Cloning and Characterization of an Alternatively Processed Human Type II Interleukin-1 Receptor mRNA*

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Two types of interleukin (IL)-1 receptors with three extracellular immunoglobulin-like domains, limited homology (28%), and different pharmacological characteristics termed type I and type II have been cloned from mouse and human cell lines. Both receptors exist in transmembrane and soluble forms; the soluble IL-1 receptor is thought to be post-translationally derived from cleavage of the extracellular portion of the membrane receptors. In preliminary cross-linking studies with radiolabeled IL-1, we found that monkey kidney COS1 cells express a soluble receptor with molecular mass of ~55–60 kDa, which is different from previously reported soluble IL-1 receptors. This soluble IL-1 receptor protein from COS1 cells was purified to homogeneity by affinity chromatography using recombinant IL-1β as the ligand and shown to have an affinity for human 125I-IL-1β (Kd ~2–3 nm) comparable to the human type II IL-1 receptor (IL-1RII). The purified protein was microsequenced, and the sequence information was used to design primers to clone the COS1 IL-1RII using reverse transcription-coupled polymerase chain reaction; the DNA comparison with monkey COS1 and human IL-1RII indicate that they are 95% identical at the nucleic acid and amino acid levels. In addition, another cdNA, which represents an alternatively processed mRNA of the IL-1RII gene, was also cloned both from monkey COS1 and human Raji cells and was shown to have ~95% sequence identity between these species. While the cDNA of the novel alternatively processed gene has a 5′ end identical to the IL-1RII, the 200 base pairs at the 3′ end are different and the sequence predicts a soluble IL-1 receptor protein of 296 amino acids. Radioligand binding studies of the alternatively processed IL-1RII mRNA demonstrated kinetic and pharmacological characteristics similar to the known type II IL-1 receptor. COS7 cells (which lack IL-1 receptor) transfected with the transmembrane form of the human IL-1RII cDNA showed 125I-IL-1β binding in both the membrane fractions and supernatant. In contrast, COS7 cells transfected with the alternatively processed human IL-1RII cDNA showed high affinity 125I-IL-1β binding (Kd ~1.2 nm) predominantly in the supernatant; a very small amount of detectable membrane IL-1 binding activity was also observed presumably due to association of the soluble IL-1 receptor and membrane-integrated proteins. In cross-linking and ligand blot studies, the alternatively processed human IL-1RII cDNA-transfected COS7 cells expressed a soluble IL-1 receptor with molecular masses ranging from 60 to 160 kDa, further indicating the association between this soluble IL-1 receptor and other soluble proteins. In summary, we report the purification and characterization of a soluble IL-1 receptor expressed by COS1 cells and the cloning of an alternatively processed type II IL-1 receptor mRNA from both human and COS1 cells. The alternative splicing of a primary transcript leading to a secreted protein provides a potentially important mechanism by which soluble IL-1RII can be produced.

Interleukin 1 (IL-1)β is a hormone-like polypeptide that performs many roles in inflammation and immunity (1–3). Currently, two forms of IL-1 (IL-1α and IL-1β) and one IL-1 receptor antagonist (IL-1ra) have been characterized (1). IL-1α and IL-1β (collectively referred to as “IL-1”) and IL-1ra elicit their biological effects by binding to specific receptor molecules on the surface of responsive cells. Two types of IL-1 receptors with three extracellular immunoglobulin-like domains, limited homology (28%), and different pharmacological characteristics termed type I (4, 5) and type II (6) have been cloned from mouse and human cell lines. IL-1α, IL-1β, and IL-1ra all bind with comparable affinity to the type I IL-1 receptor (IL-1RI), which is expressed mainly on T cells, fibroblasts, keratinocytes, endothelial cells, synovial lining cells, chondrocytes, hepatocytes, brain, and endocrine tissues (1, 5, 7). On the other hand, IL-1β binds with much higher affinity and selectivity to the type II IL-1 receptor (IL-1RII), which is expressed mainly on neutrophils and B cells, including the Raji human B cell lymphoma line (6, 8, 9). Functional characterization studies have indicated that the two receptors exert different effects. While the type I IL-1 receptor is a signal transducing molecule for IL-1-1 (10, 11), the type II IL-1 receptor is thought to be a decoy receptor (6, 12). Very recently, a third member of the IL-1 receptor family (designated as IL-1 receptor accessory protein; IL-1RaCP), which has limited homology to both type I and type II receptors, has been cloned from mouse (13) and rat (14) cells. The IL-1RaCP forms a complex with type I IL-1 receptor and either IL-1α or IL-1β but not with IL-1ra and increases the binding affinity of IL-1β for type I IL-1 receptor when the two proteins are co-expressed (13).

