine thymoma cell line (EL4 NOB-1) which has high IL1 receptor expression (29) and responds to IL1 by synthesizing IL2 (30).

EXPERIMENTAL PROCEDURES

Materials—Recombinant human IL1α (2 x 10^7 LAF units/mg) was given by the Daippon Pharmaceutical Co., Osaka, Japan; human recombinant IL1β (5 x 10^7 units/mg) was a gift from Dr. C. A. Dinarello, Tufts University School of Medicine, Boston, MA. Neu-

* This work was supported by grants from the Arthritis and Rheumatism Council and the Medical Research Council of the United Kingdom. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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The abbreviations used are: IL1, interleukin 1; IL2, interleukin 2; G protein, guanine nucleotide-binding protein; GTPγS, guanosine 5′-[γ-thio]triphosphate; App(MH)p, adenosine-5′-[β,γ-imino]-triphosphate; EGTA, ethylenebis(oxyethylenenitrilo)tetraacetic acid; PGE, prostaglandin E2.
IL-1 Stimulates GTP Binding and GTPase Activity

Purification of sheep antisera to IL-1α and IL-1β were given by Dr. S. Poole, National Institute of Biological Standards and Control, South Mimms, United Kingdom (U.K.), and Dr. C. A. Dinarello, respectively. [35S]GTPγS ([127 Ci/mmol] was from Du Pont-New England Nuclear. [32P]GTP (10 Ci/mmol) was from Amersham, U.K. GTPγS, EDTA, dithiothreitol, Lubrol, ATP, creatine phosphokinase, bovine serum albumin, dextran and EGTA, peptatin A, apotinin, leupeptin, phenylmethylsulfonyl fluoride, pertussis and cholera toxins, PGE, and epinephrine were from Sigma. Nitrocellulose and glass fiber filters were from Whatman. Norit A charcoal and App(NH)p were from ICN. Medium for cell culture was from GIBCO.

Preparation of Cell Membranes—EL4 NOB-1 cells were grown as stirred suspensions in RPMI-1640 medium containing 10% heat-inactivated fetal calf serum at 37°C in air/CO2 (95:5). Membranes were prepared as described previously (31). Briefly, 1 × 106 cells were washed in phosphate-buffered saline, suspended in hypotonic buffer (10 mM Tris, pH 7.2, 1 mM MgCl2; buffer A) containing a mixture of proteinase inhibitors (peptatin A, apotinin, and leupeptin at 10 μg/ml and 1 mM phenylmethylsulfonyl fluoride), homogenized with a “tight” Dounce pestle, mixed with 4 volumes of 0.25 M sucrose in buffer A and centrifuged for 10 min at 400 × g at 4°C to remove nuclei and unbroken cells. A membrane-enriched fraction was obtained by centrifuging the supernatant at 25,000 × g for 30 min. The resulting membrane pellet was stored at 4°C in 1 ml of phosphate-buffered saline containing 1 mM phenylmethylsulfonyl fluoride. Membrane protein was measured by the Coomassie Brilliant Blue dye binding method (129).

GTPγS Binding Assays—[35S]GTPγS ([127 Ci/mmol]) binding to EL4 membranes was measured using a standard protocol (25). The reaction consisted of 20 mM Tris-HCl, pH 8, 30 mM MgCl2, 1 mM EDTA, 1 mM dithiothreitol, 100 mM NaCl, 0.1% Lubrol, and [35S]GTPγS in a volume of 50 μl. IL-1α, IL-1β, and non-radioactive GTPγS were included as indicated. All solutions were prewarmed to 37°C. Reactions were started by the addition of membranes (incubated at 37°C), and terminated at various times with 450 μl of ice-cold buffer containing 25 mM non-radioactive GTPγS. Unbound nucleotide was separated from bound by filtration through 0.45-μm nitrocellulose filters. Filters were washed under gentle vacuum with 3 × 5-ml volumes of buffer and counted for [32P] by liquid scintillation spectrometry. Control experiments using antisera to IL-1α or β were performed by preincubating cytokine with antisera for 2 h before assay.

