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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 [^{35}S]guanosine 5′-[γ-thiol]trisphosphate (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 [^{35}S]GTPγS was varied revealed that IL1 increased the affinity of the binding sites for [^{35}S]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 [^{35}S]GTPγS binding. Half-maximal enhancement of [^{35}S]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 [^{35}S]GTPγS binding and [γ-^{32}P]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[1] 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 heterotrimeric guanine nucleotide binding proteins (G proteins) (24). These proteins transduce signals from occupied receptors to effector enzymes such as adenylyl 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-hydrolyzable 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 (28). 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 (2×10^7 LAF units/mg was given by the Dainippon Pharmaceutical Co., Osaka, Japan; human recombinant IL1β (5×10^7 units/mg) was a gift from Dr. C. A. Dinarello, Tufts University School of Medicine, Boston, MA. Neu-

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[1] The abbreviations used are: IL1, interleukin 1; IL2, interleukin 2; G protein, guanine nucleotide-binding protein; GTPγS, guanosine 5′-[γ-thio]trisphosphate; App(MH)p; adenosine-5′(β,γ-imino)-triphosphate; EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; PGE_1, prostaglandin E_1.

tralizing sheep antisera to IL1α and IL1β 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. [^{35}S]GTPγS (1272 Ci/mmol) was from DuPont-New England Nuclear, [γ-^{32}P]GTP (10 Ci/mmol) was from Amersham, U.K. GTPγS, EDTA, dithiothreitol, Lubrol, ATP, creatine phosphokinase, bovine serum albumin, dextran and EGTA, pepstatin A, aprotinin, leupeptin, and phenylmethylsulfonyl fluoride, pertussis and cholera toxins, PGE_1 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 RPM1-1640 medium containing 10% heat-inactivated fetal calf serum at 37 °C in air/CO_2 (95:5). Membranes were prepared as described previously (31). Briefly, 1×10^9 cells were washed in phosphate-buffered saline, suspended in hypotonic buffer (10 mM Tris, pH 7.2, 1 mM $MgCl_2$; buffer A) containing a mixture of proteinase inhibitors (pepstatin A, aprotonin, and leupeptin all 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 5 min at 500 × *g* 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 (32).

GTPγS Binding Assays—[^{35}S]GTPγS (1272 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 $MgCl_2$, 1 mM EDTA, 1 mM dithiothreitol, 100 mM NaCl, 0.1% Lubrol, and [^{35}S] GTPγS in a volume of 50 μl. IL1α, IL1β, 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 μM 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 ^{35}S by liquid scintillation spectrometry. Control experiments using antisera to IL1α or β were performed by preincubating cytokine with antiserum for 2 h before assay.

Experiments were also performed with a fixed concentration of IL1α and varying concentrations of [^{35}S]GTPγS. The incubation time was 4 min and the percentage of [^{35}S]GTPγS bound was calculated in the presence and absence of IL1α (10 ng/ml).

GTPase Assays—GTPase assays were performed by the method of Cassel and Selinger (33). Briefly, 10 μg of membrane protein was incubated with 250 nM [^{32}P]GTP (10–30 Ci/mmol), 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 0.5 mM $MgCl_2$. The reaction volume was 50 μl. Incubations were carried out at 37 °C and terminated at various times by the addition of 1 ml of a 5% suspension of charcoal (Norit A) containing 0.5% bovine serum albumin and 0.1% dextran in 20 mM phosphate buffer, pH 8.0. Assay tubes were centrifuged and 200-μl aliquots of the supernatant were counted for ^{32}P by liquid scintillation spectrometry.

IL1 Receptor Binding—Binding of ^{125}I-IL1α to EL4 membranes was measured as previously described (31). Briefly, 50 μg of membrane protein was incubated with a range of concentrations of ^{125}I-IL1α (7.4×10^{18} dpm/mol) dissolved in binding buffer (Hank's balanced salts supplemented with 20 mM HEPES and 0.1% bovine serum albumin) in a final volume of 50 μl. Unlabeled IL1α was added to some tubes to determine nonspecific binding. Incubations were performed for 1.5 h at 19 °C. Bound radioligand 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. All binding data were analyzed by the LIGAND program (34).

