IL-1Rrp Is a Novel Receptor-like Molecule Similar to the Type I Interleukin-1 Receptor and Its Homologues T1/ST2 and IL-1R AcP

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A novel member of the interleukin-1 receptor family has been cloned by polymerase chain reaction using degenerate oligonucleotide primers derived from regions of sequence conservation, using as template a yeast artificial chromosome known to contain both regions of sequence conservation, using as template a degenerate oligonucleotide primers derived from regions of sequence conservation, using as template a degenerate oligonucleotide primers derived from regions of sequence conservation, using as template a

The type 1 interleukin-1 receptor (IL-1R) mediates the biological effects of interleukin-1, a proinflammatory cytokine. Recently it has become apparent that the IL-1R belongs to a family of homologous proteins (1). Some family members (T1/ST2 and IL-1R accessory protein (AcP)) (2, 3) are transmembrane receptor-like molecules that bear sequence similarity to the IL-1R in both their extracellular and transmembrane domains. Others (MyD88, rsc786, and a number of Drosophila proteins, the best characterized of which is Tdi1) (4–6) contain domains homologous to the cytoplasmic part of the IL-1R, associated with other, divergent domains. In some cases this sequence homology also reflects functional homology (1).

Having recognized the existence of a family of proteins with homology to the IL-1R, we sought to ask whether there were other, as yet undetected, members of this family. We used oligonucleotide primers based on sequence motifs conserved within the family to search for new genes by PCR. In this paper we report the cloning and characterization of a novel cDNA bearing strong resemblance to the IL-1RI, T1/ST2, and the IL-1R AcP.

EXPERIMENTAL PROCEDURES

Degenerate oligonucleotides based on the amino acid motifs indicated in Fig. 1 and used in the attempt to isolate new IL-1RI family members by PCR had the sequences, 5′-T(ATT)/C(TT)/C(G/A)(G/A)T(G/A)/(G/A)T/TT(ATT)/C(TT)/A/G/TT-3′ (sense oligo) and 5′-TA(G/A)(G/A)(G/A)/C/T(A/G)TT(A/G)/C(TT)/TTT-3′ (antisense oligo).

PCR reactions (20 μl) used 0.5 μl of a 16.1 mixture of Taq (Promega) and Vent (New England Biolabs) DNA polymerases and contained 200 pmol of each primer, 200 μM dNTPs, and 5–10 μl of human YAC DNA (YAC C02133 (7)), partially purified by extraction from a pulse-field gel. Cycle conditions were 5 min at 94°C, during which time the DNA polymerase mixture was added; 40 cycles of 1 min at 94°C, 3 min at 35°C, and 1 min at 72°C, followed by 10 min at 72°C. The PCR was run on a low melting temperature agarose gel and the band containing material between 90 and 150 bp was cut out and melted, and 5 μl was used as template in a second PCR reaction performed similarly to the first PCR except that only 20 cycles were run. The reaction was again run on an agarose gel, and the 90–150 bp fraction was eluted, blunt-ended using T4 DNA polymerase, phosphorylated using T4 polydeoxyribonuclease, heated for 10 min at 65°C, ethanol-precipitated, and ligated into pcRscript (Stratagene) in the presence of SrfI. Ligation were transformed into Escherichia coli DH10. White colonies were picked from X-Gal (5-bromo-4-chloro-3-indolyl β-D-galactoside) plates, and their inserts were amplified by PCR using vector primers and a small amount spotted on nylon filters, which were subsequently hybridized at 42°C in aqueous conditions to a mixture of 32P-labeled oligonucleotide probes derived from the human and murine IL-1RIs (sequences: human, 5′-CCCAAGCTTTGATTACG; mouse, 5′-CTGTTGCCTAGGTTCTGG). Filters were washed at 50°C in 0.1 M NaCl.

The human IL-1R and human IL-1RI Fc fusion proteins, joining the extracellular portions of the receptors to the CH2 and CH3 domains of human IgG, were generated as described (8). The chimeric receptor containing the mouse IL-1RI extracellular and transmembrane region and the human IL-1Rrp cytoplasmic domain was generated as described (1).

Restriction digests and Southern blots were performed on IL-1R containing YACs CO2133 and F1150 as described (7), using PCR probes amplified from human IL-1Rrp cDNA. The 5′-probe was a 258-bp fragment whose 5′-end lies 473 bp downstream of the A of the initiating methionine codon. The 3′-probe was a 345-bp fragment whose 5′-end lies 1500 bp downstream of the A of the initiating methionine codon.

A BIAcore biosensor (Pharmacia Biosensor) was used to examine binding of IL-1 ligands to the human IL-1Rrp Fc fusion protein, essentially as described in detail in Arend et al. (10). Briefly, a goat anti-human α-serum was coupled to the derivatized matrix of a hydrogel chip was used to capture the human IL-1Rrp Fc protein. The appropriate IL-1 ligand, at several different concentrations, was re-added with the captured protein, and the change of mass per unit area over time was measured.