Experiments were also performed with a fixed concentration of IL-1α and varying concentrations of [35S]GTPγS. The incubation time was 4 min and the percentage of [35S]GTPγS bound was calculated in the presence and absence of IL-1α (10 ng/ml). GTPase Assays—GTPase assays were performed by the method of Cascal and Selinger (33). Briefly, 10 μg of membrane protein was incubated with 250 mM [35S]GTP (10–30 Ci/mmole), 0.05 mM App(NH)p, 0.1 mM ATP, 3 mM creatine phosphate, 75 units/ml creatine phosphokinase, 20 mM Tris-HCl, pH 8.0, 0.1 mM EGTA, 1 mM dithiothreitol, and 50 μCi [35S]GTPγS in a final volume of 50 μl. Unlabeled IL-1α was added to some tubes to determine nonspecific binding. Incubations were performed for 1.5 h at 19°C. Bound radioactivity was separated from free by filtration through glass fiber filters, which were then washed three times with 3 ml of binding buffer. Radioactivity was determined for each filter.

The Results—The Use of Pertussis and Cholera Toxins—[35S]GTPγS binding and GTPase activity was also assayed in EL4 NOB-1 membranes pretreated with pertussis and cholera toxins (100 ng/ml). Both toxins were activated in 25 mM potassium phosphate buffer, pH 8.0 containing 5 mM dithiothreitol and 1 mM ATP for 30 min at 37°C just prior to use. Membranes were then incubated in 150 mM potassium phosphate buffer, pH 7.5, 1 mM NAD, 0.5 mM ATP, 10 mM thymidine, and 50 mM GTP for 30 min at 37°C. Control membranes were treated similarly but without toxins. After treatment, membranes were washed three times in phosphate-buffered saline and then assayed for [35S]GTPγS binding and GTP hydrolysis in response to IL-1 (100 ng/ml) as described above. Prostaglandin E1 (10 μM) and epinephrine (10 μM) were used as positive controls.

The effect of toxins on IL-1 enhanced IL2 release from EL4 NOB-1 cells was also assayed. Unlabeled IL-1α was preincubated for 4 h in the presence of 100 ng/ml pertussis or cholera toxins at 37°C. Following preincubation, they were washed three times by centrifugation and resuspended at 1 × 107/ml in RPMI-1640 medium containing 5% fetal calf serum. Next, the cells were distributed into 96-well microtiter plates (100 μl/well), and IL-1α 0.1–100 ng/ml was added. After 24 h incubation, 50 μl of supernatant was removed from each well, transferred to a microplate, and 15,000 washed CTLL-2 cells were added to each well. After 18 h culture, 0.5 μCi of tritiated thymidine was added to each well and incubated radioactivity determined by liquid scintillation counting after a 3-h incubation. Toxin-treated cell membranes were incubated with cells preincubated in medium alone.

RESULTS

IL1 Enhances [35S]GTPγS Binding to EL4 Cell Membranes—The nonhydrolyzable analogue GTPγS was used to determine the effect of IL-1α and IL-1β on GTP binding to EL4 membranes. Both forms of IL-1 rapidly increased the amount of nucleotide that specifically bound to the membranes (Fig. 1, A and B). The effect was detectable 1 min after addition of cytokine and was most marked at the earlier times (up to 4 min), when binding was increased approximately 2-fold over controls. Because the specific binding of the nucleotide gradually increased, the effect was less obvious at the later times.

Stimulation of GTPγS binding was dependent upon the dose of IL1 (Fig. 2, A and B). Interestingly, IL-1α was more potent than IL-1β. IL-1α caused a maximal stimulation at 10 ng/ml and IL-1β at 100 ng/ml.

To ensure that the response was specific, both forms of IL-1 were inactivated by use of specific neutralizing antisera. Treatment of IL-1α and IL-1β with appropriate antisera abolished their ability to increase GTPγS binding (Table I). Irrelevant serum, for example anti-IL-1α against IL-1β, had no effect on the response (data not shown).