The IL-1RII exists in both membrane and soluble forms (6). The soluble form of IL-1RII, a glycoprotein with molecular mass ~45 kDa, is thought to be post-translationally derived

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1 The abbreviations used are: IL-1, interleukin-1; IL-1ra, IL-1 receptor antagonist; IL-1RI and IL-1RII, IL-1 receptor I and II, respectively; RT-PCR, reverse transcription-coupled polymerase chain reaction; PAGE, polyacrylamide gel electrophoresis; DMEM, Dulbecco’s modified Eagle’s medium; PBS, phosphate-buffered saline; BSA, bovine serum albumin; RACE, rapid amplification of cdNA ends.
from cleavage of the membrane form (12). In preliminary cross-linking studies with radiolabeled IL-1, we found that monkey kidney COS1 cells, a commonly used cell line for transient gene expression, express a soluble receptor with molecular mass of ~55–60 kDa, significantly larger than the reported soluble type II IL-1 receptor (12). In the present study, we purified the soluble IL-1 receptor expressed in COS1 cells and cloned a novel alternatively processed type II IL-1 receptor mRNA from both COS1 and human cells.

**EXPERIMENTAL PROCEDURES**

**Human IL-1β Expression and Purification**

The human IL-1β mature peptide coding sequence with one extra methionine codon at the N terminus was amplified by reverse transcription-coupled polymerase chain reaction (RT-PCR) using primers (P1, 5′-GCC ATG GCA CCT GTA CGA TCA CTG-3′; P2, 5′-TTT GCC CAG CCC TAG GGA TTG AGT-3′) derived from the human IL-1β cDNA sequence (15). The amplified cDNA was sequenced and cloned into a prokaryotic expression vector pET-21-d (Novagen) and transfected into Escherichia coli BL21(DE3) (Stratagene). A single colony was inoculated in 1 liter of LB broth containing 50 μg/ml ampicillin and cultured at 37°C with vigorous shaking. Isopropyl-1-thio-galactopyranoside was added to the cell culture to a final concentration of 0.5 mm when the cell culture reached an OD of 1.0 (at 600 nm), and the cells were cultured for an additional 2 h. The cell mixture was centrifuged at 5000 × g for 30 min. The cell pellet was resuspended in 5 ml of phosphate buffer (40 mM KCl, 10 mM Na3HPO4, 2 mM KH2PO4, pH 7.4) containing 5 mM EDTA. The cell suspension was frozen and thawed twice, then resuspended in 5 ml of pH 7.4 phosphate buffer containing 5 mM EDTA, 0.2% Triton X-100, and 200 μM l-lysozyme (Sigma). The suspension was mixed gently and incubated at 37°C for 20 min or until the cell lysate became clear. The cell lysate was placed on ice and sonicated for 5 min to break down the bacterial chromosomal DNA and decrease the viscosity of the cell lysate. The mixture was then added to 100 ml of 30 mM sodium citrate buffer at pH 3.5 containing 5 mM EDTA with gentle stirring. Two hundred milliliters of phosphate buffer was added to the mixture, and the pH was adjusted to 5.0. The mixture was centrifuged at 4°C, 10,000 × g for 30 min, and the supernatant was collected. Twenty milliliters of SP-Sepharose medium (Pharmacia Biotech Inc.) pre-equilibrated with phosphate buffer (at pH 5.0) was then added to the mixture, and the pH was adjusted to 5.0. The mixture was centrifuged at 4°C, 10,000 × g for 30 min with gentle agitation. The mixture was centrifuged 1000 × g at room temperature for 5 min, and the supernatant was discarded. The SP-Sepharose medium was loaded onto a column and washed extensively with phosphate buffer at pH 5.0 and eluted with 30 ml of phosphate buffer adjusted to pH 8.0. The eluant was then directly passed through the DEAE-Sepharose (Pharmacia Biotech Inc.) pre-equilibrated with flow-through fractions, which contain the recombinant human IL-1β purified to homogeneity. The sample was run on a 4–20% SDS-PAGE under non-reducing conditions and then transferred to a nitrocellulose membrane. The band of protein that corresponded to the IL-1β in the IL-1β affinity column preparation was excised from the x-ray film overnight at 70°C with an intensifying screen. The band was excised from the x-ray film overnight at 70°C with an intensifying screen.

