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Biochemistry

T-cell interleukin 1 receptor cDNA expressed in Chinese hamster ovary cells regulates functional responses to interleukin 1

(receptor structure/gene expression/signal transduction/deletion mutagenesis)

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ABSTRACT **We have cloned a cDNA encoding a receptor identical to the native M_r 80,000 glycoprotein that binds interleukin (IL) 1α and -β in murine T cells. Chinese hamster ovary (CHO) cells transfected with this T-cell IL-1 receptor (IL-1R) [CHO(IL-1R)] cDNA express ≈100,000 IL-1Rs per cell, compared to the <100 receptors present on control CHO cells. For two functional responses to IL-1, prostaglandin synthesis and cytokine secretion, CHO(IL-1R) cells were 1000 times more sensitive to IL-1α than were control CHO cells. Northern blot analysis and antibody precipitation demonstrated that one of the cytokines induced was granulocyte colony-stimulating factor and that mRNA levels for this cytokine were increased in CHO(IL-1R) cells by IL-1α concentrations that had no effect on control cells. To establish the role of the recombinant receptors in signal transduction, an IL-1R cDNA modified by deletion of the predicted cytoplasmic domain was expressed in the CHO cell line termed CHO(IL-1RΔCT). CHO(IL-1RΔCT) cells expressed ≈100,000 high-affinity IL-1 binding sites per cell, but these cells were less sensitive than control lines to IL-1, as measured by prostaglandin and cytokine release. These results show that the IL-1R cDNA encodes the entire functional receptor and that the cytoplasmic domain is required for signal transduction but not ligand binding.**

Interleukin (IL) 1α and -β are polypeptide hormones that have pleiotropic effects on a wide variety of cells (1). These effects include: T-cell activation (2), stimulation of hematopoietic cells (3, 4), and regulation of synthesis and secretion of acute-phase proteins, collagenase, and prostaglandin: part of the central role of IL-1 in the inflammatory response (1, 5). The responses to IL-1 are mediated by binding to specific receptors (6), and, in spite of significant sequence divergence (7), IL-1α and -β bind to the same cell surface receptor molecule on T cells and fibroblasts (8–10). The IL-1 receptor (IL-1R) on murine T cells has been identified by cDNA expression cloning and N-terminal sequence analysis as a M_r 80,000 integral membrane glycoprotein that binds IL-1α and IL-1β (11, 12). Although these results clearly establish the primary sequence of a polypeptide required for IL-1 binding, they do not address the question of whether the same polypeptide can function in IL-1 signal transduction. In this report, we demonstrate that IL-1R expressed from transfected cDNA in Chinese hamster ovary (CHO) cells regulates two functional responses to IL-1 in these cells, prostaglandin secretion and cytokine production, and that signal transduction requires both the extracellular IL-1 binding and cytoplasmic domains of the receptor.

MATERIALS AND METHODS

Materials. Human recombinant IL-1α was prepared from *Escherichia coli* cultures (13) and labeled with ^{125}I by a modified chloramine-T method as described (8). Fluorescein isothiocyanate (FITC)-conjugated IL-1α (FITC-IL-1α) was prepared by reacting 2.9 nmol of IL-1α with 100 nmol of FITC (Research Organics), in a total volume of 70 μl of 0.02 M sodium borate/0.15 M NaCl, pH 8.5, for 2 hr at 37°C. Protein was separated from unconjugated dye by gel filtration on Bio-Gel P6 (Bio-Rad). The FITC-IL-1α had 82% of the binding activity of unlabeled IL-1α, as determined by its capacity to compete with ^{125}I-labeled IL-1α for IL-1Rs on EL-4 cells. Human recombinant granulocyte colony-stimulating factor (G-CSF) was produced in a yeast expression system, purified to homogeneity from yeast conditioned medium, and a rabbit polyclonal antiserum to G-CSF was prepared, as described for granulocyte–macrophage (GM)-CSF (14). Human G-CSF was coupled to Affi-Gel 10 (Bio-Rad) according to the manufacturer's instructions with a coupling efficiency of 99.9%, resulting in 3 mg of G-CSF per ml of gel. Rabbit antiserum was affinity-purified by absorption to G-CSF Affi-Gel, exhaustive washing with isotonic phosphate-buffered saline (PBS), and elution with 10 mM citric acid. The affinity-purified antiserum to human G-CSF quantitatively immunoprecipitated human G-CSF but not human GM-CSF, human IL-3, or murine CSF-1 (J.E. and D. Mochizuki, unpublished observation).

