Brief Definitive Report

The Type II "Receptor" As a Decoy Target for Interleukin 1 in Polymorphonuclear Leukocytes: Characterization of Induction by Dexamethasone and Ligand Binding Properties of the Released Decoy Receptor

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Summary

Whereas the signaling function of the interleukin 1 (IL-1) receptor type I (IL-1R I) has been well documented, the type II "receptor" has been suggested to act as a decoy target for this cytokine. Since IL-1 may represent a key target of the immunomodulatory and antiinflammatory properties of glucocorticoids (GC), the aim of this study was to investigate the effects of dexamethasone (Dex) on IL-1R expression in human polymorphonuclear leukocytes (PMN), which express predominantly the type II molecule (IL-1R II). We found that Dex augments the levels of steady state transcripts encoding the IL-1R I and, most prominently, those of IL-1R II. Dex induced both transcripts via transcription-dependent mechanisms and by prolongation of the mRNAs half-lives. Inhibition of protein synthesis superinduced basal and Dex-augmented IL-1R II mRNA, whereas it completely inhibited the induction by Dex of IL-1R I transcripts. Induction of IL-1R II mRNA by Dex was associated with augmented membrane expression and release of the type II IL-1 binding molecule. This effect was mediated by the GC receptor. Other steroids (17β-estradiol, progesterone, and testosterone) were ineffective. The concentrations of IL-1α and IL-1 receptor antagonist required to displace the binding of IL-1β to the soluble form of the decoy molecule induced by Dex from PMN were, respectively, 100 and 2 times higher compared with IL-1β. The induction by Dex of the type II receptor, a decoy molecule for IL-1, may contribute to the immunosuppressive and antiinflammatory activities of Dex.

Glucocorticoids (GC) are potent modulators of the immune and inflammatory responses in humans and experimental models. IL-1, a key mediator of inflammatory reactions (1), represents an important target of GC-mediated immunosuppressive activities. GC suppress IL-1 production by monocyte-macrophages, both in vitro and in vivo (2–9). In apparent contrast with the inhibitory activities of GC on IL-1, GC were found to increase the expression of IL-1R on different cell types, including human PBMC (10, 11), PMN (11, 12), and the B cell line Raji (13).

Two IL-1Rs have been identified and cloned. The IL-1R I is an 80-kD transmembrane protein expressed predominantly or exclusively in T lymphocytes, fibroblasts, and endothelial cells, whereas the most represented receptor on myelomonocytic cells and B lymphocytes is the 68-kD (type II) IL-1R II (14, 15). Although the IL-1R I has a demonstrated transmembrane signaling function (16–22), the role of IL-1R II, which has a short (29-aa) cytoplasmic tail, is still a matter of investigation. Efforts aimed at defining a signaling function for IL-1R II using blocking mAbs have failed (22). Moreover, studies on regulation of PMN survival by IL-1 and IL-4 have suggested that the IL-1R II acts as a decoy target for IL-1 (23).

Given the importance of GC as antiinflammatory and immunosuppressive agents, this study was designed to conduct an in depth analysis of the regulation by GC of IL-1 receptors in human PMN.

Materials and Methods

Cells. Human PMN were separated from the peripheral blood of human healthy donors by Percoll gradient centrifugation (23). Briefly, whole blood was fractionated by Ficoll gradient centrifugation (Seromed-Biochem KG, Berlin, Germany), and PMN, collected from the pellet, layered on top of 62% Percoll (Pharmacia, Uppsala, Sweden). PMN (≥98% pure as assessed by morphology)

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were resuspended at 5–10 × 10^6/ml in RPMI 1640–10% FCS (Hyclone Laboratories, Logan, UT).

Reagents. Dexamethasone (Dex), cortexolone (17-hydroxyl-11-deoxycorticosterone), progesterone, 17β-estradiol, and testosterone were from Sigma Chemical Co. (St. Louis, MO). RU 486 was a kind gift of Dr. D. Di Lorenzo, Brescia, Italy. IL-1α and IL-1β were from Immunex Research and Development Corp. IL-1 receptor antagonist (IL-1ra) was from Cetus Corp. (Berkeley, CA).

IL-1 Binding Assay. After treatment with 10^{-7} M Dex for 12–14 h at 37°C, 1–2 × 10^6 PMN were incubated with decreasing concentrations of ^{125}I-IL-1β (180 μCi/μg; NEN, Bad Homburg, Germany) in the presence or absence of a 200-fold molar excess of cold cytokine in 0.1 ml binding buffer (PBS-0.1% BSA-0.02% sodium azide; Sigma Chemical Co.) at room temperature for 1 h. To separate bound from free radiolabeled IL-1, cells were centrifuged over a cushion of silicon oil. Scatchard analysis was performed by the LIGAND program (24).