**IL-1β Affinity Column Preparation**

Human recombinant IL-1β purified as described above was cross-linked to a CNBr-activated Sepharose-4B matrix (Pharmacia) as described by the manufacturer.

**Ligand Receptor Affinity Cross-linking**

Monkey kidney COS1 cells cultured under serum-free conditions (DMEM with 10 mM Hepes, 50 units/ml ampicillin and streptomycin) at 37°C and 5% CO2, were centrifuged at 4°C, 10,000 × g for 1 h, and the supernatant was concentrated 20-fold with Centricon-30 (Amicon). One hundred microliters of concentrated supernatant was incubated with 100 pm 125I-labeled human recombinant IL-1β (125I-IL-1β) (DuPont NEN, specific activity of ~1800 Ci/mmol), either in the presence or absence of 100 nM unlabeled competitors (recombinant human IL-1β, recombinant human IL-1α, or recombinant human IL-1α, at 4°C overnight. The cross-linker ethylene glycol bis succinimidyl succinate (Pierce) was then added to the mixture at a final concentration of 2 mM, and the reaction mixture was incubated at room temperature for an additional 20 min. The reaction mixture was then run on to a 4–20% SDS-PAGE under reducing conditions and the gel was dried and exposed to a x-ray film overnight at ~70°C with an intensifying screen (Kodak).

**Soluble IL-1 Receptor Purification**

COS1 cells were cultured in serum-free DMEM supplemented with glutamine, sodium pyruvate, penicillin, streptomycin, and 15 mM Hepes. The cell culture medium was collected and fresh medium was added every 2 days. In total, 2 liters of medium were collected. The medium was centrifuged at 4°C, 10,000 × g for 1 h and then passed through the human IL-1β affinity column at a rate of 20 ml/h. The column was first washed with PBS plus 0.1% Triton X-100, then washed with PBS alone, and eluted with a 0.5–4 M guanidine HCl gradient, and 1 ml of fractions were collected. Aliquots (5 μl) of eluant from each fraction were directly spotted on to a dry nitrocellulose membrane (Schleicher & Schüll), blocked with 20 μl Tris-HCI, pH 7.5, 0.15 μl NaCl, 0.05% Tween 20 (TBST), 1% BSA, and blotted with human 125I-IL-1β as described above. Fractions with human 125I-IL-1β-binding activity were then pooled and dialyzed against PBS buffer then against water overnight at 4°C. The dialyzed sample was then lyophilized and redissolved in 200 μl of water. An aliquot (5 μl) was used for the IL-1β ligand blot in order to determine the recovery of the soluble IL-1 receptor. Aliquots (20 μl) were run on a 4–20% SDS-PAGE under non-reducing conditions. The gel was then stained with Coomassie Brilliant Blue. The band of protein that corresponded to the IL-1β-binding activity was sequenced by the Edman Degradation method (Protein Chemistry Laboratory, UC Davis).