In the experiments measuring GTPγS binding at varying IL1α concentrations, IL1 receptor occupancy was estimated as follows. Membranes (50 μg) were incubated with a constant concentration of ^{125}I-IL1α (0.5 ng/ml) mixed with varying concentrations of unlabeled IL1α for 4 min. Reactions were stopped by rapidly separating bound from free radioligand by filtration as before. The amount of IL1 binding to the membranes at each concentration was then expressed as a % of the total binding capacity determined at equilibrium, to give the % occupancy of receptors.

The Use of Pertussis and Cholera Toxins—[^{35}S]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 assessed for [^{35}S]GTPγS binding and GTP hydrolysis in response to IL1 (100 ng/ml) as described above. Prostaglandin E_1 (10 μM) and epinephrine (10 μM) were used as positive controls.

The effect of toxins on IL1-enhanced IL2 release from EL4 NOB-1 cells was also assessed. Cells were 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 1×10^6/ml in RPMI-1640 medium containing 5% fetal calf serum. Next, the cells were distributed into 96-well microtiter plates (100 μl/well), and IL1α 0.1–100 pg/ml was added. After 24 h incubation, 50 μl of supernatant was removed from each well and transferred to a copy plate. 5×10^3 washed CTLL-2 cells (an IL2-dependent murine line) were then added to each copy well. After 18 h culture 0.5 μCi of tritiated thymidine was added to each well and incorporated radioactivity determined by liquid scintillation counting after a 3-h incubation. Toxin-treated cells were compared with cells preincubated in medium alone.

RESULTS

IL1 Enhances [^{35}S]GTPγS Binding to EL4 Cell Membranes—The nonhydrolyzable analogue GTPγS was used to determine the effect of IL1α and IL1β on GTP binding to EL4 membranes. Both forms of IL1 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, IL1α was more potent than IL1β. IL1α caused a maximal stimulation at 10 ng/ml and IL1β at 100 ng/ml.

To ensure that the response was specific, both forms of IL1 were inactivated by use of specific neutralizing antisera. Treatment of IL1α and IL1β with appropriate antisera abolished their ability to increase GTPγS binding (Table I). Irrelevant serum, for example anti-IL1α against IL1β, had no effect on the response (data not shown).

To determine whether IL1 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). IL1α (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, IL1α caused a greater proportionate enhancement of GTPγS binding than at higher concentrations. For example, at 0.09 nM [^{35}S]GTPγS, 8% of the nucleotide was bound in untreated membranes as compared with 15% in the IL1α-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 IL1α causing an increase in the affinity of GTP-binding sites.

IL1 Stimulates Hydrolysis of [γ-^{32}P]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 IL1α. Fig. 4 shows that IL1α (100 ng/ml) increased the GTPase activity and after 8 min there was more than a 2-fold increase. The

