

Primary structure and functional expression from complementary DNA of a human interleukin-1 receptor antagonist

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Human monocytes induced with adherent IgG secrete an interleukin-1 receptor antagonist which could be important for the *in vivo* regulation of IL-1 activity. A complementary DNA for this molecule has been isolated from a human monocyte library. Analysis of monocyte RNA indicates that the gene is transcriptionally regulated. The sequence of the receptor antagonist indicates that it is structurally similar to IL-1 β . Expression of the cDNA in *Escherichia coli* yields IL-1 receptor antagonist activity.

INTERLEUKIN-1 (IL-1) is a cytokine¹ produced primarily by mononuclear phagocytes. There are two forms of IL-1, and although they have only slightly homologous primary structures², they have similar effects on a variety of target cells³. They both stimulate the production of prostaglandin and collagenase by fibroblasts, induce the synthesis of interleukin-2 by T cells, and induce adhesion proteins and a procoagulant-antifibrinolytic character in vascular endothelial cells. On the basis of these activities and the increased serum levels of IL-1 seen in several disease states⁴, IL-1 is implicated in a range of inflammatory diseases, such as rheumatoid arthritis. Also, injections of IL-1 in rabbit knee joints causes swelling and the loss of proteoglycan⁵, and intravenous injections of IL-1 into rabbits lowers blood pressure and dramatically reduces the number of circulating white blood cells⁶.

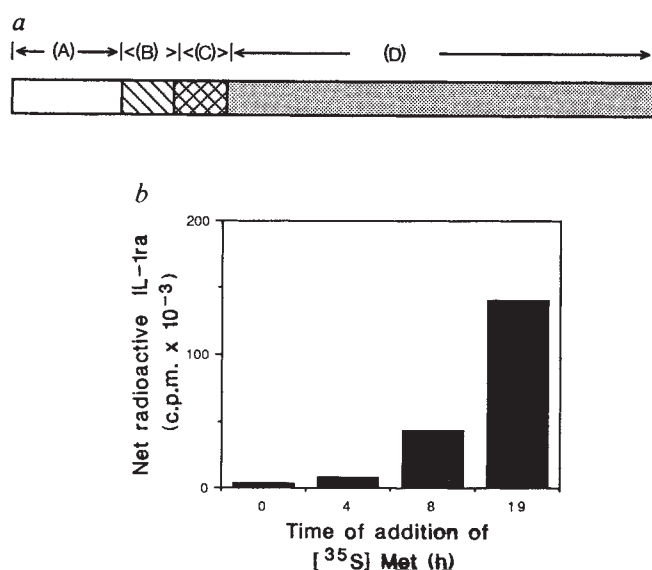
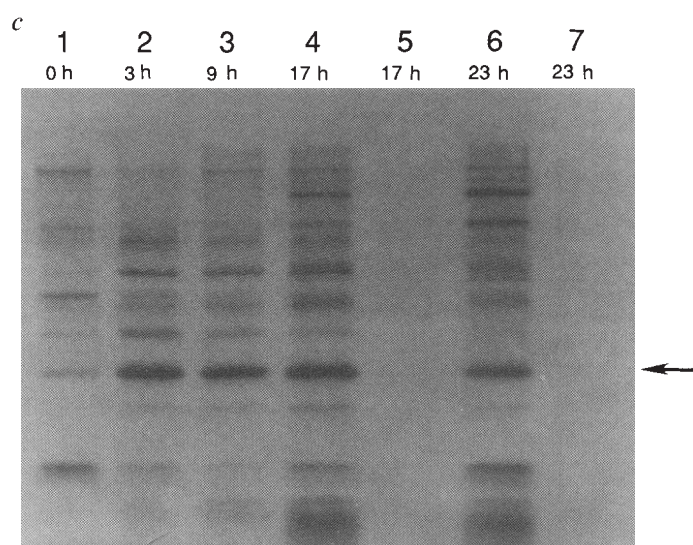


FIG. 1 Analysis of pulse-labelled proteins from IgG-stimulated monocytes. *a*, Design of labelling protocol. Mononuclear leukocytes (which include monocytes and lymphocytes) were isolated from normal donor blood by Lymphoprep gradient centrifugation. These cells were plated on IgG-coated culture dishes at a density of 6.5×10^6 cells ml^{-1} in RPMI medium containing $\frac{1}{50}$ normal concentration of methionine ('low Met RPMI')¹² and incubated at 37 °C in 5% CO₂. The schedule for metabolically labelling the cells is indicated: (A) 0–*x* hours, prelabelling period; (B) 2-h pulse labelling, [³⁵S]methionine (50 $\mu\text{Ci ml}^{-1}$) added to the medium; (C) 2-h chase, excess cold methionine (100-fold) added to the medium, medium is collected at the end of (C) and is replaced with fresh RPMI medium; and (D) (20–*x*) hour recovery period, medium is collected at the end of this period. *b*, [³⁵S]IL-1 receptor antagonist identified by column chromatography. Supernatants taken after periods (C) or (D) were applied to a Mono-Q column (Pharmacia) and eluted using a NaCl gradient from 0 to 0.1 M, in 25 mM Tris-HCl buffer, pH 7.6, 0.1% sucrose. Fractions were collected and the amount of radio-