To determine whether IL-1 was enhancing GTPγS binding to membranes by increasing the affinity of G protein for nucleotide rather than increasing the number of binding sites, an experiment was performed using varying concentrations of GTPγS (Fig. 3). IL-1α (5 ng/ml) was incubated with 20 μg of membranes for 4 min in the presence of different concentrations of GTPγS. At lower concentrations of GTPγS, IL-1α caused a greater proportionate enhancement of GTPγS binding than at higher concentrations. For example, at 0.09 nm [35S]GTPγS, 8% of the nucleotide was bound in untreated membranes as compared with 15% in the IL-1α-treated ones. At 0.75 nm, the difference was less, with 7% binding in untreated membranes as compared with 9.5% in treated ones. Such an effect was indicative of IL-1α causing an increase in the affinity of GTP binding site.

IL1 Stimulates Hydrolysis of [γ-32P]GTP in EL4 Membranes—As a consequence of GTP binding, activated G proteins exhibit enhanced GTPase activity. We therefore looked for this effect in EL4 membranes treated with IL-1α. Fig. 4 shows that IL-1α (100 ng/ml) increased the GTPase activity and after 8 min there was more than a 2-fold increase. The
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FIG. 1. Time dependence of the effect of IL-1 on specific binding of [35S]GTPyS to EL4 membranes. EL4 membranes (20 μg) were incubated in the presence of 2.5 nM [35S]GTPyS at 37 °C for the indicated times in the presence (O) or absence (●) of IL-1. Specific binding was in the range 5-15% of total radioactivity added. Nonspecific binding, determined in the presence of 25 μM GTPyS was always <2% of the total radioactive material added. Results are means ± S.D. of triplicate determinations.

A, IL-1α, 10 ng/ml; 2 × 10⁶ cpm [35S]GTPyS added. B, IL-1α, 100 ng/ml; 2 × 10⁶ cpm [35S]GTPyS added.

Response was concentration-dependent. At 10 ng/ml IL-1, the maximal rate of GTP hydrolysis was 83.2 ± 3 pmol/mg/min compared with 62.2 ± 4 pmol/mg/min in control membranes (p < 0.005). The range of concentrations over which IL-1 increased GTP hydrolysis (Fig. 5) was similar to that over which it enhanced binding of GTPyS to the membranes.

Relationship between Binding of GTPyS and IL-1 Receptor Occupancy—The concentration range over which IL-1 increased binding of GTPyS and hydrolysis of GTP was 1-100 ng/ml. This was three orders of magnitude higher than the range used for eliciting responses in intact cells. For instance, the dose-response curve for IL-1-induced IL2 synthesis by EL4 NOB-1 is in the range 1-100 pg/ml (see Fig. 6). We therefore examined the relationship between IL-1 receptor occupancy and stimulation of binding of GTPyS in the membranes (Fig. 5). Maximal specific binding of IL-1α to the membranes was measured at equilibrium. The amount of IL-1 bound 4 min after addition to the membranes was measured for a range of concentrations of the cytokine and expressed as a percentage of the maximal binding capacity determined at equilibrium (Fig. 5). An IL-1 concentration of 250 ng/ml was required to achieve apparent 100% occupancy of available receptors by 4 min. It will be seen from Fig. 5 that IL-1 stimulated nucleotide binding at very low levels of receptor occupancy. Half-maximal stimulation of [35S]GTPyS binding was found to occur when only 5% of the receptors were occupied (1 ng/ml IL-1) and the effect was maximal at 30% occupancy. These results suggest that maximal receptor occupancy is not necessary to trigger a maximal enhancement of [35S]GTPyS binding and that fractional occupancies of less than 5% were adequate to generate a signal.

Effect of Pertussis and Cholera Toxins on IL-1 Action—Covalent modification of G proteins by pertussis and cholera toxins has been shown to prevent their normal coupling with receptors (35,36).

