# INDUCTION OF HIGH-AFFINITY INTERLEUKIN 1
# RECEPTOR ON HUMAN PERIPHERAL BLOOD
# LYMPHOCYTES BY GLUCOCORTICOID HORMONES

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Glucocorticoids (GCs)[1] are well established as modulaters of the immune and inflammatory responses in man and in animal models. Most studies of the effects of GCs on the immune responses have demonstrated that GCs are generally immunosuppressive. The suppressive effects include GC-induced rodent thymocyte lysis (1), inhibition of antigen- and mitogen-induced lymphocyte proliferation (2–4), inhibition of production of and response to lymphokines (5, 6), and inhibition of NK activity (7). Recent studies also report that GC inhibits IL-2-R gene expression (8). Conflicting data have been published concerning the effect of GCs on B lymphocytes. The production of polyclonal Ig is significantly enhanced in the presence of GCs in vitro (9–11). The magnitude of the enhancing effect was similar to that observed with other polyclonal B cell activators such as PWM. Since this effect by GCs did not require proliferation, GCs might accelerate B cell maturation and differentiation. However, despite extensive investigation, the precise mechanism of these contrasting effects (inhibitory and enhancing) of GC remains to be clarified.

IL-1, a hormone-like polypeptide, functions as a fundamental mediator of immune and inflammatory responses. Although IL-1 has been defined as a thymocyte comitogenic factor (12), it has become evident that IL-1 exhibits a diverse range of biological activities. These include augmentation of B lymphocyte proliferation (13) and antibody production (14), fibroblast proliferation (15), acute-phase protein-inducing activity (16), prostaglandin-inducing activity (17), and endogenous pyrogen activity (18).

GCs have been reported to suppress both the production and effects of IL-1. GCs suppress IL-1 production by LPS-stimulated mouse peritoneal macrophages (19, 20). Recently we have extended this observation and shown that GC inhibits the production of both IL-1α and IL-1β by LPS-stimulated human monocytes (Lew, W., J.J. Oppenheim, and K. Matsushima, submitted for publication). Consequently, this suppressive effect of GC on IL-1 production may contribute

to the immunosuppressive and the antiinflammatory effect of GC. The effects of IL-1 are also downregulated by GC since GC suppresses the thymocyte comitogenic activity of IL-1 (20). We also observed that GC suppresses IL-1–induced proliferation of human dermal fibroblasts.

Dower et al. (21) have recently reported the existence of specific receptors for IL-1 on a variety of both human and murine cell types. We have also demonstrated high-affinity receptors for IL-1 on an EBV-transformed human B lymphocyte cell line that binds IL-1$\alpha$ and IL-1$\beta$ equally well (22). Since GC downregulates the effects of IL-1, we speculated that this may be based on effects of GC on the expression of receptors for IL-1. Therefore we investigated the effect of GC on IL-1-R expression of human PBMCs. Unexpectedly, our data reveal that GC dramatically increased IL-1-R expression on PBMCs within several hours without any loss in the binding affinity.

## Materials and Methods

*Reagents.* Prednisolone, dexamethasone, cortexolone (17-hydroxy-11-deoxycorticosterone), progesterone, 17$\beta$-estradiol, and testosterone were purchased from Sigma Chemical Co. (St. Louis, MO). Cycloheximide and actinomycin D were also obtained from Sigma Chemical Co. Human rIL-1$\alpha$ was a generous gift from Dr. M. Yamada (Dainippon Pharmaceutical Co., Osaka, Japan). Human rIL-1$\beta$ was a generous gift from Dr. Y. Hirai (Otsuka Pharmaceutical Co., Tokushima, Japan).

*Cell Cultures.* PBMCs were obtained from PBLs isolated from healthy volunteers by leukapheresis. Mononuclear cells were separated by centrifugation on a Ficoll-Hypaque gradient. To separate T lymphocytes, PBMCs were mixed with 2-aminoethylisothiouromium bromide (AET; Sigma Chemical Co.)-treated sheep erythrocytes (SRBCs) for 1 h on ice. The rosetting and nonrosetting cells were separated by Ficoll-Hypaque gradient centrifugation. The SRBCs were removed from the rosetting cells by lysis with $NH_4Cl$-lysing buffer. The nonadherent rosetting population obtained by removal of plastic adherent cells was demonstrated to consist of 92% Leu 4$^+$ and 5% Leu 11$^+$ cells as determined by flow cytometry and will be referred to as "T cells" population. The nonrosetting cells included B lymphocytes (12% Leu 12$^+$), monocytes (71% Leu M3$^+$), large granular lymphocytes (LGLs; 13% Leu 11$^+$), and rosette-positive cells (<4%) and will be referred to as "non-T cells."