Northern Blot Analysis. RNA isolation and analysis were as described (23). Probes were a EcoRI–HindIII 477-bp fragment and a EcoRI–SalI 750-bp fragment from IL-1R I and IL-1R II cDNAs, respectively. Membranes were washed twice with 2× SSC/1% SDS (Merck & Co., Inc., Rahway, NJ) at 60°C and exposed for 24–36 h for IL-1R I or 4–6 h for IL-1R II expression at −80°C. RNA transfer to membranes was checked by UV irradiation.

Affinity Cross-linking. Cross-linking experiments were described in detail (23). Briefly, for surface affinity cross-linking, 30 × 10^6 PMN treated with 10^{-7} M Dex for 14 h were incubated in binding buffer with 1 nM ^{125}I-IL-1β. After addition of 1 mM disuccinimidyl suberate (DSS; Pierce Chemical Co., Rockford, IL), the cell pellet was lysed in 100 μl lysis buffer (0.5% Triton X-100, 25 mM Hepes, 1 mM PMSF, 100 μg/ml aprotinin and leupeptin; Sigma Chemical Co.). The debris-free supernatant was analyzed by 8% SDS-PAGE under reducing conditions and dried gels were exposed to autoradiography for 1–3 d. For soluble covalent cross-linking, 30 × 10^6 PMN were cultivated with 10^{-7} M Dex for 14 h in RPMI 1640 without serum at 37°C. Medium was recovered and concentrated 10 times by membrane filtration (cut-off 10,000; Amicon, Beverly, MA). 200 μl were added with 1 nM ^{125}I-IL-1β, with or without a 2–2000-molar excess of cold competitors (IL-1α, IL-1β, and IL-1ra), and incubated at 4°C for 1 h. After addition of 1 mM DSS at 4°C for 30 min, samples were analyzed by gel electrophoresis as above. Densitometric analysis of autoradiographic signals has been performed with a scanning densitometer (GS 300; Hoefer Scientific Instruments, San Francisco, CA), calculating the area under the curve.

Results and Discussion

Dex Stimulates Predominantly IL-1R II mRNA Expression. Untreated PMN expressed abundant levels of the type II mRNA and these were considerably increased (4–10-fold, six donors) by Dex, with peak levels at 4–5 h (Fig. 1). Visualized by Northern analysis, PMN have barely detectable or undetectable levels of type I IL-1R mRNA (11, 23). Dex augmented also IL-1R I transcripts (three- to sixfold, six donors), but constitutive and inducible transcripts coding for IL-1R I were evident only after long times of exposure (the membranes shown in Fig. 1 were exposed to autoradiography for 25 h for the type I R and 5 h for type II R transcripts). Thus, the predominant IL-1R transcripts induced by Dex in PMN are those coding for the IL-1R II.

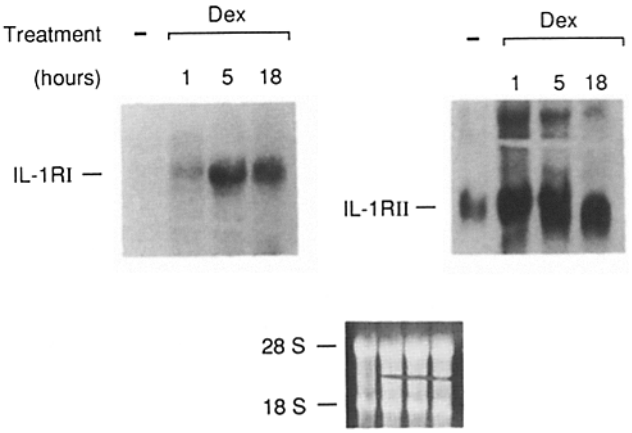

Figure 1. Induction of IL-1R I and IL-1R II transcripts in Dex-treated PMN. Cells were incubated with 10^{-7} M Dex for 1, 5, and 18 h. Total RNA was extracted and analyzed by Northern blotting. The same membrane was hybridized, first with the IL-1R I probe, and then with the IL-1R II probe. The lower part of the figure shows the ethidium bromide–stained membrane. The membrane was exposed to autoradiography for 25 and 5 h for type I and type II transcripts, respectively.