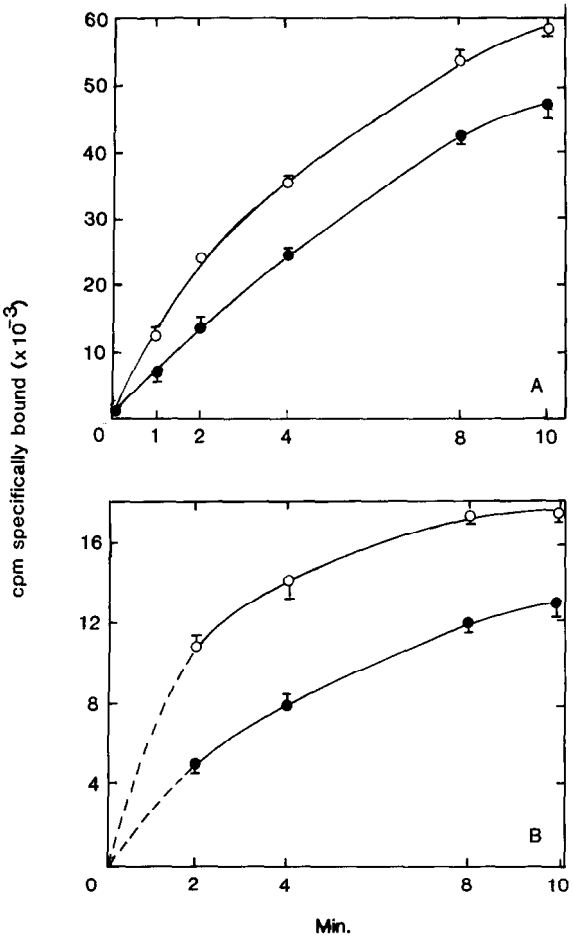

FIG. 1. **Time dependence of the effect of IL1 on specific binding of [^{35}S]GTPγS to EL4 membranes.** EL4 membranes (20 μg) were incubated in the presence of 2.5 nM [^{35}S]GTPγS at 37 °C for the indicated times in the presence (○) or absence (●) of IL1. Specific binding was in the range 5–15% of total radioactivity added. Nonspecific binding, determined in the presence of 25 μM GTPγS was always <2% of the total radioactive material added. Results are means ± S.D. of triplicate determinations. *A*, IL1α, 10 ng/ml; 4×10^5 cpm [^{35}S]GTPγS added. *B*, IL1β, 100 ng/ml; 2×10^5 cpm [^{35}S]GTPγS added.

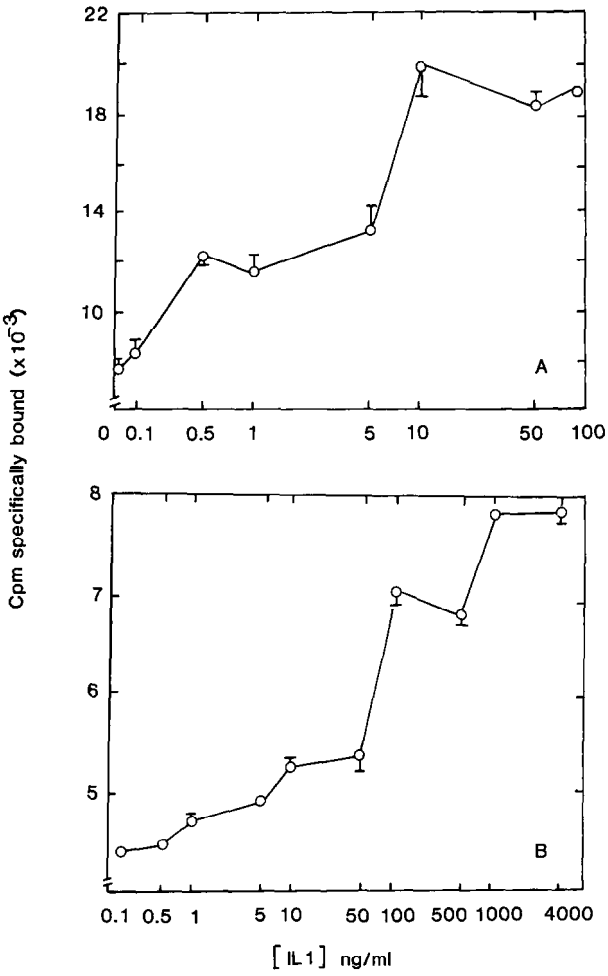

FIG. 2. **Effect of increasing concentrations of IL1α (*A*) and β (*B*) upon specific binding of GTPγS to EL4 membranes.** EL4 membranes (20 μg) were incubated with 2.5 nM [^{35}S]GTPγS (2.7×10^5 cpm) at 37 °C for 4 min in the presence of increasing concentrations of IL1. Nonspecific binding was determined in the presence of 2.5 μM GTPγS. Results are means ± S.D. of triplicate determinations.

response was concentration-dependent. At 10 ng/ml IL1, 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 IL1 increased GTP hydrolysis (Fig. 5) was similar to that over which it enhanced binding of GTPγS to the membranes.

Relationship between Binding of GTPγS and IL1 Receptor Occupancy—The concentration range over which IL1 increased binding of GTPγS 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 IL1-induced IL2 synthesis by EL4 NOB-1 is in the range 1–100 pg/ml (see Fig. 6). We therefore examined the relationship between IL1 receptor occupancy and stimulation of binding of GTPγS in the membranes (Fig. 5). Maximal specific binding of IL1α to the membranes was measured at equilibrium. The amount of IL1 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 IL1 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 IL1 stimulated nucleotide binding at very low levels of receptor occupancy. Half-maximal stimulation of [^{35}S]GTPγS binding was found to occur when only 5% of the receptors were occupied (1 ng/ml IL1α) and the effect was maximal at 30% occupancy. These results suggest that maximal receptor occupancy is not necessary to trigger a maximal enhancement of [^{35}S]GTPγS binding and that fractional occupancies of less than 5% were adequate to generate a signal.