Large numbers of cells enriched in B lymphocytes can be obtained by using a technique known as counter-current centrifugal elutriation. The "lymphocyte" fraction, which consists of small lymphocytes, and an "intermediate" monocyte fraction, which consists of monocytes and large lymphocytes, were isolated from PBMCs by counter-current centrifugal elutriation as described elsewhere (23). Subsequently, the cells in each fraction were separated into T and B cell populations by two times AET-SRBC rosetting of lymphocytes. The nonrosetting cells from the lymphocyte fraction was shown to consist of 65% Leu 12$^+$ and 4% Leu M3$^+$ cells and will be referred to as the "small B cells," and the cells from intermediate monocyte fraction consisting of 58% Leu 12$^+$, 23% Leu M3$^+$, 22% Leu 11$^+$, and 5% Leu 4$^+$ cells and will be referred to as the "large B cells." We chose not to use surface Ig as a marker because anti-Ig (even F[ab]'2) reacts with both human B lymphocytes and monocytes.

LGLs were separated from PBMCs as previously described (24). Briefly, PBMCs were depleted of monocytes by adherence on a plastic surface and applied to a nylon wool column, and the eluted cells were then fractionated on a seven-step discontinuous gradient of Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden) at concentrations between 40 and 60%. LGL-enriched preparations collected from the low-density fraction (fraction 2 and 3) were demonstrated to contain 75–80% Leu 11$^+$ cells.

A monocyte-enriched population was obtained by detaching adherent PBMCs from a plastic surface, and comprised 82% Leu M3$^+$ cells. A human LGL cell line YT (25) was

maintained in RPMI 1640 (Cellgrow; Mediatech, Washington, DC) supplemented with 10% FCS (HyClone Laboratories, Logan, UT), 2 mM of L-glutamine, 100 U/ml penicillin, and 100 $\mu$g/ml streptomycin at 37°C in 5% $CO_2$.

The cells were resuspended at a density of 5 × 10⁵ cells/ml in RPMI 1640 medium with 10% FCS. All cultures were performed at a final volume of 20 ml in 100 × 20-mm culture dishes (model 3100; Costar, Data Packaging Corp., Cambridge, MA). The cells were incubated in a humidified atmosphere at 37°C with 5% $CO_2$ as described. The various steroid hormones were dissolved in 95% ethanol and added to the culture media to yield the indicated final concentration. An equivalent volume of ethanol was added to control cultures. Cell viability after drug treatment was >95% by trypan blue dye exclusion test.

Human dermal fibroblasts (CRL 1507; American Type Culture Collection, Rockville, MD) were grown in Eagle's MEM (Cellgrow; Mediatech, Washington, DC) supplemented with 10% FCS, 2 mM L-glutamine, and antibiotics. Fibroblasts (5 × 10⁵ cells) were plated in 35-mm diam dishes (model 3506; Costar, Data Packaging Corp.) containing 2 ml of medium. After 2 d, the medium was then replaced with 2 ml of new medium with steroid hormones.

*Preparation of Iodinated IL-1α.*    Human rIL-1α was labeled with ¹²⁵I using the Bolton-Hunter reagent as described previously (22). Briefly, 4 $\mu$g of human rIL-1α (2 × 10⁷ U/mg) without carrier protein in 50 $\mu$l of 0.1 M borate buffer, pH 8.5, was conjugated to the 1 mCi of ¹²⁵I-labeled Bolton-Hunter reagent (2,200 Ci/mmol; monoiodinated; New England Nuclear, Boston, MA) (26) for 1 h on ice. After stopping the reaction with 0.5 ml of 0.05 M glycine in 0.1 M borate buffer, pH 8.5, ¹²⁵I-IL-1α was separated from free iodine by chromatography on a Sephadex G-25 column (1 × 20 cm) equilibrated with Dulbecco's PBS (D-PBS) and 10% glycerol. The biological activity of radiolabeled IL-1α was measured by a thymocyte comitogenic assay. The labeling efficiency of IL-1α was estimated by determining the number of units of unlabeled rIL-1α required to inhibit 50% of the binding of the ¹²⁵I-IL-1α to YT cells. This indicated that IL-1α contained 9.9 × 10⁶ cpm of ¹²⁵I per 1 $\mu$g of IL-1. There was no significant loss of thymocyte comitogenic activity by ¹²⁵I-labeled human rIL-1α.