RESULTS AND DISCUSSION

In order to search for novel cDNAs similar to the IL-1RI, we designed PCR primers based on the alignment of IL-1RI family members presented in Mitcham et al. (1). We chose primers from the first and third conserved regions (Fig. 1; see “Experimental Procedures”) because those regions offered the highest degree of sequence conservation. In addition, no intron lies between these in the genomic structures of the IL-1RI (7) and fit-1 genes (fit-1 is the rat homologue of T1/ST2) (11). If other
family members were to have the same genomic structure, this would mean that we could predict the size of an expected PCR product from genomic DNA and be less dependent on making the proper selection of mRNA source to use as a PCR substrate.

Initially, appropriate conditions for PCR amplification with the degenerate primers were determined by use of cDNA clones of the human and mouse type I IL-1 receptors and the mouse T1/ST2 receptor-like protein. Using conditions that allowed an amplification product to be obtained from each of these cDNAs, a 500-kilobase human YAC that contained the genes for type I and type II IL-1 receptors as well as the T1/ST2 gene (7) was used as template. PCR amplification produced a band of the expected size from the YAC DNA, which was easily visible on an ethidium bromide-stained gel and which after Southern transfer hybridized to an oligonucleotide probe derived from the human IL-1RI gene. The amplification product was gel-purified and cloned into a plasmid vector. The inserts were then amplified by PCR, spotted on nitrocellulose, and probed with a mixture of IL-1RI oligonucleotides. Only 5 out of 180 inserts hybridized, and indeed, random DNA sequencing of nine of the non-hybridizing inserts revealed that they derived from yeast DNA. One of the five hybridizing inserts gave a strong hybridization signal, and DNA sequencing revealed it to be amplified from the IL-1RI gene. Of the four weakly hybrid-

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**Fig. 1.** Degenerate oligonucleotides, derived from the sequences indicated by the arrows and specified under “Experimental Procedures,” served as primers in PCR amplifications using YAC DNA from the human chromosome 2q12 region as template. The amplification products were cloned, and the DNA sequence of one of them, IL-1Rrp, predicted the amino acid sequence shown in the figure. Black- and gray shading indicates regions of great and lesser sequence conservation, respectively. The sequence of the IL-1R Acp was not available at the time the experiment was done.

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**Fig. 2.** Comparison of the predicted amino acid sequences of human and mouse IL-1Rrp with the sequences of murine IL-1 receptor (12), T1/ST2 (2), and AcP (3). The alignment was performed using the UWGCG program Pileup (13) and subsequently optimized subjectively by the authors. The predicted signal peptides are underlined, as well as being separated from the postulated extracellular regions by a space. The predicted transmembrane regions are doubly underlined and separated by spaces on either side from the postulated extracellular and cytoplasmic domains. Asterisks mark the positions of the cysteines predicted to form the typical intradomain disulfide bonds of the three Ig domains of the extracellular region. Black- and gray shading indicates regions of great and lesser sequence conservation, respectively.

The human IL-1Rrp sequence is derived from three cDNA clones from a peripheral blood lymphocyte library and four PCR clones from the epidermal carcinoma line KB. The codon for alanine 317 is polymorphic, being present in the PBL clones and two of the KB clones and absent from the other two KB clones. It is also absent from the two mouse clones derived from an EL4 T cell library. The IL-1Rrp sequences can be retrieved from GenBank using accession numbers U43672 (human) and U43673 (murine). Detailed comparison of the IL-1Rrp sequences with those of other family members reveals great overall similarity but some interesting changes. For example, all of the IL-1R-like proteins (including the type II IL-1 receptor (14)) have a proline following the first cysteine of the typical immunoglobulin domain cysteine pair in the first two of the three Ig domains; these prolines are missing in mouse and human IL-1Rrp. Second, like all IL-1R-like proteins except T1/ST2 (but including the type II IL-1 receptor), mouse and human IL-1Rrp have a cysteine residue just a few residues C-terminal to the point of signal peptide cleavage. However, unlike the type I and type II IL-1R and the AcP, they do not have a corresponding cysteine 8 residues C-terminal to the second "Ig" cysteine of the first domain. (Human, but not mouse, IL-1Rrp does have two additional cysteines lying between the "Ig" cysteine of the first domain.) Third, like all family members except the type II IL-1R, the IL-1Rrp proteins have an extra cysteine pair in the second Ig domain. Finally, human IL-1Rrp lacks the typical "Ig" tryptophan in the second domain, and human and mouse IL-1Rrp both lack the second cysteine of the typical intradomain cysteine pair in the first domain.
IL-1Rrp Is a Novel Receptor-like Molecule

Fig. 3. RNA analysis of IL-1Rrp expression. Northern blots (purchased from Clontech), stated to contain 2 μg of human poly(A)+ RNA in each lane, were probed overnight with a 32P-labeled antisense IL-1Rrp riboprobe at 63 °C in 0.75 M NaCl, 50% formamide and washed at 63 °C in 0.3 M NaCl. To ascertain evenness of loading as well as effectiveness of RNA removal, the filters were subsequently probed for glyceraldehyde-phosphate dehydrogenase (GAPDH) and 28 S rRNA.