**Ligand Blot**

The sample was first run on a 4–20% SDS-PAGE under non-reducing conditions and then transferred to a nitrocellulose membrane (Schleicher & Schüll). The membrane was blocked with TBST containing 1% BSA at room temperature for 30 min. The blocked membrane was incubated at 4°C overnight with gentle agitation either in the presence or absence of 100 nM human unlabeled IL-1β with TBST containing 1% BSA and 30 μm human 125I-IL-1β. The membrane was then washed four times (5 min each) with ice-cold TBST and exposed to a x-ray film overnight at ~70°C with an intensifying screen.

**Cell Transfection**

COS7 cells were cultured in six-well cell culture dishes (3.5 cm) with DMEM containing 10% fetal calf serum and transfected using LipofectAMINE (Life Technologies, Inc.) as described by the manufacturer.

**Whole Cell Binding Assay**

The cell culture medium was removed, and the cells in 3.5-cm cell culture dishes were directly incubated with 1 ml of DMEM, 15 mM Hepes buffer, plus 1% BSA and 60 μM 125I-IL-1β, either in the presence or absence of 200 μM unlabeled IL-1β at room temperature for 2 h and washed three times with ice-cold PBS. The cells were then lysed with 4x guanidine HCl, and the cell lysate was counted in a γ counter (Packard).

**Solid Phase Binding Assay for Soluble IL-1 Receptor**

Twenty-fold concentrated serum-free cell culture supernatant (concentrated by Centricron-30) was incubated in a 96-well plate (100 μl) with high protein-binding, Costar) at 4°C for 2 h, and the medium aspirated. The cell pellets were washed three times with PBS at 4°C for 2 h followed by two washes with PBS. The plates were then incubated with 100 μl/well of DMEM containing 1% BSA with various concentrations of human 125I-IL-1β or human 125I-IL-1α (1 μl to 20 nm) either in the presence or absence of 500 μl unlabeled IL-1β at 4°C overnight. The binding medium was then aspirated, and the wells were washed three times with 200 μl of ice-cold PBS containing 0.1% Triton X-100. The bound 125I-IL-1β was then eluted from wells by adding 200 μl of 2% SDS, and samples were counted in a γ counter.
Alternatively Processed Type II IL-1 Receptor

AGG ACA CAG-3′) corresponding to portions of the human IL-1RII cDNA sequence (6) were synthesized and used to amplify a cDNA pool made from COS1 total RNA using a oligo(dT)-adapter primer (5′-GAC TCG ACT GAG CAT CAG TTT TTT TTT TTG TTT T-3′) (17) for reverse transcription. The resultant cDNA fragment was cloned, sequenced, and shown to have 95% DNA sequence identity to human IL-1RII cDNA, suggesting that this cDNA fragment is COS1 cell IL-1RII cDNA. One oligonucleotide (P3, 5′-GAA CGG GTG CTC TGT GCT TCT G-3′) designed from the cloned COS1 IL-1RII cDNA fragment sequence was used to amplify the 3′ end of COS1 IL-1RII cDNA together with the 3′ end adapter primer (5′-GAC TCG ACT GAG CAT CG-3′) described as 3′ RACE by Frohman (17). Two different clones with identical 5′ end regions (about 600 base pairs) were identified. One clone has 95% overall homology to human IL-1RII cDNA and is designated as COS1 mL-1RII. The second clone, which has a different 3′ end region, is designated as COS1 sL-1RII.

The complete coding sequence for COS1 mL-1RII was amplified using two primers: 5′ primer (5′-CTC TGG AAG TTG TCA GGA GCA ATG-3′; P4) was derived from the published human IL-1RII cDNA sequence (6), and 3′ primer (5′-CAT GTG TGA TGT GGG TCA TAG TG-3′; P5) was designed to flank the 3′ end of COS1 mL-1RII cDNA (Fig. 1). The complete coding sequence for COS1 sL-1RII was amplified using two primers: 5′ primer (5′-CTC TGG AAG TTG TCA GGA GCA ATG-3′; P4) and 3′ primer (5′-CAT GTG TGA TGT GGG TCA TAG TG-3′; P6), which was derived from the 3′ end sequence of COS1 sL-1RII cDNA cloned by 3′ RACE.