Effect of Pertussis and Cholera Toxins on IL1 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 IL1 could be modified by ADP-ribosylation, we investigated the effect of pertussis and cholera toxins on [^{35}S]GTPγS binding and GTPase activity in EL4 membranes. As can be seen in Table II, pretreating the membranes with pertussis toxin partially decreased the IL1-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 G_i and G_s (37), but was without effect on PGE_1-stimulated GTPase activity. Cholera toxin had no effect on IL1-stimulated GTPase activity but inhibited that due to PGE_1 (indicating that the toxin was active).

The effects of the toxins on [^{35}S]GTPγS 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 [^{35}S]GTPγS to EL4 membranes

EL4 membranes were incubated with 2.5 nM [^{35}S]GTPγS (4.1 × 10^5 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.

Addition	Bound cpm [^{35}S]GTPγS	*p* value
None	31,151 ± 1,736	
IL1α 10 ng/ml	43,153 ± 2,901	
IL1α 10 ng/ml + Anti-IL1α 1/5,000	29,198 ± 1,392	<0.01
IL1α 10 ng/ml + Anti-IL1α 1/10,000	25,493 ± 1,726	<0.001
IL1α 10 ng/ml + Anti-IL1α 1/100,000	28,123 ± 2,100	<0.01
IL1β 100 ng/ml	51,908 ± 3,016	
IL1β + Anti-IL1β 1/5,000	28,567 ± 1,672	<0.001
IL1β + Anti-IL1β 1/10,000	20,085 ± 3,126	<0.001
IL1β + Anti-IL1β 1/100,000	46,337 ± 3,001	not significant

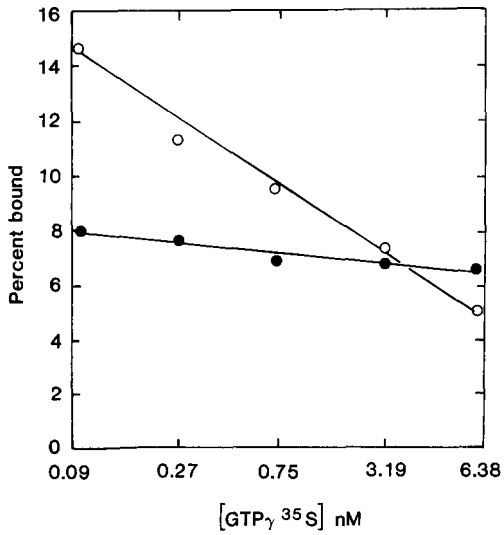

FIG. 3. **Effect of IL1α on the binding of increasing concentrations of [^{35}S]GTPγS added to EL4 membranes.** EL4 membranes (20 μg) were incubated with varying concentrations of [^{35}S]GTPγS for 4 min at 37 °C in the presence (○) or absence (●) of IL1α (5 ng/ml). % bound was calculated by expressing the number of counts specifically bound as a percentage of total counts added. Total counts added ranged from 9,483 cpm for 0.09 nM [^{35}S]GTPγS to 665,908 cpm for 6.38 nM. Results shown are means of duplicate determinations.