To determine whether the G protein associated with IL-1 could be modified by ADP-ribosylation, we investigated the effect of pertussis and cholera toxins on [35S]GTPyS binding and GTPase activity in EL4 membranes. As can be seen in Table II, pretreating the membranes with pertussis toxin partially decreased the IL-1-induced stimulation of GTPase activity from 159% in controls to 119% in pretreated membranes. The pertussis toxin also inhibited the response to epinephrine, an agonist known to stimulate Gi and Gβ (37), but was without effect on PGE1-stimulated GTPase activity. Cholera toxin had no effect on IL-1-stimulated GTPase activity but inhibited that due to PGE1, (indicating that the toxin was active).

The effects of the toxins on [35S]GTPyS binding paralleled those on GTPase activity (Table III). Pretreatment of mem-
**TABLE I**

**Effect of antisera to IL1α and IL1β on the enhanced specific binding of [³⁵S]GTPγS to EL4 membranes**

EL4 membranes were incubated with 2.5 nM [³⁵S]GTPγS (4.1 x 10⁶ cpm) with the indicated additions for 4 min at 37 °C. Nonspecific binding was determined in the presence of 25 μM GTPγS. IL1α or β were incubated with sera at the indicated dilutions for 2 h at 37 °C before assay. Results are the means ± S.D. of triplicate determinations. p values show the level of significance when compared with IL1 alone treated membranes.

<table>
<thead>
<tr>
<th>Addition</th>
<th>Bound cpm [³⁵S]GTPγS</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>31,151 ± 1,736</td>
<td></td>
</tr>
<tr>
<td>IL1α 10 ng/ml</td>
<td>43,153 ± 2,901</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>IL1α 10 ng/ml + Anti-IL1α 1/5,000</td>
<td>29,198 ± 1,392</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL1α 10 ng/ml + Anti-IL1α 1/10,000</td>
<td>25,493 ± 1,726</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>IL1α 10 ng/ml + Anti-IL1α 1/100,000</td>
<td>28,123 ± 2,100</td>
<td></td>
</tr>
<tr>
<td>IL1β 100 ng/ml</td>
<td>51,908 ± 3,016</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL1β + Anti-IL1β 1/5,000</td>
<td>28,567 ± 1,672</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL1β + Anti-IL1β 1/10,000</td>
<td>20,085 ± 3,126</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL1β + Anti-IL1β 1/100,000</td>
<td>46,337 ± 9,001</td>
<td>not significant</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The signal transduction mechanisms by which IL1 regulates cellular functions are unknown. The receptor which has been cloned (12) is a transmembrane protein possessing a short single membrane-spanning domain and a cytoplasmic portion consisting of 217 amino acids having no sequence homology to other known proteins. The IL1 receptor has no clearly recognizable regulatable enzymic function. Many cell surface receptors appear to be coupled to their effectors by G proteins, and our experiments were carried out to investigate the possibility that the IL1 receptor may also activate such a molecule. The experiments were performed on membranes made from a strain (NOB1) of the murine EL4 thymoma line that expresses the IL1 receptor in relatively high numbers and which we have used formerly as a source for its purifica-
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The experiments showed that IL1 caused activation of GTP-binding proteins in the membranes within 1 min as judged by both enhanced binding of GTPγS and GTPase activity. IL1α caused these changes over a similar concentration range and with a similar time dependence. The effects were due to IL1 rather than contaminants in our cytokine preparations since the increased binding of GTPγS was abolished in the presence of the appropriate specific neutralizing antiserum. Human IL1α was found to be 10 times more potent than human IL1β in its effect on GTPγS binding, but this difference was consistent with previous studies showing that

![Graph](image_url)

**Fig. 5.** Correlation between IL1α-receptor occupancy and IL1α enhanced [35S]GTPγS binding. EL4 membranes were analyzed either for [3H]IL1 binding (O) or IL1α-enhanced [35S]GTPγS binding (C) after addition of IL1. IL1 receptor occupancy was the amount of IL1α specifically bound at 4 min expressed as a percentage of the total specific binding capacity for IL1 at equilibrium (see "Materials and Methods"). Maximum binding of [3H]IL1 corresponded to 3897 cpm, which represented 10% of the total cpm added. Results shown are means + S.E. from three separate experiments carried out in triplicate. Specific binding of [35S]GTPγS was measured after incubation of membranes in 2.5 nM [35S]GTPγS and varying concentrations of IL1α for 4 min at 37 °C. Results are expressed as a percentage of the maximum enhancement in [35S]GTPγS binding. Maximum enhancement corresponded to an increase in binding from 5039 cpm in non-IL1α-treated membranes to 12902 cpm at 100 ng/ml IL1α. Results are expressed as mean ± S.E. from triplicate determinations.