activity was determined by liquid scintillation counting. The times at which labelling began (beginning of (B)) and the net radioactivity (total – background (6,000 c.p.m. per fraction)) in the IL-1ra peak¹² from each of the labelled samples collected after period (C) are indicated. In each case, supernatants collected after period (D) contained no detectable [³⁵S]IL-1 antagonist, indicating that the 2-h chase was effective. *c*, Polyacrylamide gel electrophoresis of [³⁵S]-labelled supernatants from pulse-labelled monocytes. Supernatants were collected after periods (C) and (D), and 25 μl of each sample was loaded on a 15% SDS-polyacrylamide gel³³. After electrophoresis, the gel was fixed, treated with Enhance (NEN), dried and exposed to Kodak XAR film to produce the fluorogram shown. The times at which labelling began are indicated at the top of each lane. Lanes 1, 2, 3, 4 and 6 are samples taken after period (C); lanes 5 and 7 are samples taken after recovery period (D). The arrow indicates the putative IL-1ra band at a position corresponding to an *M_r* of 22K. The absence of radioactivity in lanes 5 and 7 indicates that the cold methionine chase was effective.

The potent pleiotropic inflammatory effects of IL-1 are likely to be stringently controlled *in vivo*. There are many ways in which this regulation could be achieved. One of the more interesting mechanisms would be through the synthesis of a protein inhibitor of IL-1 that could regulate IL-1 action after its synthesis. In addition to its potential physiological importance, this inhibitor could be used in the appropriate animal models of human disease to determine whether IL-1 is pathophysiologically significant in human disease. An IL-1 inhibitor could also be useful as a therapeutic agent for treatment of inflammatory diseases.

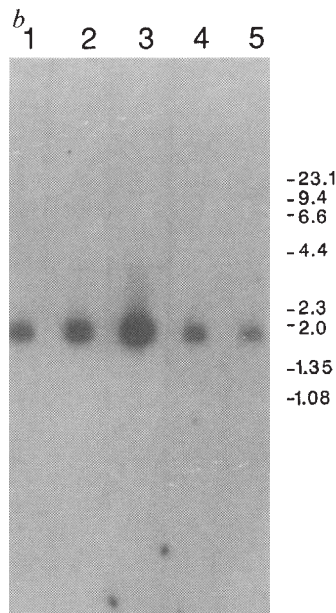
Several IL-1 inhibitory activities have been described^{7,8}. Most of these seem to inhibit the actions of IL-1 only in the thymocyte proliferation assay⁸. Only two IL-1 inhibitors have been shown

to inhibit IL-1 action in all of the commonly used cellular assays^{9,10}, suggesting that they act directly on IL-1 or on its receptor. We decided to clone the monocyte-derived inhibitor, because the cell that synthesizes this protein is known, and the conditions necessary to induce it have been described^{9,11}. In the accompanying article, Hannum *et al.*¹² describe the purification and properties of a monocyte-derived IL-1 inhibitor that acts as a receptor antagonist (termed IL-1ra) and describe peptide sequences that we used for the synthesis of oligonucleotide probes.

Isolating a cDNA clone for IL-1ra

We performed a preliminary experiment to estimate the levels of functional IL-1ra messenger RNA in monocytes at different

<i>a</i>	Peptide sequence	RNA coding sequence	Oligodeoxyribonucleotide probe sequence (probe no.)
	KMQAF	5'–AARAUGCARGCNUUY–3'	5'–RAANGCYTGCATYTT–3' (1)
	KFYFQED	5'–AARUUYUAYUYCARGARGA–3'	5'–TCYTCYTGRAARTARAAYTT–3' (2)
	MVTKFYF	5'–AUGGUNACNAARUUYUAYUU–3'	5'–AARTARAAYTTNGTNACCAT–3' (3)
	DVNQKT	5'–GAYGUNAAACARAARAC–3'	5'–GTYTTYTGRTTNACRTC–3' (4)
	NQKTFY	5'–AAYCARAARACNUUYUA–3'	5'–TARAANGTYTTYTGRTT–3' (5)