Construction of IL-1R Expression Vectors. The plasmid pDC201 (Fig. 1*C*) was assembled from (*i*) the simian virus 40 (SV40) origin of replication, enhancer, and early and late promoters; (*ii*) the adenovirus 2 major late promoter and tripartite leader; (*iii*) SV40 polyadenylylation and transcription termination signals; (*iv*) adenovirus 2 virus-associated RNA genes (VAI and VAII); and (*v*) pMSLV (15). Details of the construction of pDC201 are available on request. The IL-1R cDNA clone containing the entire coding region in the expression vector pDC201 has been described (ref. 11; and Fig. 1*A*). The variant lacking all but the membrane proximal 23 amino acids of the cytoplasmic region was constructed by deleting all IL-1R sequences downstream of the *Hin*dIII site (at nucleotide 1085 of figure 4 in ref. 11). Subsequent to Ala-363 encoded by the filled-in *Hin*dIII site, the amino acid sequence continues with 9 amino acids (Gly-His-Arg-Ser-Thr-Arg-Phe-Asp-Val) encoded by the vector before encountering a translational stop codon (Fig. 1*B*).

Cell Lines and Transfections. CHO-K1 cells (provided by T. J. Moehring, University of Vermont) were maintained in Ham's F-12 medium supplemented with 5% (vol/vol) fetal bovine serum, penicillin (50 units/ml), streptomycin (50 μg/ml), and 2 mM glutamine and passaged at confluency. Cells were transfected with pSV2Neo, which confers G418

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Abbreviations: IL (prefix), interleukin; IL-1R, IL-1 receptor; CHO, Chinese hamster ovary; CSF, colony-stimulating factor; PGE_2, prostaglandin E_2; FITC, fluorescein isothiocyanate; G, granulocyte; GM, granulocyte–macrophage; SV40, simian virus 40; BSA, bovine serum albumin.

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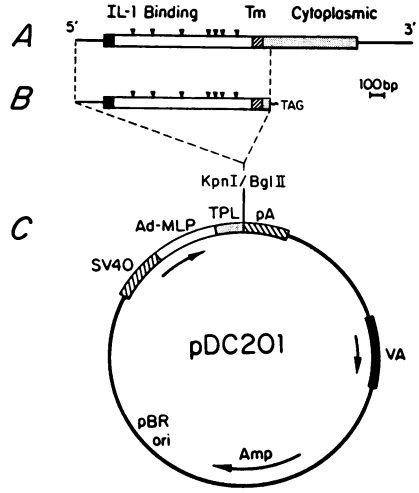

FIG. 1. IL-1R expression constructs. (*A*) IL-1R cDNA expression construct (11). Untranslated regions are lines; the IL-1R coding region is a box. The signal peptide is a solid box, the extracellular portion is an open box, the transmembrane region (TM) is a cross-hatched box, and the cytoplasmic segment is a stippled box. Inverted triangles above the box show potential N-glycosylation sites. The IL-1R cDNA was inserted into pDC201 as shown. (*B*) Schematic diagram of the IL-1R cytoplasmic deletion (ΔCT) expression construct. IL-1R cDNA encoding Leu-1 to Ala-363 followed by Gly-His-Arg-Ser-Thr-Arg-Phe-Asp-Val and a translational stop codon was constructed and inserted into pDC201 as shown. (*C*) pDC201 expression vector. SV40, SV40 origin of replication, enhancer, and promoters; Ad-MLP, adenovirus 2 major late promoter; TPL, adenovirus 2 tripartite leader; pA, SV40 polyadenylylation signal; VA, adenovirus 2 virus-associated RNA genes; bp, base pairs.