Cloning of the Human Soluble IL-1RII cDNA—Human soluble IL-1RII (hsIL-1RII) cDNA containing the complete coding sequence was amplified using two primers: 5′ primer (5′-CTC TGG AAG TTG TCA GGA GCA ATG-3′; P4) and 3′ primer (5′-CAT GTG TGA TGT GGG TCA TAG TG-3′; P6) were derived from the published human IL-1RII cDNA sequence (6). The complete coding sequence for COS1 mL-1RII cDNA was derived from the published human membrane IL-1RII cDNA sequence (6); 3′ end primer (5′-CAT GTG TGA TGT GGG TCA TAG TG-3′; P6) was derived from COS1 mL-1RII cDNA sequence. The complete coding sequence for human membrane IL-1RII (hml-1RII) was amplified using primers: 5′ end primer (5′-CTC TGG AAG TTG TCA GGA GCA ATG-3′; P4) and 3′ end primer (5′-CAT TCC ATT TAT TTC ACT TGG GAT AGG-3′; P7), which was derived from the published human IL-1RII cDNA sequence (6).

**RESULTS**

Preliminary Characterization of the Soluble IL-1 Receptor Expressed by COS1 Cells—Monkey kidney COS1 cells were found to express a soluble IL-1 receptor as determined by radioligand receptor affinity cross-linking studies with 125I-IL-1b. Since the molecular mass of cross-linked 125I-IL-1b-IL-1 receptor complex is ~75 kDa (Fig. 1) and the molecular mass of IL-1b is ~17–18 kDa, the estimated molecular mass of this soluble receptor is 55–60 kDa. This soluble IL-1 receptor bound 125I-IL-1b and could be displaced by 100 nM unlabeled IL-1b or IL-1α but not appreciably by IL-1α (Fig. 1). We also examined the IL-1 receptor expressed intracellularly and on the cell surface of COS1 cells. COS 1 cells were lysed and IL-1 receptors were precipitated with IL-1b-Sepharose 4B as described under "Experimental Procedures." The precipitated products were analyzed by a 12% SDS-PAGE gel. Several specific bands (i.e., displaced by 100 nM unlabeled human IL-1b) with molecular masses of 60, 85, and 120 kDa were seen in the ligand blot assay (Fig. 2). Human 125I-IL-1α was also used in a parallel ligand blot assay under similar conditions, but no detectable signal was seen (data not shown).

Purification and Characterization of the Soluble IL-1 Receptor Expressed by COS1 Cells—A recombinant human IL-1α affinity column was prepared and COS1 cell culture medium was passed through the column in order to identify proteins with affinity for IL-1α. After passage of the cell culture medium, the column was washed with PBS containing 0.1% Triton X-100, followed by PBS, and the bound protein was eluted with a 0.5–4 M guanidine HCl gradient. The eluted fractions were directly spotted on a nitrocellulose membrane and blotteded with 125I-IL-1b. The fractions (nos. 13–20) with highest IL-1b-binding activity were pooled, and an aliquot of the pool (20 μl) was run on a 4–20% SDS-PAGE under reducing conditions. The gel was dried and exposed to x-ray film overnight.
ligand, no binding activity was seen (Fig. 3B). Human recombinant soluble type I IL-1 receptor (hslL-1RI) was included as a control in both ligand blot assays. The results demonstrated that, while hslL-1RI bound both 125I-IL-1α and 125I-IL-1β, the purified soluble receptor has a strong preference for 125I-IL-1β.

A solid phase binding assay was also performed using 125I-IL-1α and 125I-IL-1β. The binding of 125I-IL-1β to the purified soluble IL-1 receptor was saturable and of high affinity with a K_D value of 2.8 nM (Fig. 3C). The purified soluble IL-1 receptor also binds human 125I-IL-1α, but with a significantly lower affinity compared with that of human 125I-IL-1β.