*Receptor Binding Assay.*    After the treatment with steroid hormones, the cells were harvested and washed twice with cold D-PBS, and resuspended with 0.2 ml of binding medium (RPMI 1640 supplemented with 1 mg/ml BSA) in Eppendorf tubes. ¹²⁵I-IL-1α (13 ng/ml) was incubated with cells in the presence and absence of unlabeled IL-1α at a concentration of 2 $\mu$g/ml. After incubation at 4°C for 1 h, the cells were centrifuged at 10,000 g for 10 s. The pellets were washed with 1 ml of cold binding medium and centrifuged again. The cell suspensions with 0.2 ml of binding medium were layered onto 0.8 ml of 10% sucrose in D-PBS and centrifuged again at 10,000 g for 1 min. After aspiration of the supernatants, the cell pellets in the tube were excised and the radioactivity was counted in a gamma counter. Nonspecific binding, defined as the amount of ¹²⁵I-IL-1α bound to the cells in the presence of 2 $\mu$g/ml of human rIL-1α, was <2% of the total radioactivity. In the case of human dermal fibroblasts, the cells in 35-mm dishes were washed twice with cold D-PBS and were transferred to 0.5 ml of binding medium containing ¹²⁵I-IL-1α. After a 1-h incubation at 4°C, the cultures were rinsed four times with cold D-PBS and solubilized in 0.5 ml of 0.1 N NaOH containing 2% $Na_2CO_3$ and 1% SDS. The total cell-associated radioactivity was determined by counting this volume in a gamma counter.

*Treatment with Metabolic Inhibitors.*    The cells were cultured with cycloheximide (10⁻⁵ M) or actinomycin D (10⁻⁶ M) in the presence of prednisolone (10⁻⁶ M). After 6 h of culture, the effects of metabolic inhibitors on the expression of IL-1-Rs were measured. The viability of the treated cells was not changed as compared with the control cells.

## Results

*Kinetics and Affinity of ¹²⁵I-IL-1α Binding to GC-treated PBMCs.*    The specific binding of ¹²⁵I-IL-1α to cells increased rapidly and maximal binding was reached

after 1 h at 4°C and remained stable for up to 3 h (data not shown). In all subsequent experiments, incubations were therefore carried out for 1 h. The results in Fig. 1 illustrate the effect of prednisolone ($10^{-6}$ M) on $^{125}$I-IL-1$\alpha$ binding to human PBMCs. Each bar represents the mean of the triplicate measurements of the specific $^{125}$I-IL-1$\alpha$ binding as assessed by incubation with 150-fold excess of unlabeled human rIL-1$\alpha$. The specific binding increased rapidly with time after exposure to prednisolone; the maximal effect occurred after 6 h of exposure, and by 48 h declined toward control levels. A dose of 13 ng $^{125}$I-IL-1$\alpha$, used in this experiment, was sufficient to saturate the binding sites.

Scatchard plot analysis was performed using PBMCs treated with $10^{-6}$ M prednisolone for 6 h to determine whether the increased binding can be accounted for by an increase in the number of high-affinity receptors. As illustrated in Fig. 2, treatment with prednisolone led to an increase in the number of $^{125}$I-IL-1$\alpha$ binding sites on PBMCs without any reduction in affinity. Receptor numbers increased from 130 on control cells to 1,480 on prednisolone-treated cells. When PBMCs were treated with $10^{-5}$ M prednisolone, ~2,000 high-affinity