Fig. 4. Biosensor analysis demonstrating easily measurable binding of human IL-1α to a human IL-1RI Fc fusion protein (left panel) but no detectable binding to a comparable human IL-1Rrp Fc fusion protein (right panel). Similar results were obtained using human IL-1α and human IL-1ra as ligands (data not shown). 1, 10, and 100 nM refer to the ligand concentration used in generating each of the curves. The left arrow indicates the time of addition of ligand, while the right arrow indicates the start of the buffer wash. RU, resonance units.

Fig. 5. A, NF-κB gel shift assay. COS cells were transfected with receptor constructs, treated with blocking antibody to the primate IL-1 receptor, and stimulated (30 min, 1 ng/ml) with human IL-1α as indicated. Nuclear extracts were incubated with 32P-labeled NF-κB oligonucleotides and electrophoresed. Cassette vector refers to the expression vector containing the extracellular and transmembrane portions of the murine IL-1R but no cytoplasmic domain. The arrow points to the position of the induced NF-κB complex with DNA. B, IL-8 promoter assay. COS cells were transfected with the indicated receptor plasmid together with a reporter plasmid containing the IL-8 promoter driving expression of the IL-2 receptor α chain cDNA. Twenty-four hours later, the cells were stimulated overnight, in the presence of blocking antibody to the primate IL-1 receptor, with medium (solid bars) or 1 ng/ml IL-1α (hatched bars). They were then incubated with mouse monoclonal antibody 2A3 against IL-2Rα followed by 125I-labeled goat anti-mouse Ig serum and counted. The blocking antibody to the primate IL-1 receptor was a 1/100 dilution of sheep anti-human IL-1RII serum P3 (1), which at this concentration blocks binding of IL-1 to the endogenous COS cell IL-1 receptors but has no effect on binding to the transfected mouse IL-1RII extracellular region.
was detected to the IL-1Rrp fusion protein for any of the IL-1s (Fig. 4, right panel; data not shown). We conclude that despite its sequence resemblance, IL-1Rrp is not an IL-1 receptor.

In order to ask about the signal transduction capability of the IL-1Rrp cytoplasmic domain, we constructed a chimeric receptor containing the extracellular and transmembrane portions of the mouse type I IL-1 receptor fused to the cytoplasmic portion of the human IL-1Rrp. The chimeric receptor was expressed in COS cells, and the ability of IL-1 to activate the transcription factor NF-xB was examined. A polyclonal serum raised to the human IL-1 receptor was used to block the endogenous (cross-reactive) monkey IL-1R, without affecting IL-1 stimulation of the transfected murine IL-1R receptor chimera (1). In Fig. 5A, it can be seen that the IL-1Rrp cytoplasmic domain is capable of inducing NF-xB DNA binding ability in response to IL-1 stimulation of the chimeric receptor molecule. The induction is comparable in magnitude with that mediated via a transfected murine IL-1R. Parenthetically, it is unclear why such a chimera is functional, although a similar chimera between the IL-1R extracellular domain and the T1/ST2 cytoplasmic domain also signals in response to IL-1. One possibility that is subsequent to ligand binding, the chimeric receptor associates with the IL-1R AcP and that the AcP cytoplasmic domain is then capable of performing the same role in IL-1Rrp signaling as it does in IL-1 signaling.

The signaling capability of IL-1Rrp was also examined in a second assay, namely stimulation of transcription from the IL-8 promoter. A reporter plasmid carrying a partial human IL-8 promoter fused to the coding region of the human IL-2 receptor chain was transfected into COS cells along with the IL-1R/IL-1Rrp receptor chimera. The cells were then stimulated with human IL-1x, and expression of IL-2Rx on the cell surface was measured. Transcription of the reporter construct can be induced by IL-1 stimulation of the IL-1Rrp-containing chimera to about half the level mediated by an intact mouse IL-1RI (Fig. 5B).

In a third assay, the IL-1R/IL-1Rrp chimera was transfected into KB human epidermal carcinoma cells. In the presence of the polyclonal antiserum to block human IL-1 receptors, IL-1 stimulation of the IL-1Rrp chimera resulted in the synthesis of prostaglandin E2 (data not shown). Thus, the IL-1Rrp cytoplasmic portion was capable of eliciting a broad spectrum of responses, comparable with those induced via the IL-1RI.

There are now three proteins known to be homologous to the type I IL-1 receptor throughout their entire length: T1/ST2 (2), IL-1R AcP (3), and IL-1Rrp. The chromosomal map position of the AcP is not known. The others all cluster at human chromosome 2q12–13 (Ref. 7 and this study), as does the type II IL-1R, which has only a very short cytoplasmic domain but is homologous to the others in its extracellular portion. Whether IL-1Rrp has a ligand of its own or functions instead as a subunit of a heteromeric receptor as AcP is proposed to do is a question that remains to be answered.

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