resistance on mammalian cells (16), or co-transfected with pDC201IL-1R and pSV2Neo or pDC201 and pSV2Neo by calcium phosphate precipitation (17). Cells were then selected in Ham's F-12 medium containing G418 (GIBCO) (0.5 mg/ml). Drug-resistant colonies (>200 colonies per experiment) were selected for high levels of receptor expression by fluorescence-activated cell sorting using FITC-IL-1α. Cells (10^6 cells per ml) were suspended in PBS containing 2% (wt/vol) bovine serum albumin (BSA) and 1 nM FITC-IL-1α. Cells were incubated for 1 hr at which time the cells were pelleted by centrifugation and washed three times in PBS with 2% BSA. Stained cells were then analyzed and sorted using an EPICS C flow cytometer (Coulter), utilizing an argon laser at an excitation of 488-nm and a constant power of 300 mW. The top 1% of the viable FITC-IL-1α stained cells were collected at each sort. Transfected cell lines were maintained in supplemented Ham's F-12 medium with G418 and passaged at confluency.

^{125}I-Labeled IL-1α Binding Assay. CHO cells were harvested by scraping after a 10-min treatment at 37°C with 5 mM EDTA in PBS following at least 48 hr of growth after passage. After two washes with fresh culture medium, cells were resuspended in binding medium (RPMI 1640 medium/10% BSA/0.1% sodium azide/20 mM Hepes, pH 7.4). Binding incubations with ^{125}I-labeled IL-1α with or without unlabeled IL-1α were performed as described (8). Data analysis and curve fitting were done using RS/1 (Bolt, Beranek, and Newman) and a VAX 11/750 computer running under the VMS operating system.

Prostaglandin and CSF Assays. Confluent cultures of CHO cells (10^6 cells per 35-mm dish) were washed with medium and then treated with various concentrations of human IL-1α for the times indicated. Conditioned medium was filtered (0.45 μm) and assayed for prostaglandin E_2 (PGE_2) levels using a commercially available RIA kit (New England Nuclear). PGE_2 concentrations were expressed per mg of cellular protein by determining the protein concentration for an aliquot of harvested cells. The conditioned medium was also assayed for its capacity to induce mouse bone marrow cell proliferation (3). In all proliferation assays, one unit is defined to be the amount of sample necessary to stimulate to half the maximal thymidine incorporation observed with the samples. Time courses and IL-1 dose–response curves were reproducible, but the absolute number of units/ml varied between assays, and in the absence of hamster CSF standards, the number of units/ml were calculated based on sample values. The control supernatant, which always gave values close to background, was medium plus 10 nM human IL-1α.

Northern Blot Analysis. Confluent cultures of CHO(SVNeo) and CHO(IL-1R) cells were washed with Ham's F-12 medium and treated for 16 hr with medium containing 10 pM IL-1α or medium alone. Total RNA was prepared (18) and 20 μg per lane was loaded on a 1% formaldehyde/agarose gel (19). After electrophoresis, RNA was blotted onto Hybond-N (Amersham), the filter was hybridized at 50°C in 50% (vol/vol) formamide/1% $NaDodSO_4$/5× saline sodium citrate (SSC)/50 mM potassium phosphate, pH 6.5/Ficoll (1 mg/ml)/BSA (1 mg/ml)/polyvinylpyrolidone (1 mg/ml)/salmon sperm DNA (300 μg/ml)/0.05% sarcosyl, with an SP6 RNA polymerase transcript of a human G-CSF cDNA (5×10^5 cpm/ml; specific activity, 10^8 cpm/μg), and washed at 60°C with 0.1× SSC/1% $NaDodSO_4$. (1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0.)