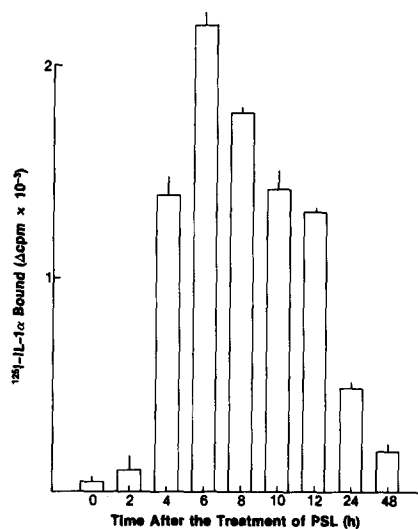FIGURE 1. Time course of $^{125}$I-IL-1$\alpha$ binding to GC-treated PBMCs. The cells ($10^7$ PBMCs) were incubated in culture media (RPMI 1640 with 10% FCS) in the presence of prednisolone ($10^{-6}$ M) for the period as indicated. Subsequently, cells were harvested and specific binding of $^{125}$I-IL-1$\alpha$ at 0.72 nM ligand was determined as described in Materials and Methods. Each bar represents the mean (and indicated SD) of triplicate measurements.
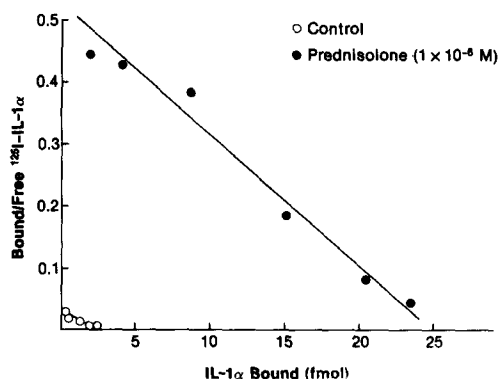


FIGURE 2. Scatchard plot analysis of $^{125}$I-IL-1$\alpha$ binding to control and prednisolone-treated PBMCs. The cells ($10^7$ PBMCs) were incubated for 6 h in the presence or absence of $10^{-6}$ M prednisolone. Subsequently, the cells were incubated with various dilutions of $^{125}$I-IL-1$\alpha$ to determine the specific binding.

receptors were expressed. $K_d$ values of $4.1 \times 10^{-10}$ M and $2.6 \times 10^{-10}$ M were obtained for control and treated cells, respectively. Thus, prednisolone markedly increased the expression of high affinity IL-1-R on PBMCs. Furthermore, as shown on Fig. 3, the prednisolone-induced IL-1-R on PBMCs are bound equally well by IL-1$\alpha$ and IL-1$\beta$.

*Effect of Various Steroids on Specific $^{125}I$-IL-1$\alpha$ Binding.*    Human PBMCs were exposed to different doses of various steroid hormones over a physiological and pharmacological concentration range ($10^{-5}$–$10^{-10}$ M) for 6 h. Fig. 4 demonstrates the specific binding of $^{125}$I-IL-1$\alpha$ to treated PBMCs. GCs increased IL-1$\alpha$ binding in a dose-dependent manner, and maximal effects were obtained with $10^{-7}$ M dexamethasone and $10^{-5}$–$10^{-6}$ M prednisolone. We also evaluated the effect of other types of steroid hormones. Progesterone, 17-$\beta$-estradiol, and testosterone had no effect on $^{125}$I-IL-1$\alpha$ binding to PBMCs. In addition, a mineralocorticoid,
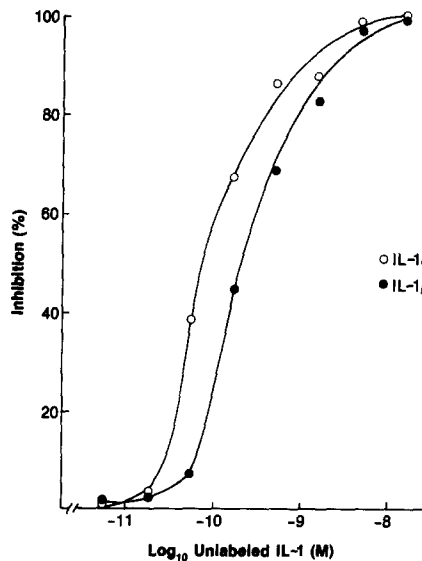