A sample of the purified soluble IL-1 receptor was lyophilized, redissolved in 200 μl of water, run onto a 4–20% SDS-PAGE, and stained with Coomassie Brilliant Blue. The protein band corresponding to the IL-1-binding activity was then sequenced as described under “Experimental Procedures.” The protein sequence indicated that among 21 amino acids sequenced, 20 amino acids were identical to the human type II IL-1 receptor (underlined in Fig. 4). The Phe at position 132 in the human IL-1RII was replaced by a Ser in the soluble IL-1 receptor purified from COS1 cell culture supernatant. These results indicated that COS1 cells express type II IL-1 receptor.

cDNA Cloning and Characterization of Alternatively Processed IL-1RII mRNAs from COS1 Cells and Human Raji Cells—We cloned a cDNA fragment corresponding to this IL-1 receptor using RT-PCR from COS1 cells as described under “Experimental Procedures.” The resultant 427-base pair DNA fragment was sequenced and showed 95% sequence identity to human IL-1RII cDNA. In addition, the soluble IL-1 receptor protein sequence obtained from the purification and microsequencing studies matched the deduced amino acid sequence from the DNA fragment, including a Ser, which is different from the human IL-1RII sequence. These results indicated that we had cloned an IL-1RII cDNA fragment from COS1 cells, and that the purified protein was the soluble type II IL-1 receptor.

The 3' end of the COS1 cell IL-1RII cDNA was cloned using the 3' RACE approach (17). Two different cDNA clones with identical 5' regions but different 3' regions were identified. One of these clones had an overall sequence identity of 95% to human IL-1RII cDNA spanning the entire coding region. The second clone had a 5' end region that was homologous to human IL-1RII cDNA, but the 3' region was different. Using a 5' primer derived from the human IL-1RII cDNA sequence and two 3' end primers (one from each clone described above; for details see “Experimental Procedures”), the complete coding sequences for the two different clones were amplified using RT-PCR and the DNA sequence was obtained (GenBank accession nos. U64092 and U64093). The human counterparts of these COS1 IL-1RII cDNAs were also cloned from Raji cells (a B cell line) using RT-PCR. One of these two clones from Raji cells was identical to the published human IL-1RII cDNA sequence, while the second one had the same 5' end but a different 3' end (GenBank accession no. U64094).

From the deduced amino acid sequence, one IL-1RII clone encodes a longer protein comprising a signal peptide, an extracellular domain, a transmembrane domain, and a very short intracellular domain, and is designated as mIL-1RII (hmIL-1RII for human and COS1 mIL-1RII for the COS1 clone). Another clone predicts a peptide with an identical signal peptide and extracellular domain but no transmembrane or intracellular domain, and is designated as sIL-1RII (hsIL-1RII for protein binding, Costar). The plates were blocked with 1% nonfat milk and then incubated with human 125I-IL-1α or human 125I-IL-1β at different concentrations either in the presence or absence of 400 nM unlabeled IL-1β as the competitor.
human and COS1 sIL-1RII for COS1 receptor). The deduced amino acid sequence demonstrated that COS1 mIL-1RII and hmIL-1RII share 95% sequence identity, but COS1 mIL-1RII is 5 amino acids shorter at the C terminus (Fig. 4A). COS1 sIL-1RII and hsIL-1RII have similar homology to that of COS1 mIL-1RII and hmIL-1RII, respectively. When compared with mIL-1RII, the sIL-1RII (for both COS1 and human) is much shorter. While mIL-1RII has almost 400 amino acids (393 amino acids for COS1 and 398 amino acids for human), sIL-1RII (both COS1 and human) is only 296 amino acids in length terminating at amino acid Q296 (Fig. 4B). The predicted protein structures for hsIL-1RII, hmIL-1RII, COS1 mIL-1RII, and COS1 sIL-1RII are illustrated in Fig. 4C. An analysis of the sequence around the splice site of hsRII revealed a sequence that is very close to the splice consensus sequence (Fig. 4D). This splicing event creates an in-frame stop codon in hsRII resulting in a soluble form of the IL-1 receptor.