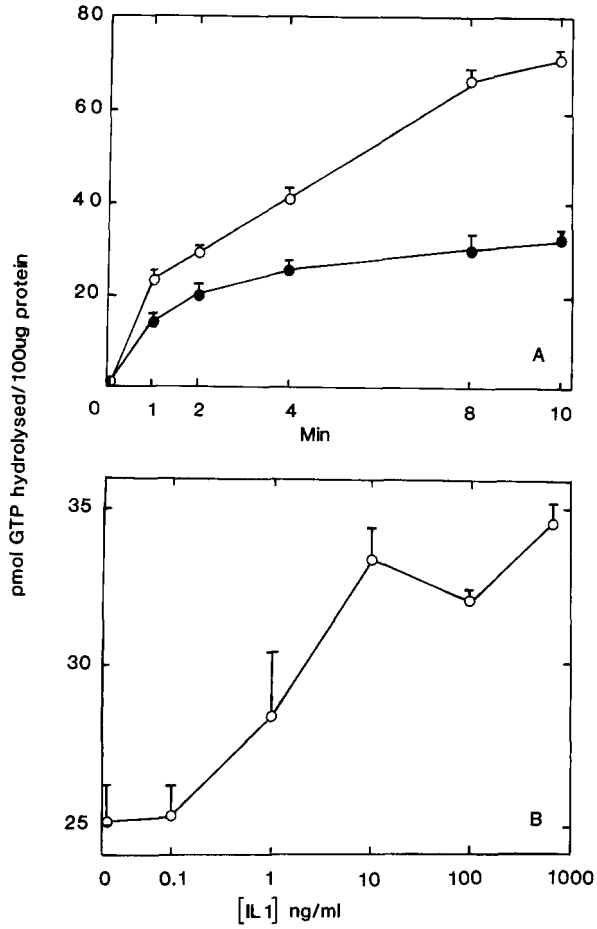

FIG. 4. **Effect of IL1α on [γ-^{32}P]GTP hydrolysis by EL4 membranes.** *A*, EL4 membranes (10 μg) were incubated with 250 nM [γ-^{32}P]GTP at 37 °C for the indicated times in the presence (○) and absence (●) of 100 ng/ml IL1α. GTP hydrolysis was determined by the release of $^{32}PO_4$. Results are expressed as the mean ± S.D. of triplicate determinations. *B*, EL4 membranes (10 μg) were incubated for 4 min with 250 nM [γ-^{32}P]GTP of 37 °C in the presence of varying concentrations of IL1α. GTP hydrolysis was determined as in *A*.

branes with pertussis toxin reduced the stimulation of [^{35}S]GTPγS binding from 153 to 135%. Pertussis toxin also inhibited epinephrine-stimulated [^{35}S]GTPγS binding but was without effect against PGE_1. Cholera toxin again had no effect on the IL1 response but was inhibitory to PGE_1. Since pertussis toxin could partially inhibit IL1α-mediated G protein activation a G_i-like component may be involved.

Experiments were then carried out to see whether the toxins affected IL1-induced release of IL2 by EL4 cells. Pretreatment with either toxin strongly inhibited the response to IL1 (Fig. 6).

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-

tion (31). These cells produce IL2 in response to IL1 and are used in a standard bioassay (30).

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

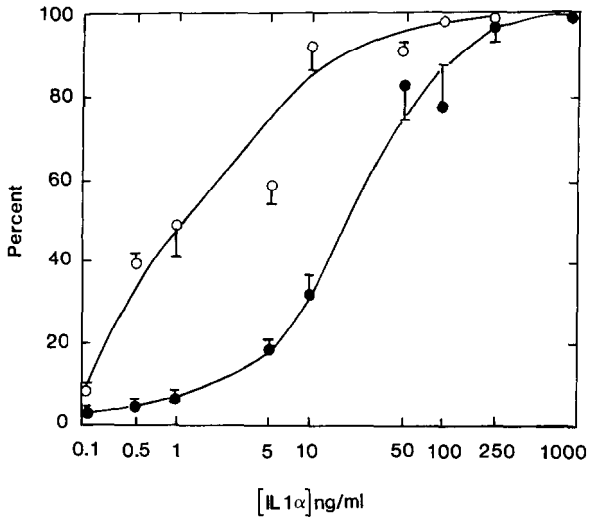

FIG. 5. **Correlation between IL1α-receptor occupancy and IL1α enhanced [^{35}S]GTPγS binding.** EL4 membranes were analyzed either for ^{125}I-IL1α binding (●) or IL1α-enhanced [^{35}S]GTPγS binding (○) after addition of IL1. IL1 receptor occupancy was the amount of IL1α specifically bound at 4 min expressed as a percentage of the total specific binding capacity for IL1 at equilibrium (see "Materials and Methods"). Maximum binding of ^{125}I-IL1 corresponded to 3,897 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 [^{35}S]GTPγS was measured after incubation of membranes in 2.5 nM [^{35}S]GTPγS and varying concentrations of IL1α for 4 min at 37 °C. Results are expressed as a percentage of the maximum enhancement in [^{35}S]GTPγS binding. Maximum enhancement corresponded to an increase in binding from 5,039 cpm in non-IL1α-treated membranes to 12,902 cpm at 100 ng/ml IL1α. Results are expressed as mean ± S.E. from triplicate determinations.