FIGURE 3.    Competitive effect on the binding of $^{125}$I-IL-1$\alpha$ to prednisolone-treated PBMCs of unlabeled human rIL-1$\alpha$ and IL-1$\beta$. The cells ($10^7$ PBMCs) were incubated for 6 h in the presence of $10^{-6}$ M prednisolone. Subsequently, the cells were incubated with $^{125}$I-IL-1$\alpha$ in the presence of various concentrations of IL-1$\alpha$ or IL-1$\beta$ to determine the inhibitable binding of $^{125}$I-IL-1$\alpha$.
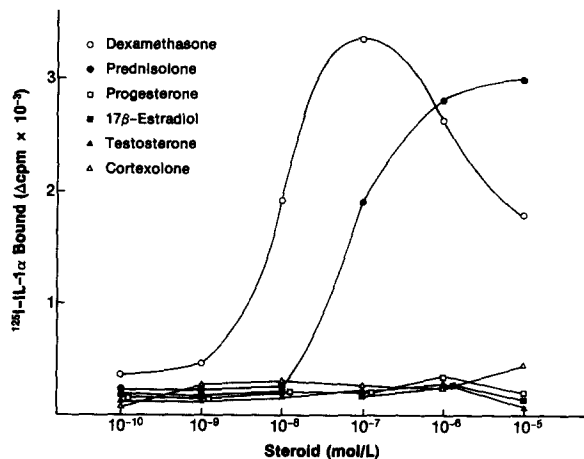


FIGURE 4.    Effect of steroids on specific $^{125}$I-IL-1$\alpha$ binding on human PBMCs. The cells ($10^7$ PBMCs) were incubated in culture media in the presence of varying concentrations of steroids. After culture for 6 h at 37°C, specific binding of $^{125}$I-IL-1$\alpha$ was determined. Nonspecific binding, defined as the amount of $^{125}$I-IL-1$\alpha$ bound to the cells in the presence of 0.11 $\mu$M of human rIL-1$\alpha$, was subtracted from total binding to yield specific binding.

aldosterone, did not have any effect on $^{125}$I-IL-1$\alpha$ binding to PBMCs (data not shown). Thus the capacity to enhance the binding of $^{125}$I-IL-1$\alpha$ to PBMCs appeared to be limited to GC.

*Induction of IL-1-R by GC Was Mediated by the Receptor for GC.* To further ensure that the induction of IL-1-R was due to the physiologic effect of GC, the competitive effects of an analogue cortexolone (17-hydroxy-11-deoxycorticosterone) were evaluated. Although cortexolone itself had a minimal inducing effect on IL-1-Rs on PBMCs, it acted as a competitive antagonist of GC-induced IL-1-R expression (Fig. 5). The dose-dependent induction of $^{125}$I-IL-1$\alpha$ binding to GC-treated PBMCs was shifted to the right in the presence of 3 × 10$^{-5}$ M cortexolone. At this concentration, cortexolone blocked about half of the induction of IL-1-R by 10$^{-6}$ M prednisolone (data not shown). These data suggest that the effect of GC on IL-1-R expression is dependent on the interaction of GC with its own receptor.

*Effect of Metabolic Inhibitors on GC-induced IL-1-R Expression.* To elucidate whether GC-induced IL-1-R expression required newly synthesized protein and RNA, we analyzed the effects of metabolic inhibitors, namely cycloheximide and actinomycin D on the induction process. PBMCs cultured with cycloheximide (10$^{-5}$ M) or actinomycin D (10$^{-6}$ M) in addition to an active concentration of prednisolone (10$^{-6}$ M) for 6 h, were not induced to express IL-1-R (Table I). Cycloheximide (10$^{-5}$ M) and actinomycin D (10$^{-6}$ M) inhibited ~90% of net protein synthesis and RNA synthesis of normal human PBMCs, respectively (data not shown). These metabolic inhibitors did not reduce cell viability over a 6-h period. Thus the induction of IL-1-R expression in PBMCs by GC required both protein and RNA synthesis.