Different Mechanisms Are Involved in Induction of Type I and Type II IL-1R Expression. Next we examined the mechanisms involved in the induction of IL-1R I and IL-1R II transcripts by Dex. Actinomycin D (ActD) completely abolished the augmented expression of both type I and type II transcripts induced by Dex (Fig. 2, representative of four donors), indicating that gene transcription is involved in this phenomenon. We encountered considerable difficulties in performing run-off analysis in human circulating PMN. In one experiment with the lymphoblastoid B cell line Raji, Dex induced augmented levels of type II IL-1R transcription (data not shown). When transcript stability was examined, the half-lives of type I and type II R transcripts in untreated cells

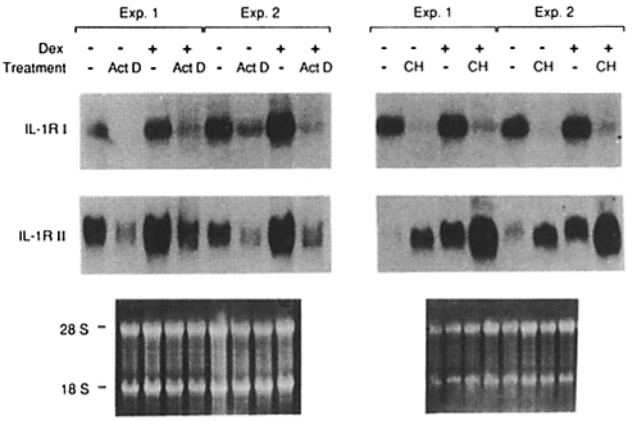

Figure 2. Effects of metabolic inhibitors on Dex-induced expression of IL-R transcripts. PMN from two different donors, representative of four, were incubated with Dex (10^{-7} M), with or without ActD (1 μg/ml) or CH (10 μg/ml), for 4 h and then analyzed for IL-1R I and IL-1R II transcripts. Each membrane (*bottom*) was hybridized with both probes and exposed to autoradiography as detailed in Fig. 1.

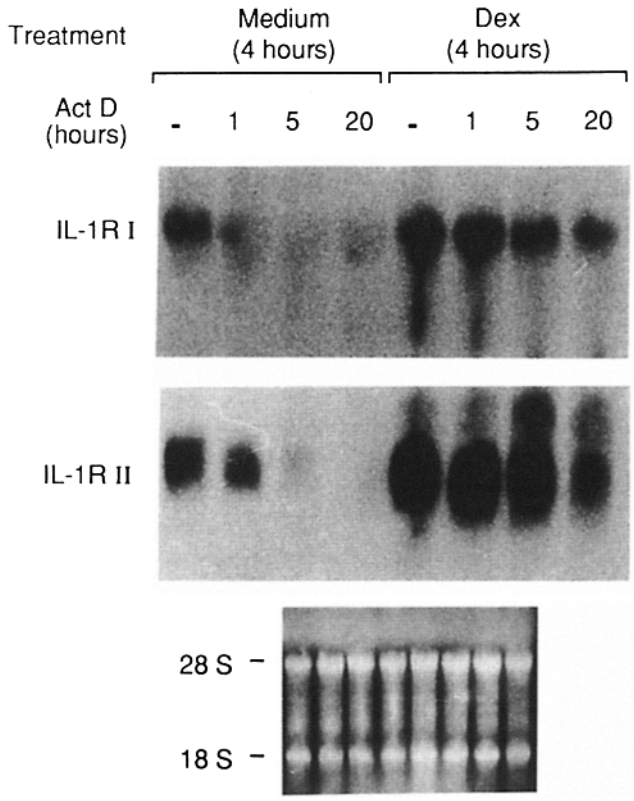

Figure 3. Half-life of IL-1R transcripts in PMN untreated or treated with Dex. PMN were cultivated with or without Dex (10^{-7} M) for 4 h. Then ActD was added (1 μg/ml) to block gene transcription and cells examined for IL-1R transcripts after various time points. The same membrane (*bottom*) was hybridized with both probes and exposed to autoradiography as detailed in Fig. 1.

were ~2 and 3 h, respectively (Fig. 3, representative of three donors). Dex increased IL-1R transcript stability, prolonging the half-lives of type I and type II R to ~15 and 7 h, respectively.