RESULTS

Expression of Murine IL-1R in CHO Cells. The parental CHO-K1 cell line, as well as CHO cells selected in bulk for G418 resistance after transfection with pSV2Neo [CHO(SVNeo)] or cotransfection with pSV2Neo and control pDC201 vector [CHO(pDC201)], bound human IL-1α with a K_a of 3×10^9 M^{-1} and had very low levels of IL-1Rs per cell [30 ± 10 IL-IRs per cell (n = 6), Fig. 2*B*]. CHO cells cotransfected with pSV2Neo and pDC201 containing the IL-1R cDNA had ≈7000 IL-1Rs per cell after G418 selection, and, after cell sorting with FITC-conjugated IL-1α, CHO(IL-1R) cells had ≈100,000 IL-1Rs per cell that bound IL-1α with a K_a of 3×10^9 M^{-1} (Fig. 2*A*).

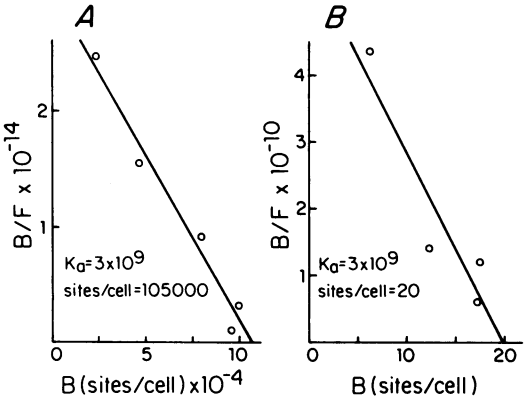

FIG. 2. Comparison of ^{125}I-labeled IL-1α binding to CHO(IL-1R) and CHO(SVNeo) cells. CHO(IL-1R) (*A*) and CHO(SVNeo) (*B*) cells were prepared, selected, and harvested. Cells were incubated with 1–30×10^{-9} M ^{125}I-labeled IL-1α for 2 hr at 4°C in binding medium, and duplicate aliquots of cells with bound IL-1 were separated from free IL-1 by centrifugation through a phthalate oil mixture (8). Nonspecific binding in the presence of a 100-fold molar excess of unlabeled IL-1α was subtracted from the total binding, the specific binding was converted to the number of molecules per cell, and this number was plotted as a least squares fit in the Scatchard coordinate system. B, bound IL-1α; F, free IL-1α.

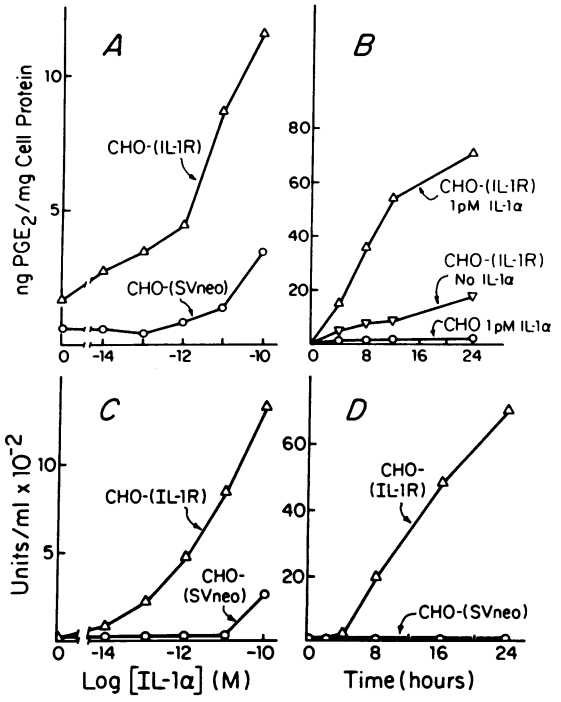

FIG. 3. Comparison of IL-1α-induced prostaglandin and CSF production by CHO(IL-1R) and CHO(SVNeo) cells. Dose–response curves for IL-1 induction of PGE_2 (*A*) and CSF (*C*). Confluent cultures containing equivalent numbers of cells were washed with medium and treated with the indicated concentrations of IL-1α for 8 hr (*A*) or 24 hr (*C*). Time course of IL-1 induction of PGE_2 (*B*) and CSF (*D*). Confluent cultures were treated with 1 pM (*B*) or 10 pM (*D*) IL-1α for the times indicated. Medium was harvested and assayed for PGE_2 production by using an RIA standardized with synthetic PGE_2 and values were corrected for total cellular protein, by determining the protein concentration of an aliquot of harvested cells. Conditioned medium was assayed for CSF production by a mouse bone marrow cell proliferation assay, and the results are expressed as units/ml. Individual points represent the data from a single representative experiment that was repeated on two cell lines with similar results.