*Identification of Lymphocyte Subpopulations That Express IL-1-R.* PBMCs were fractionated into T cells, B cells, LGLs, and monocyte-enriched populations to elucidate the subpopulation on which GC induced to express IL-1-R. Table II represents the effect of GC on each subpopulation. The T cell–enriched population (AET-SRBC rosette–positive) was induced to express only a small increase
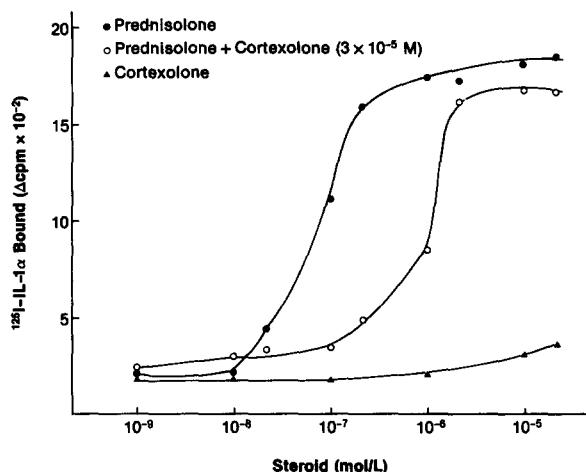


FIGURE 5. Inhibition of GC effect on IL-1 binding by cortexolone in PBMCs. The cells (10$^7$ PBMCs) were incubated for 6 h at 37°C with various concentrations of prednisolone alone, various concentrations of cortexolone alone, and various concentrations of prednisolone with 3 × 10$^{-5}$ M cortexolone. The cells were sedimented and washed, and specific $^{125}$I-IL-1$\alpha$ binding was performed as described.

TABLE I

Effects of Metabolic Inhibitors on the Induction of IL-1-R by GC

| | Agents added | | | Radioactivity |
|---|---|---|---|---|
| | Predniso-lone (10$^{-6}$ M) | Cyclohexi-mide (10$^{-5}$ M) | Actinomy-cin D (10$^{-6}$ M) | |
| | | | | cpm ± SD/10$^7$ cells |
| 1 | − | − | − | 619 ± 13 |
| 2 | − | + | − | 470 ± 28 |
| 3 | − | − | + | 198 ± 36 |
| 4 | + | − | − | 3,270.5 ± 184 |
| 5 | + | + | − | 724 ± 92 |
| 6 | + | − | + | 166.5 ± 0.5 |

The cells (10$^7$ PBMCs) were incubated with 10$^{-5}$ M cycloheximide or 10$^{-6}$ M actinomycin D in the presence or absence of 10$^{-6}$ M prednisolone for 6 h. The data represents the total binding of $^{125}$I-IL-1α to the cells.

TABLE II

Effects of GC on the Induction of IL-1-R on Various Cells Types

| Exp. | Cells | Radioactivity | |
|---|---|---|---|
| | | Without prednisolone | With prednisolone (10$^{-6}$ M) |
| | | cpm ± SD/10$^7$ cells | |
| A | PBMC | 31 ± 3 | 4,284 ± 395 |
| | T cells | 4 ± 1 | 19.5 ± 1.5 |
| | Non-T cells | 133 ± 29 | 19,340 ± 157 |
| | LGL | 12 ± 3 | 81 ± 25 |
| | Monocytes | 45 ± 1.0 | 30 ± 6 |
| B | Small B cells | 82 | 565 ± 32 |
| | Large B cells | ND | 4,262 |
| C | PMN | 86 ± 20.7 | 79 ± 20.8 |
| D | Fibroblasts | 417 ± 32.5 | 1,214 ± 92 |
| | YT cells | 3,085 ± 148 | 4,206 ± 125 |

Each subpopulation of PBMCs (10$^7$ cells) was incubated with 10$^{-6}$ M prednisolone for 6 h. YT cells (4 × 10$^6$ cells) and human fibroblasts (at confluent state in 35-mm dishes) were incubated with prednisolone (10$^{-6}$ M) for 18 h. The cells were harvested and the specific $^{125}$I-IL-1α binding was determined as described.

in IL-1-R by prednisolone. On the other hand, the non-T cell population (AET-SRBC rosette–negative) was induced to express IL-1-R by prednisolone to a much greater extent than unfractionated PBMCs. Neither purified LGLs nor monocyte-enriched populations were induced by GC to express IL-1-R. Prednisolone induced a significant enhancement of IL-1-R expression only on PBMC populations enriched in small and large B lymphocytes. In addition, when PBMCs were first cultured in the presence of 10$^{-6}$ M prednisolone at 37°C for 6 h and then fractionated into T cells and non-T cells by E rosette formation, 10$^7$ T cells and non-T cells specifically bound 425 ± 45 and 6,912 ± 198 cpm $^{125}$I-IL-1α, respectively. These data suggest that high level of IL-1-R are induced by GC predominantly on B lymphocytes.