**TABLE II**

Inhibition of G protein activation by pertussis and cholera toxins: effects on GTPase activation

<table>
<thead>
<tr>
<th>Control membranes</th>
<th>Pertussis toxin-treated membranes</th>
<th>Cholera toxin-treated membranes</th>
</tr>
</thead>
<tbody>
<tr>
<td>pmol GTP hydrolyzed/100 µg protein</td>
<td>pmol GTP hydrolyzed/100 µg protein</td>
<td>pmol GTP hydrolyzed/100 µg protein</td>
</tr>
<tr>
<td>Control</td>
<td>8.3 ± 0.05 (100%)</td>
<td>8.4 ± 0.3 (100%)</td>
</tr>
<tr>
<td>IL1 (100 ng/ml)</td>
<td>13.2 ± 0.1 (159%)</td>
<td>10 ± 0.2 (119%)</td>
</tr>
<tr>
<td>PGE₂ (10 µM)</td>
<td>13.3 ± 0.02 (160%)</td>
<td>13.4 ± 0.1 (159%)</td>
</tr>
<tr>
<td>Epinephrine (10 µM)</td>
<td>19.7 ± 0.02 (237%)</td>
<td>10 ± 0.1 (119%)</td>
</tr>
</tbody>
</table>

**TABLE III**

Inhibition of G protein activation by pertussis and cholera toxins: effects on [35S]GTPγS binding

EL4 membranes were preincubated with toxins as described in Table II and were then assessed for enhanced [35S]GTPγS binding by IL1 (100 ng/ml), PGE₂ (10 µM), or epinephrine (10 µM) by incubating 20 µg of membranes with 2.5 nM [35S]GTPγS at 37 °C for 4 min. Non-specific binding was determined in the presence of 2.5 µM GTPγS. Results are means ± S.D. of triplicate determinations. The data are also expressed as a % of control.

<table>
<thead>
<tr>
<th>Control membranes</th>
<th>Pertussis toxin-treated membranes</th>
<th>Cholera toxin-treated membranes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12,355 ± 1,506 (100%)</td>
<td>10,998 ± 673 (100%)</td>
</tr>
<tr>
<td>IL1 (100 ng/ml)</td>
<td>18,924 ± 552 (153%)</td>
<td>14,831 ± 1,234 (135%)</td>
</tr>
<tr>
<td>PGE₂ (10 µM)</td>
<td>19,898 ± 1,840 (190%)</td>
<td>20,901 ± 109 (190%)</td>
</tr>
<tr>
<td>Epinephrine (10 µM)</td>
<td>29,514 ± 970 (239%)</td>
<td>15,477 ± 650 (140%)</td>
</tr>
</tbody>
</table>

**Fig. 6.** Effect of pertussis and cholera toxins on IL1 action: EL4 NOB 1 bioassay. EL4 cells were preincubated for 4 h at 37 °C with pertussis toxin (0, 100 ng/ml), cholera toxin (0, 100 ng/ml), or no addition (O). Cells were washed then incubated with increasing concentrations of IL1 for 24 h, after which supernatants were removed for assay of IL2 on CTLL-2 cells (see "Materials and Methods"). Results are expressed as cpm tritiated thymidine incorporated and are representative of three separate experiments.
the IL1 receptor on EL4 cells has a higher affinity for human IL1α than β (28, 39).

The effect of IL1 on GTPγS binding was due to an increase in the affinity, rather than an increase in the number, of GTP-binding sites. This is generally the case in activation of G proteins by ligand receptor complexes (24).