IL-1 Induction of PGE_2 Production in CHO(SVNeo) and CHO(IL-1R) Cells. Fig. 3*A* compares the dose–response curves for IL-1-induced PGE_2 production from CHO(IL-1R) and CHO(SVNeo) cells. CHO(SVNeo) cells demonstrated a stimulation of basal PGE_2 production by 10 pM IL-1α and a substantial increase by 100 pM. In contrast, CHO(IL-1R) responded to 10 fM IL-1α. The CHO(IL-1R) cell dose–response curve demonstrates a 1000-fold increase in sensitivity to IL-1α compared to CHO(SVNeo) cells. Fig. 3*B* shows the time course of PGE_2 production in CHO(IL-1R) cells in response to 1 pM IL-1α. IL-1-dependent PGE_2 production in CHO(IL-1R) cells was detected at the earliest time point (4 hr) and began to plateau at 24 hr (data not shown). No stimulation of PGE_2 production was seen in CHO(SVNeo) cells with 1 pM IL-1α. There was significantly higher PGE_2 production from CHO(IL-1R) cells in the absence of IL-1α than from CHO(SVNeo) cells.

IL-1 Induction of CSF Production in CHO(SVNeo) and CHO(IL-1R) Cells. CSF production induced by IL-1 was monitored by a mouse bone marrow cell proliferation assay. In CHO(SVNeo) cells the minimal IL-1α concentration for stimulation of CSF production was 10 pM (n = 6, Fig. 3*C*). The concentration of IL-1α required for half of the maximum CSF production observed (the EC_{50}) was $9 \pm 1.2 \times 10^{-10}$ M (n = 7). In contrast to control cell lines, two independently derived lines of CHO(IL-1R) cells produced CSF in response to 10 fM IL-1α (n = 7, Fig. 3*C*). As the IL-1α concentration was increased, the induction of CSF production in CHO(IL-1R) cells had two components. The initial IL-1-induced increase in CSF (Fig. 5*C*) plateaued between 0.1 and 1 nM. The concentration of IL-1α required for half of the initial component of IL-1-induced CSF production was $2 \pm 0.8 \times 10^{-12}$ M (n = 4).

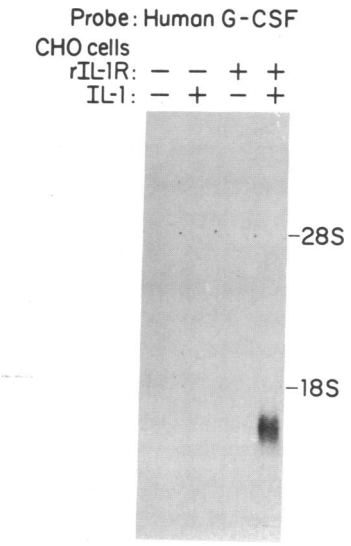

FIG. 4. Induction of G-CSF mRNA in CHO(IL-1R) and CHO-(SVNeo) cells. CHO(IL-1R) (+ IL-1R) and CHO(SVNeo) (− IL-1R) cells (5×10^8 cells) were washed and cultured for 16 hr with (+) or without (−) 10 pM IL-1α as indicated. Total RNA was prepared and a Northern blot analysis was performed. The autoradiogram is of a filter washed at 60°C in 0.1× SSC. The signal is diminished but remains present after washing at 63°C in 0.1× SSC.