GCs also increased the binding of $^{125}$I-IL-1α to human dermal fibroblasts and to a limited extent on YT cells as shown in Table II. Thus, these data indicate that GCs are able to induce IL-1-R expression on several cell types.

## Discussion

GCs play a critical regulatory role in cell differentiation and in the physiological response to stress. GCs may cause their diverse effects directly by modulating intracellular enzyme systems of target cells or indirectly by regulating heterologous hormone receptor systems, which is the so-called "permissive" action (27). GC hormones have been shown to upregulate heterologous hormone and cytokine receptors including those for β adrenalin (28), insulin (29), cholecystokinin (30), and epidermal growth factor (31) as permissive actions.

In this study, we have shown that GC elevates the number of IL-1-R on human PBMCs by >10-fold at 6 h of incubation. The high affinity of IL-1-R binding remains unaltered after treatment with GC. The upregulation of IL-1-R levels as assessed by the binding of $^{125}$I-IL-1α is also confirmed by data showing increases in the binding of $^{125}$I-IL-1α to the receptors as detected by chemical crosslinking (32). The functional capacity of the GC-induced IL-1-R on PBMCs was studied by evaluating induction of protein phosphorylation by IL-1. Carrier-free human rIL-1α induced phosphorylation within 5 min of an acidic cytosolic 65-kD protein at serine residue in GC-treated PBMCs (32). Furthermore, only GC-treated human PBMCs responded to rIL-1 with increases in the number of Ig-producing cells in reverse hemolytic plaque-forming cell assays. GC-induced polyclonal B cell activation could be inhibited by monoclonal anti–IL-1β (Tosata, G., T. Akahoshi, J.J. Oppenheim, and K. Matsushima, manuscript in preparation). These data suggest that GC induces high-affinity IL-1-R that are functional in increasing B cell Ig production.

The upregulation of IL-1-R level is specific for GC. This is supported by the lack of effect of other steroid hormones. Maximal effects were obtained with $10^{-7}$ M dexamethasone and $10^{-6}$ M prednisolone. This 10-fold higher potency of dexamethasone on the enhancement of $^{125}$I-IL-1α binding correlates with the greater antiinflammatory potencies of this GC (33). The effect of GC on IL-1-R expression is mediated via the interaction of GC with its own receptor. This is supported by the inhibitory effect of cortexolone, the competitive antagonist for GC, which can bind to the GC receptor but has no GC activity (34).

The finding that cycloheximide and actinomycin D abolished the increase in the level of IL-1-R indicates that GC stimulate *de novo* synthesis of protein and RNA for IL-1-R. A requirement for RNA and protein synthesis has been also demonstrated in the regulation of insulin and epidermal growth factor receptors by GC (29, 31). However, whether GC directly regulates gene expression for IL-1-R remains to be established by cloning of IL-1-R gene(s).

A number of reports highlight the complex interactions between GC and IL-1. Woloski et al. (35) showed that IL-1 could stimulate a mouse pituitary tumor cell line to secrete adrenocorticotropic hormone which is followed by an increase in the serum cortisol level. GCs in turn have been shown to inhibit the production of IL-1 by murine peritoneal Mφ (20) and human monocytes (Lew, W. J.J. Oppenheim,and K. Matsushima, submitted for publication). GC and IL-1 also

regulate each others' receptor expression. Hill et al. (36) reported that IL-1 could decrease the expression of GC receptors in murine hepatocytes. On the other hand, we have shown upregulation of IL-1-R on several cell types by GC. Overall these observations suggest that GC and IL-1 mutually regulate each others' production and effects. The capacity of GC to upregulate IL-1-R suggests that IL-1 and GC can interact in agonistic as well as in an antagonistic manner.