Expression and Characterization of the Protein Encoded by sIL-1RII cDNA—In order to determine if the sIL-1RII mRNA encoded a soluble IL-1 receptor, both human and COS1 sIL-1RII cDNAs were subcloned into a eukaryotic expression vector pcDNAI/Amp (Invitrogen). The membrane forms of both human and COS1 cell mIL-1RII cDNAs were cloned into pcDNAI/Amp as well. These clones were then transfected into COS7 cells, which do not express IL-1 receptors as determined by the IL-1b-Sepharose-4B affinity precipitation studies followed by radioligand blot assays (data not shown). Our results indicated that mIL-1RII cDNA-transfected COS7 cells expressed a high level of membrane IL-1 receptor as detected by 125I-IL-1 binding; there was no detectable binding to mock-transfected COS7 cells and low but detectable levels of binding to sIL-1RII cDNA-transfected COS7 cells (Fig. 6A). We assayed the IL-1 binding activity of the soluble receptor in the cell culture medium by a solid phase binding assay. As expected, in both sIL-1RII- and mIL-1RII-transfected COS7 cell culture supernatant, a high amount of soluble IL-1 receptor was detected (Fig. 6B).

RNase protection assays indicated that the two mRNAs (sIL-1RII and mIL-1RII) were present in both COS1 cell and Raji cells (Fig. 5). Raji cells expressed high levels of mIL-1RII mRNA, while COS1 cells expressed high levels of sIL-1RII mRNA. The ratio of sIL-1RII to mIL-1RII RNA in the COS1 cells was estimated to be 1:3 to 1:4. This ratio of RNAs is consistent with the ratio of protein of greater than 80 kDa (spliced) versus that at 60 kDa (processed) as seen in Fig. 2. U937, THP1, HepG2, and U118 cell lines did not show any specific signal, while 293 cells had detectable mIL-1RII but no detectable sIL-1RII mRNA. Since sIL-1RII contains almost the entire extracellular domain of mIL-1RII but no transmembrane or intracellular domain, sIL-1RII mRNA might encode a soluble receptor for IL-1.

Alternatively Processed Type II IL-1 Receptor
IL-1 receptor was also detected in mIL-1RII cDNA-transfected cultures. Cell culture supernatant (100 μl of 20-fold concentrated) from transfected COS7 cells was immobilized to 96-well plates as described. The plates were then blocked with 1% nonfat milk and incubated with 100 pM 125I-IL-1α as a competitor. The cells were then washed with cold PBS and bound 125I-IL-1α was expressed in counts/min. A, solid phase binding assay for soluble IL-1 receptor expressed by mRII and sRII cDNA-transfected COS7 cells. Cell culture supernatant (100 μl of 20-fold concentrated) from transfected COS7 cells was immobilized to 96-well plates as described. The plates were then blocked with 1% nonfat milk and incubated with 100 pM human 125I-IL-1α either in the presence (open columns) or absence (solid columns) of 200 nM unlabeled human IL-1α as a competitor. The cells were then washed with cold PBS and bound 125I-IL-1α was expressed in counts/min. B, solid phase binding assay for soluble IL-1 receptor expressed by mRII and sRII cDNA-transfected COS7 cells. Cell culture supernatant (100 μl of 20-fold concentrated) from transfected COS7 cells was immobilized to 96-well plates as described. The plates were then blocked with 1% nonfat milk and incubated with 100 pM human 125I-IL-1α either in the presence (open columns) or absence (solid columns) of 200 nM unlabeled human IL-1α at 4°C overnight. The plates were then washed and bound 125I-IL-1α counted. All results are the mean of three parallel experiments. Ct, control; mRII, mL-1RII; sRII, sIL-1RII.