Fig. 3*D* shows the time course of CSF production in CHO(IL-1R) cells. In the presence of 10 pM IL-1α, CSF production was detected in some assays after 2 hr and was always apparent after 4 hr. CSF levels were maximal at 24 hr and slowly declined after 2 or 3 days of treatment (data not shown). In CHO(SVNeo) cells stimulated with 10 pM IL-1α, CSF activity was not detectable after 24 hr. The CSF activity measured in the mouse bone marrow assay was not due to IL-1 synergism with a constitutively produced CSF, as addition of 10 nM IL-1α to medium conditioned without IL-1 from CHO(IL-1R) or CHO(SVNeo) cells caused no bone marrow cell proliferation.

Comparison of G-CSF Induction by IL-1 in CHO(SVNeo) and CHO(IL-1R) Cells. To identify a specific CSF induced by IL-1 in CHO(IL-1R) cells, total RNA was prepared from CHO(IL-1R) and CHO(SVNeo) cells after 16 hr of culture with or without 10 pM IL-1α, conditions that gave a clear difference in cytokine induction (Fig. 3*D*). Total RNA was analyzed by probing Northern blots for RNAs of several human and mouse cytokines. A human G-CSF RNA probe hybridized to a 1.7-kilobase mRNA after moderately high-stringency washes (63°C, 0.1× SSC). This RNA is similar in size to the 1.5-kilobase mRNA reported for mouse G-CSF (20). G-CSF mRNA was present in CHO(IL-1R) cells stimulated with IL-1, but not in unstimulated CHO(IL-1R) or in CHO(SVNeo) cells with or without stimulation by 10 pM IL-1 (Fig. 4). To determine whether G-CSF mRNA was also translated, conditioned medium from CHO(IL-1R) cells stimulated for 24 hr with 10 pM IL-1α was treated with an affinity-purified polyclonal antiserum to human G-CSF. The anti-G-CSF antiserum neutralized up to 90% of the CSF activity. The same antiserum inhibited 96% of CSF activity produced by human G-CSF (2 μg/ml) added to the assay (data not shown). Thus, stimulation of CHO(IL-1R) cells with low concentrations of IL-1α induced G-CSF mRNA and stimulated secretion of G-CSF activity. With the same

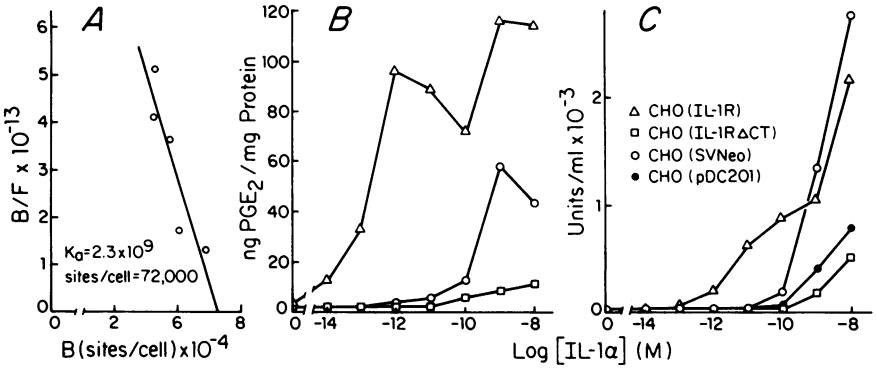

FIG. 5. Binding and responses in CHO(IL-1RΔCT) cells. (*A*) CHO(IL-1RΔCT) cells were incubated with ^{125}I-labeled IL-1α, bound IL-1 was separated from free IL-1, and the specific number of molecules of IL-1α bound per cell was expressed as a Scatchard plot. (*B* and *C*) Confluent cultures of 10^6 cells in 35-mm dishes were washed and stimulated with the indicated concentration of IL-1α. Medium was harvested after 8 hr and assayed for PGE_2 (*B*) or harvested after 24 hr to assay for CSF activity in the bone marrow cell proliferation assay (*C*). CHO(IL-1RΔCT) cells are shown with CHO(IL-1R), CHO(SVNeo), and CHO(pDC201) cells assayed in the same experiment for comparison. Points represent data from a single representative assay that was repeated twice on two CHO(IL-1RΔCT) cell lines with similar results.