Thus, Dex affects the expression of IL-1R I and IL-1R II at transcriptional and posttranscriptional levels. However, different mechanisms seem to underlie the Dex-mediated induction of these transcripts since inhibition of protein synthesis by cycloheximide (CH) superinduced the Dex-augmented expression of type II IL-1R mRNA but completely inhibited the induction of type I IL-1R transcripts (Fig. 2, representative of four donors).

Dex-induced Surface Expression and Release of IL-1R II. As expected (11, 12), Dex caused a three- to sixfold increase in specific binding of IL-1β to PMN, with unchanged K_d values (Table 1). Surface affinity cross-linking demonstrated an IL-1 receptor with a molecular mass consistent with the type II IL-1 binding protein (68 kD) (Fig. 4 *A* illustrates a representative donor out of three tested).

Augmented surface expression of the type II IL-1R was associated with release of a soluble version of this IL-1 binding protein. Cross-linking revealed that the supernatants of Dex-treated PMN contained an IL-1-binding protein of apparent

Table 1. *Binding of Radiolabeled IL-1β on the Surface of Dex-treated PMN*

Exp.	Dex	K_d	Receptors/cell
		(10^{-10} M)	
1	–	5.98 ± 0.7	111 ± 17
	+	6.72 ± 0.8	381 ± 61
2	–	8.1 ± 1.6	220 ± 55
	+	7.9 ± 1.5	854 ± 154
3	–	7.5 ± 1.1	124 ± 31
	+	8.4 ± 1.3	794 ± 190

PMN were purified from three donors and incubated with or without Dex (10^{-7} M) for 14 h. Cells were then incubated with various concentrations of radiolabeled IL-1β (from 1.2 to 0.05 nM). Specific binding was obtained after subtraction of counts in the presence of 200 M excess of unlabeled IL-1β.

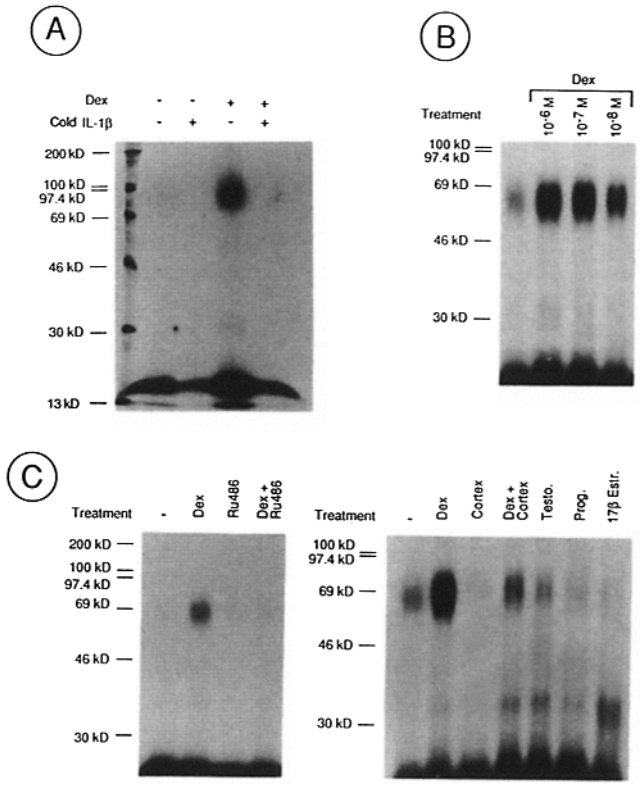

Figure 4. Surface expression and release of IL-1R II from PMN treated with Dex, GC receptor antagonists, and other steroids. (*A*) Surface affinity cross-linking of radiolabeled IL-1β to Dex (10^{-7} M, 14 h)-treated PMN. The first lane on the left shows molecular weight markers. (*B*) Affinity cross-linking of radiolabeled IL-1β to conditioned supernatants of PMN treated with Dex at various concentrations. Cells were incubated for 14 h with Dex from 10^{-6} to 10^{-8} M. Conditioned media were then concentrated and incubated with radiolabeled IL-1β. After treatment with DSS, cross-linked products were analyzed by SDS-PAGE under reducing conditions. (*C*) Affinity cross-linking radiolabeled IL-1β to conditioned media of PMN treated for 14 h with the following steroid and steroid-receptor antagonist: Dex (10^{-7} M), RU 486 (10^{-6} M), cortexolone (cortex, 10^{-5} M), progesterone (10^{-7} M), testosterone (10^{-7} M), 17β-estradiol (10^{-7} M).