**DISCUSSION**

In the present study, we have identified and purified to homogeneity a soluble IL-1 receptor from monkey kidney COS1 cell culture supernatant with radioligand binding, molecular weight characteristics, and protein sequence identity comparable to the previously characterized soluble human type II IL-1 receptor (6). Radioligand binding studies with increasing concentrations of 125I-IL-1β demonstrate saturable high affinity specific binding with a Kd value of ~2–3 nM for the purified COS1 soluble protein. The pharmacological characteristics of the purified protein were determined in cross-linking, ligand blot, and solid phase radioligand binding studies. In contrast to 125I-IL-1β, 125I-IL-1α did not show any appreciable signal in the ligand blot assay and bound only to a very limited extent in the solid phase assay; saturaibility was not seen even at a 20 nM concentration of radioligand. However, in cross-linking studies, IL-1α at a concentration of 100 nM did compete for 125I-IL-1β binding suggesting that it bound the receptor with low affinity. In contrast, IL-1ra was unable to appreciably compete for 125I-IL-1β binding even at a 100 nM concentration. Taken together, the radioligand binding data demonstrate characteristics of the type II IL-1 receptor with a clear cut preference of the protein for IL-1β and the following rank order of potency: IL-1β > IL-1α > IL-1ra. The molecular mass of the soluble COS1 IL-1 receptor as determined in cross-linking and ligand blot assays is ~55–60 kDa. This molecular mass is somewhat higher than the 45 kDa reported by Sims et al. (12). This may be due to glycosylation differences in the cell types analyzed or the association of the receptor with other proteins. A report by Svenson et al. (18) identified a soluble IL-1 binding activity in human...
by human sIL-1RII-transfected COS7 cells demonstrated the presence of multiple bands ranging in molecular mass from 60 to 160 kDa. The higher molecular mass bands probably correspond, in part, to complexes of the soluble receptor with other secretory proteins. This soluble receptor may also form complexes with membrane proteins, since sIL-1RII-transfected COS7 cells show membrane IL-1β binding when compared with background binding of mock-transfected COS7 cells. COS1 cells express relatively high levels of sIL-1RII mRNA, and an IL-1 binding protein with similar binding properties of type I IL-1 receptor but with large molecular weight (>100 kDa) was seen in ligand blot assays. These large IL-1 binding proteins may represent complexes formed between sIL-1RII and other proteins or with each other through the formation of disulfide bonds, since sIL-1RII contains an unpaired cysteine.

While both the human mIL-1RII and the alternatively processed sIL-1RII forms of the receptor result in high levels of soluble IL-1RII in the supernatant, they most likely occur by different mechanisms. The mIL-1RII has been postulated to be post-translationally processed to the soluble form of the receptor resulting from cleavage of the extracellular protein. The novel sIL-1RII, on the other hand, is produced intracellularly and secreted as a soluble protein. This is the first demonstration that alternative splicing of the primary transcript can be used to generate a soluble IL-1RII protein. The soluble proteins derived from both forms of the type II IL-1 receptor most likely serve similar roles as decoy receptors and inhibitors of IL-1 function. The relative contribution of the two receptors to the soluble receptor pool is unknown and is probably dependent on a variety of factors. These include cellular expression patterns, the level of basal expression and most importantly the regulation of the two proteins. A survey of the expression pattern of the two forms of the receptor in various cell lines demonstrates that some cells (e.g. Raji cells) express both forms, while other cells (e.g. 293 cells) only express the membrane form of the receptor.

In summary, we have purified and characterized a soluble IL-1 receptor from COS1 cell culture supernatant with comparable pharmacological characteristics and a different molecular mass (55–60 kDa) to type I IL-1 receptor. In addition, we have cloned cDNAs from a novel alternatively processed mRNA from both COS1 cells and human cells, which encodes a protein of 296 amino acids with pharmacological characteristics of the soluble type II IL-1 receptor. The contribution of the newly identified type II IL-1 receptor mRNA to the pool of soluble IL-1 receptors as well as its regulation and physiological role in limiting the actions of IL-1 await future studies.

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