GCs induced only subsets containing B lymphocytes to express more IL-1-R. Since it was impossible to obtain purified B cells in sufficient numbers, all the other cell types present in PBMCs were carefully evaluated. The non-T cell subset expressed more IL-1-R after GC treatment, whereas T cells, LGLs, and monocyte populations could not be induced to express more IL-1-R. Only B cell–enriched populations (58–65% Leu 12$^+$ cells) responded well to GC and expressed more IL-1-R. In addition, only GC-treated PBMCs responded to IL-1 with increases in the Ig-producing cells. Consequently, these data suggest that B lymphocytes are the predominant non-T cell type that can be induced by GC to express IL-1-R. But, at present, we cannot negate the possibility that minor subpopulations in PBMCs such as dendritic cells also express more IL-1-R after GC treatment. The availability of antibody against IL-1-R should enable us more precisely to identify the subpopulations that respond to GC with increased levels of IL-1-R expression.

A number of studies have shown the lymphoid cell compartment to exhibit heterogeneous responses to GC (37). T lymphocytes and monocytes are reported to be sensitive to GCs, since GCs inhibit several functions of these cell types. GCs suppress thymocyte comitogenic responses (2), antigen- and mitogen-induced T cell proliferation (2–5), mixed lymphocyte reaction (38), and IL-2 production by T cells (6). GCs also inhibit antigen presentation (39), tumor cytotoxicity (40), and IL-1 production of monocytes (19, 20). This depression of monocyte and T lymphocyte functions may account for the immunosuppressive activities of GC on cell-mediated inflammatory responses.

On the other hand, B lymphocytes are reported to be extremely resistant to GC. Several studies have shown biphagic effects of GC on serum Ig level and specific antibody production in vivo. Administration of high dose of GC slightly diminishes the total serum Ig levels (41). This effect is explained in part by decreased Ig synthesis and increased protein catabolism. Some studies have demonstrated the enhancement of specific antibody production in patients treated with GC (42), but others have showed no effect of GC on specific antibody responses (43, 44). Many studies have demonstrated that GC stimulates dramatic increase in polyclonal Ig production by human PBMCs in vitro (9–11), comparable to those obtained with PWM. This stimulation of B cells by GC was reported to be dependent on both T cells and monocytes. These observations suggest that humoral immune responses can be stimulated by GC.

Enhancement of IL-1 production during the course of immune responses and inflammation is subsequently followed by an increase in serum cortisol levels. This favors immune deviation with potentiation of humoral immune responses at the expense of cellular immune responses. Thus, we hypothesize that GC-induced increases in antibody production may be mediated by IL-1 stimulation

of B cells bearing more functional receptors for IL-1. Studies in progress are aimed at investigating this hypothesis.

## Summary

The in vitro effect of glucocorticoids (GCs) on IL-1-R expression of human PBMCs was investigated. Both physiological and pharmacological concentration ranges of GC increased the specific binding of $^{125}$I-labeled human rIL-1$\alpha$ to PBMCs. This enhancement was specific for GC, since other steroid hormones, such as progesterone, 17$\beta$-estradiol, and testosterone failed to elevate the binding of $^{125}$I-IL-1$\alpha$ to PBMCs. The effect was time dependent with maximal effect occurring 6 h after treatment and dose dependent with half-maximal effect elicited by 100 nM prednisolone. Scatchard plot analysis indicated that $^{125}$I-IL-1$\alpha$ binding increased from ~100 IL-1-R per cell to $2 \times 10^3$ receptors per cell without a major change in affinity ($K_d = 2.6 \times 10^{-10}$ M). The subpopulation of PBMCs induced by GC to express higher levels of IL-1-R consisted predominantly of B lymphocytes, but not T lymphocytes, large granular lymphocytes, or monocytes. GCs also induced the expression of IL-1-R on some other cell types, including normal human dermal fibroblasts and the human large granular lymphocyte cell line YT. Since cycloheximide and actinomycin D inhibited the induction of IL-1-R by GC, synthesis of both new RNA and protein seems to be required for IL-1-R induction. This study presents the first evidence of upregulation of the receptors for IL-1 by GC, and may account for the reported enhancement of in vitro and in vivo humoral immune responses by GCs.

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