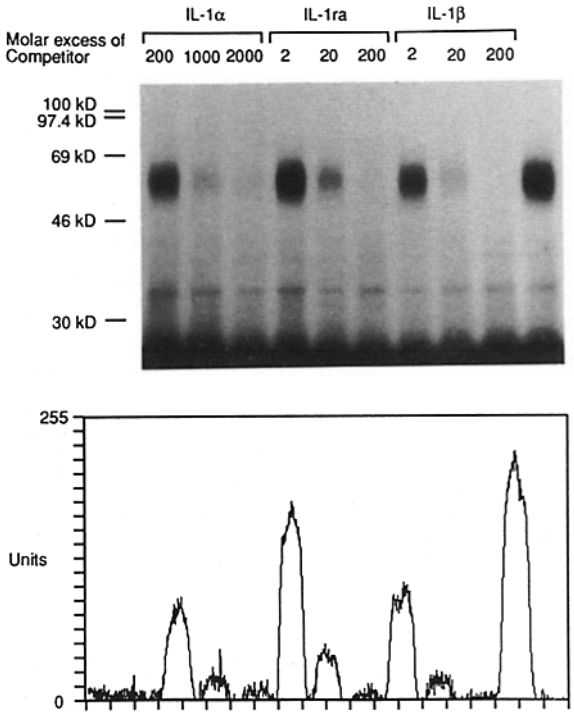

Figure 5. Competition with different concentrations of IL-1β, IL-1α, and IL-1ra of affinity cross-linking of radiolabeled IL-1β to Dex-treated PMN-conditioned media. Cells were incubated for 14 h with Dex (10^{-7} M). The conditioned media were next concentrated and incubated with radiolabeled IL-1β and the different competitors at increasing molar excess (as indicated). After treatment with DSS, products were analyzed by SDS-PAGE under reducing conditions. The densitometric analysis of the autoradiographic film is illustrated below.

molecular weight of ~45 kD (Fig. 4 *B*). This finding was confirmed in 10 different donors. Soluble IL-1 binding proteins of comparable size have been described in the supernatants from human mononuclear cells and from the B cell line Raji (25–27). Using mAbs, we recently established that the 45-kD IL-1 binding molecule released from PMN is a version of the type II IL-1R (23). Moreover, in preliminary experiments, Dex-induced release of the type II R as assessed using a specific ELISA assay (six donors, increase from 2.3–3.75-fold). A toxic effect of 10^{-6}–10^{-8} M Dex can be ruled out, since neither LDH release nor cell viability (trypan blue dye exclusion) were affected by the treatment (data not shown).

The release of a soluble form of the type II IL-1 binding protein by Dex was mediated by the glucocorticoid receptor, as two different competitive analogs (cortexolone and RU 486) prevented the Dex-mediated release of this IL-1 binding protein from PMN (Fig. 4 *C*). RU 486 inhibited the induction of IL-1R II transcripts by Dex (not shown). Other steroids, including progesterone, 17-β-estradiol, and testosterone, were ineffective (Fig. 4 *C*).

Relative Affinities of the Soluble Type II Molecule to IL-1α, IL-1β, and IL-1 Receptor Antagonist (IL-1ra). Since the membrane form of the decoy receptor binds IL-1α, IL-1β, and IL-1ra with different affinities (28–30), the binding properties of the corresponding soluble version were examined by competing the affinity cross-linking of radiolabeled IL-1β to the soluble IL-1R II with various concentrations of unlabeled IL-1α, IL-1β, and IL-1ra. In a preliminary experiment, the competitive system used here was validated using recombinant soluble IL-1R I (data not shown). Although formal binding analysis requires the purified soluble protein, the densitometric analysis of cross-linked products was used to demonstrate that the concentrations of IL-1ra and IL-1α capable of inhibiting binding of radiolabeled IL-1β by 50% were 2 and 100 times higher, respectively, compared with IL-1β (Fig. 5).

Concluding Remarks. GC have a wide range of effects on various components of immune and inflammatory responses. The precise mechanisms underlying the immunosuppressive and anti-inflammatory activities of GC have not been completely defined. GC have been shown to inhibit the synthesis of a series of cytokines involved in the regulation of inflammatory reactions, including IL-1 (2–9). The finding that GC, a major class of immunosuppressive and antiinflammatory agents, induce expression and release of the type II IL-1 binding molecule is consistent with the hypothesis that this molecule, perhaps inappropriately called a receptor, may indeed serve as a decoy target for IL-1 (23).

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