concentration of IL-1α, CHO(SVNeo) cells produced no detectable G-CSF mRNA or significant cytokine activity. Although both experiments utilized human reagents to detect hamster G-CSF, G-CSF is highly conserved among CSFs with 70% identity between murine and human G-CSF at both the nucleotide and amino acid sequence level (20). No RNA was detected by using as probes cDNA clones encoding murine IL-6, murine IL-7, murine GM-CSF, and human CSF-1.

Comparison of IL-1 Responsiveness in CHO(IL-1R) and CHO(IL-1RΔCT) Cells. To prove that the increase in response to IL-1 of the CHO(IL-1R) cells was due to signal transduction by the recombinant receptor, we cotransfected CHO cells with pSV2Neo and an IL-1R cDNA construct whose cytoplasmic domain had been deleted to give CHO(IL-1RΔCT) cells. All IL-1 binding properties are maintained in constructs containing the N-terminal 316 amino acids of the receptor (S.K.D. and J.E.S. unpublished observations). After sorting the CHO(IL-1RΔCT) cells expressed 70,000–100,000 receptors per cell that bound IL-1α with a K_a of 2.2 × 10^9 M^{-1}, similar to CHO(IL-1R) cells (compare Figs. 5*A* and 2*A*). In Fig. 5 the responses of CHO control, CHO(IL-1R), and CHO(IL-1RΔCT) cells to IL-1 for PGE_2 and CSF secretion are shown. Despite the presence of a high level of IL-1 binding sites, the CHO(IL-1RΔCT) cells were poorly responsive to IL-1. Indeed, comparison of the effect of IL-1 on these cells with that on the control cell lines showed that expression of the truncated receptor led to inhibition of the IL-1 response. The extent of inhibition was dependent on whether CHO(SVNeo) or CHO(pDC201) are used for comparison. At 10 nM IL-1α, CSF production in CHO(IL-1RΔCT) cells was 56 ± 25% (n = 3) and 90 ± 10% (n = 3) of that in CHO(SVNeo) and CHO(pDC201) cells, respectively. Similar relative extents of inhibition were seen for the PGE_2 response.

DISCUSSION

The full IL-1 binding activity of the murine T-cell IL-1R resides within a M_r 80,000 cell surface glycoprotein (11, 12). To determine whether this molecule is also capable of transducing the IL-1 transmembrane signal, we have performed transfection experiments with CHO cells. A line expressing ≈100,000 IL-1 binding sites per cell was isolated by fluorescence-activated cell sorting from CHO cells tranfected with a vector encoding the IL-1R. CHO cells transfected with control vector yielded lines with few IL-1 receptors (50–150 sites per cell).

In fibroblasts, IL-1 induces GM-CSF, G-CSF, IL-6 (21–23), and cyclooxygenase, (24) the synthesis of which accounts for IL-1 induction of PGE_2 formation (21, 24). To test IL-1-mediated signal transduction by the recombinant receptor in CHO cells, we selected PGE_2 and cytokine production as two physiological responses to IL-1. Comparison of IL-1 induction of PGE_2 production from CHO(SVNeo) and CHO(IL-1R) cells revealed that a 1000-fold increase in IL-1R expression (to 100,000 receptors per cell) resulted in a 1000-fold increase in sensitivity to IL-1. With 1 pM IL-1α, CHO(IL-1R) cells demonstrated a time-dependent increase in PGE_2 levels in conditioned medium similar to the time course of IL-1 action on diploid fibroblasts (24), whereas there was no significant response from control cells. The analysis of CSF production showed that low concentrations of IL-1α (10 pM) produced a time-dependent accumulation of G-CSF in medium conditioned by CHO(IL-1R) cells, while the same conditions generated insignificant levels of activity in control cells. Similar results were found for RNA, as G-CSF mRNA was detectable only in CHO(IL-1R) cells stimulated with IL-1. This is consistent with the observation that induction of GM-CSF, G-CSF, and IL-6 activity in fibroblasts and endothelial cells correlates in each case with increased levels of the corresponding mRNA, and, where examined, the higher mRNA levels are apparently the result of an increased rate of gene transcription (22, 23, 25).