When the activation of G protein was related to the number of IL1 receptors occupied it was found that the increase in GTPγS binding was half-maximal at about 5% occupancy and maximal at 30%. This is consistent with the usually catalytic nature of G protein activation by receptors. It is likely that the magnitude of the effect is limited by the amount of G protein rather than by there being a high percentage of receptors unable to activate it.

It is difficult to relate biological responses to receptor occupancy. IL1 acts on EL4 NOB-1 cells (see Fig. 6) at concentrations three orders of magnitude below the apparent dissociation constant (10^{-10} M) of the receptor (29). Catalytic activation of a G protein could explain why the cytokine is active at concentrations giving very low receptor occupancy.

Following submission of the present findings a report appeared showing that pertussis toxin inhibited both the action of IL1 on cells and an IL1-stimulated GTPase activity in membranes of the pre-B cell line 70Z/3 (39). The cytokine-stimulated changes in GTP binding and hydrolysis in membranes of our EL4 strain were only about 50% inhibited by pertussis toxin. Furthermore, a longer exposure and 50-fold higher concentration of IL1 were needed to stimulate GTPase activity in the EL4 membranes than those of 70Z/3 (39).

Since both bacterial toxins strongly inhibited the response of EL4 cells to IL1 we were unable to conclude that IL1 signaled through a pertussis-sensitive G protein. Both toxins may have affected the response to IL1 via mechanisms not directly involving a G protein coupled to the cytokine receptor.

Although IL1 activates G proteins in isolated cell membranes, our results do not provide evidence for their direct interaction with the IL1 receptor. The partial sensitivity to pertussis toxin suggests that a Gi-like protein is involved.

The signaling system that may be coupled to the IL1 receptor via a G protein is uncertain. The recent experiments of Chedid et al. (39) implicate adenylyl cyclase. Previously, these authors found that IL1 increased cAMP in YT cells, thymocytes, and fibroblasts (13) and they have now shown that IL1 stimulates adenylyl cyclase, in a pertussis-toxin-sensitive manner, in membranes of YT, 3T3, and 70Z/3 cells (39). These findings prompted the suggestion that IL1 activates a novel G protein that is sensitive to pertussis toxin yet stimulatory to adenylyl cyclase, although known pertussis-sensitive G proteins are inhibitors, not stimulators, of cyclase.

Whether or not the increases in cAMP are sufficient to cause significant activation of protein kinase A remains to be shown. Other workers have found that IL1 has no measurable effect on cAMP levels in synovial fibroblasts (16) or Jurkat cells (15).

Similar uncertainty exists with regard to the ability of IL1 to activate phospholipase C. For instance, one group found that IL1 rapidly increased diacylglycerol levels by causing hydrolysis of phosphatidyldicholine in Jurkat cells (17) while another found no change in diacylglycerol (15). IL1 has been reported to stimulate hydrolysis of phosphoinositides in macrophages (18) but not lymphocytes (15, 17, 19), and of phosphatidylethanolamine in mesangial cells (20). Whether either adenyl cyclase or phospholipase C play a role in IL1 signaling remains to be established.

There is some evidence for early changes in protein kinase activity in IL1-stimulated cells. IL1 rapidly increases phosphorylation of a 27-kDa cytosolic protein (21) and the epidermal growth factor receptor (22) in fibroblasts and of a 55-kDa cytosolic protein in leukocytes (23). While these changes could be due to activation of protein kinase A or C, it is also possible that IL1 activates a novel kinase (22) and that G protein coupling could be involved.

In conclusion, the results presented here show that IL1 activates a G protein in EL4 membranes. The nature of the G protein and its effecter remain to be elucidated.

Acknowledgments—We thank Dr. Ihashimoto of the Dainippon Pharmaceutical Company of Japan for generously supplying us with human recombinant IL1α, Dr. Charles Dinarello of Tufts University School of Medicine, Boston for the gift of human recombinant IL1β and antiserum to IL1β, and Dr. Steve Poole and Judith Cartwright of the National Institute for Biological Standards and Control, South Mimms, United Kingdom for the gifts of antisera to IL1α and β, and help in performing the EL4 NOB-1 bioassay, respectively.

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IL-1 Stimulates GTP Binding and GTPase Activity