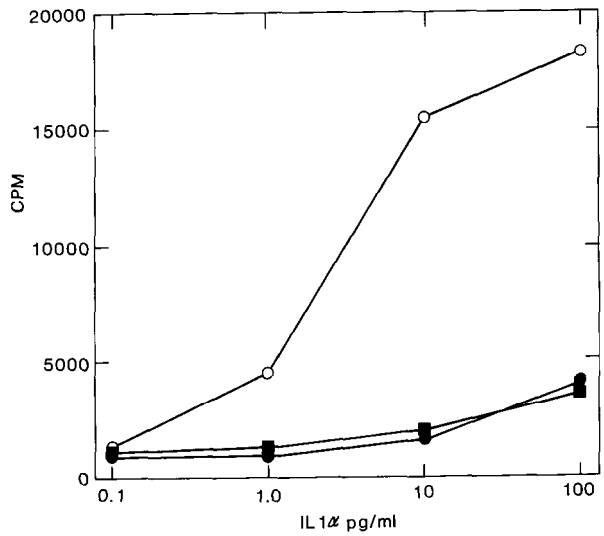

FIG. 6. **Effect of pertussis and cholera toxins on IL1 action: EL4 NOB 1 bioassay.** EL4 cells were preincubated for 4 h at 37 °C with pertussis toxin (●, 100 ng/ml), cholera toxin (■, 100 ng/ml), or no addition (○). Cells were washed then incubated with increasing concentrations of IL1 for 24 h, after 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.

TABLE II

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

EL4 membranes were preincubated for 30 min at 37 °C with activated pertussis toxin (100 ng/ml), cholera toxin (100 ng/ml), or buffer as control. Membranes (10 μg) were then assessed for GTPase activation by IL1 (100 ng/ml), PGE_1 (10 μM), or epinephrine (10 μM) by incubation with 250 nM [^{32}P]GTP at 37 °C for 4 min. GTP hydrolysis was determined by the release of $^{32}PO_4$. Results are expressed as mean ± S.D. of triplicate determinations. The data are also expressed as a % of control.

	Control membranes	Pertussis toxin-treated membranes	Cholera toxin-treated membranes
		pmol GTP hydrolyzed/100 μg protein	
Control	8.3 ± 0.05 (100%)	8.4 ± 0.3 (100%)	8.5 ± 0.15 (100%)
IL1 (100 ng/ml)	13.2 ± 0.1 (159%)	10 ± 0.2 (119%)	13.2 ± 0.5 (155%)
PGE_1 (10 μM)	13.3 ± 0.02 (160%)	13.4 ± 0.1 (159%)	8.4 ± 0.03 (99%)
Epinephrine (10 μM)	19.7 ± 0.02 (237%)	10 ± 0.1 (119%)	15.1 ± 0.3 (177%)

TABLE III

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

EL4 membranes were preincubated with toxins as described in Table II and were then assessed for enhanced [^{35}S]GTPγS binding by IL1 (100 ng/ml), PGE_1 (10 μM), or epinephrine (10 μM) by incubating 20 μg of membranes with 2.5 nM [^{35}S]GTPγS at 37 °C for 4 min. Nonspecific 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.

	Control membranes	Pertussis-toxin-treated membranes	Cholera toxin-treated membranes
		cpm [^{35}S]GTPγS specifically bound	
Control	12,355 ± 1,506 (100%)	10,998 ± 673 (100%)	13,460 ± 394 (100%)
IL1 (100 ng/ml)	18,924 ± 552 (153%)	14,831 ± 1,234 (135%)	19,907 ± 986 (145%)
PGE_1 (10 μM)	19,880 ± 1,846 (160%)	20,931 ± 109 (190%)	12,767 ± 509 (95%)
Adrenaline (10 μM)	29,514 ± 970 (239%)	15,477 ± 650 (140%)	22,909 ± 540 (170%)

the IL1 receptor on EL4 cells has a higher affinity for human IL1α than β (29, 38).

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 G_i-like protein is involved. Receptors which are coupled to the best characterized G proteins, G_i, G_s, and G_t, possess seven membrane-spanning domains (41). It would be surprising if the IL1 receptor, which has only a single membrane-spanning domain, were directly coupled to G_i, but other receptors with single membrane-spanning domains, such as those for IL2 and CSF 1 (42, 43), activate pertussis-sensitive G proteins whose nature is unknown (27, 26).

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 phosphatidylcholine 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 adenylyl 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 65-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 effector remain to be elucidated.

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