Based on the total number of receptors and the observed K_a and EC_{50}, CHO(SVNeo) cells require occupancy of 7 receptors per cell to show a response and of 22 receptors per cell to show a half-maximal response. CHO(IL-1R) cells with 100,000 receptors respond when 3 receptors per cell are occupied and require occupancy of 500 receptors per cell for 50% of the response seen at 1 nM. CHO(IL-1R) cells thus have 99,000 spare receptors per cell, and the increased sensitivity of these cells can be accounted for only if all of the recombinant receptors are functional in signal transduction. A number of biological systems express spare receptors that allow generation of a biological response at ligand levels several orders of magnitude below the receptor affinity constant (26), including IL-1 induction of IL-2 production (10).

Results with CHO(IL-1R) and CHO(SVNeo) cells did not absolutely eliminate the possibility that expression of the recombinant receptor simply increased the efficiency of signal transduction by the endogenous CHO receptors, for example, by increasing the cell surface concentration of IL-1 above that in the medium. [For a more detailed discussion of this phenomenon, see Abbott and Nelsestuen (27).] To address this issue an IL-1R variant, truncated 23 residues

beyond the putative membrane-spanning region, was expressed in CHO cells. The predicted large cytoplasmic domain of the IL-1R is highly conserved between human and mouse (unpublished observation), suggesting that it has an important role in IL-1R function. We reasoned that the deletion of the cytoplasmic domain might eliminate coupling to IL-1 responses without influencing IL-1 binding. Analysis of the truncated receptor in CHO(IL-1RΔCT) cells established that IL-1 binding to the extracellular portion of the recombinant receptor was not sufficient to modulate responsiveness and that the increased sensitivity of CHO(IL-1R) was not due to presentation of IL-1 to endogenous receptors. High-level expression of the truncated receptor inhibited the endogenous response of the CHO cells to IL-1, rather than increasing the sensitivity of these cells. Expression of nonfunctional insulin receptors created by mutations that abolish kinase activity also inhibit the endogenous response of CHO cells to insulin (28, 29). When the extracellular portion of the insulin receptor is fused to the transmembrane and cytoplasmic domain of the bacterial aspartate chemoreceptor, the resulting chimera is also nonfunctional and inhibits endogenous CHO insulin receptor responses (30). These results are consistent with the formation of aggregates of the extracellular domains of endogenous and nonfunctional insulin receptors that are not effective in transmembrane signaling. Both the native IL-1R from EL-4 C10 cells and recombinant IL-1R expressed in C-127 cells have an apparent molecular weight of ≈200,000 by gel filtration after solubilization consistent with a dimeric form of the intact receptor (B.M.C. and S.K.D., unpublished observation). Thus, in CHO(IL-1RΔCT) cells association of the extracellular domains of truncated and endogenous receptors might create nonfunctional dimers, inhibiting IL-1 signaling. Understanding of this inhibitory effect will require a more detailed examination of the importance of receptor aggregation and internalization in IL-1R signal transduction.

Comparison of the IL-1 responses in CHO(IL-1R) and CHO(IL-1RΔCT) cells clearly established the importance of the cytoplasmic domain of the IL-1R for signal transduction. The data presented also indicate that expression of the full-length recombinant receptor polypeptide is sufficient to mediate IL-1 signal transduction in the context of normal cellular proteins. Our results strongly support the conclusion that the IL-1R cDNA encodes the entire functional IL-1R. Demonstration of a system where low IL-1 concentrations mediate signal transduction through the recombinant receptor will allow a more detailed analysis of the functional roles of various domains of the receptor by mutagenesis, as well as studies on the mechanism by which the receptor regulates the transcription of IL-1-inducible genes.

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