C

1 GAATTCGGGCTGAGTACACAGA 23

ATG GAA ATC TGC AGA GGC CTC CGC AGT CAC CTA ATC ACT CTC CTC CTC TTC CTG TTC CAT 83
M E I C R G L R S H L I T L L L F L F H

TCA GAG ACG ATC TCC CGA CCC TCT GGG AGA AAA TCC AGC AAG ATG CAA GCC TTC AGA ATA 143
S E T I C R P S G R K S S K M Q A F R I₁₅

TGG GAT GTT AAC CAG AAG ACC TTC TAT CTG AGG AAC AAC CAA CTA GTT GCT GGA TAC TTG 203
W D V N Q K T F Y L R N N Q L V A G Y A₁₅

CAA GGA CCA AAT GTC AAT TTA GAA GAA AAG ATA GAT GTG GTA CCC ATT GAG CCT CAT GCT 263
Q G P N V N L E E K I D V V P I E P H A₅₅

CTG TTC TTG GGA ATC CAT GGA GGG AAG ATG TGC CTC TCC TGT GTC AAG TCT GGT GAT GAG 323
L F L G I H G G G K L S Q V K S D E₇₅

ACC AGA CTC CAG CTG GAG GCA GTT AAC ATC ACT GAC CTG AGC GAG AAC AGA AAG CAG GAC 383
T R L Q L E A V N I T D L S E N R K Q D₉₅

AAG CGC TTC GCC TTC ATC CGC TCA GAC AGT GGC CCC ACC ACC AGT TTT GAG TCT GCC GCC 443
K R F A F I R S D S G P T T S F G S D A₁₁₅

TGC CCC GGT TGG TTC CTC TGC ACA GCG ATG GAA GCT GAC CAG CCC GTC AGC CTC ACC AAT 503
G P G W F L C T A M E A D Q P V S L T N₁₃₅

ATG CCT GAC GAA GGC GTC ATG GTC ACC AAA TTC TAC TTC CAG GAG GAG TAG 567
M P D E G V M V T K A F Y F Q E D E₁₅₂

TACTGCCAGGCGCTGCTGTTCCTTCCTGATGGCAAGGACTGCGGACTGCCAGTCCCCCTGCCAGGCTCCCG 637

GCTATGGGGGCACTGAGGACGACGCTTGGGGGTGGACCTCAGAGGCGTCACAAACCTGCTCAGAGGACTCTGCC 717

TCCTCTTCAACTGACCAAGCTCCATGCTGCTCCAGAAATGCTCTTCTAATGTGTGAATCAGAGCACAGCCCTGCA 797

CAAAGCCCTTCCATGTGCGCTCTGCATTGAGGATCAAAACCCGACCACTGCCCAACCTCTCTCTTGCACCTGCCT 877

CTTCTCTCCATTCACCTTCCATGCTGCTGATTCATGAGGACCTTGATGACCCCAACCAAGTGGCTCCGACACCC 957

TGTTTACAAAAAGAAAGACCACTCCATGAGGAGGTTTAAAGGTTTGTGGAAATGAAATAGGATTTTCATGAT1037

TTTTTTTTTTCAGTCCCCGTGAAGGAGGCGCTTCATTGGAGATTATGTTCTTTCGGGAGAGGCTGAGGACTTAAAT1117

ATTCCTGCATTGTGAAATGATGGTGAAGTAAGTGTAGCTTTTCCCTCTCTTTTCTCTTTTGTGATGTCCCAAC1197

TGTGAAATTAAGATTTATGGTACTATGTTAGCCCAATAATTTTTTTCCTTTTAAACACTTCATATCTGAGACT1277

CCTCTGTGCGAGCACTGCTGCCAGGCTCCAGCTCCATCTCCACTCCAGATTTTACAGCTGCTGCACTACTTACCT1357

TCCTATCAGAAATTTCTCAGCTCCCAAGGCTCTGAGCAATGTGGCTCTGCGGGTCTTCTCTCTCTGCTGAGGAAT1437

AAATGCTCTTACATTTAGAGCTTCTGGCACTGGAGACTTGATGAAGATGGCTGCTGCTGCTGCTGCTGCTGCTGCT1517

ACCAGGCTGGGAGCTCTGAGAGCAGGAAACATGACTGCTATATGCTCAGGTCCTGAGGCGCAAGCACTAGCCTCG1597

CTCTTGGCAGGTACTCAGCAATGAATGCTGATATGTTGGGTGCAAGTTCCTACTCTGCTGCTGCTGCTGCTGCTGCT1677

TACAATAAATCTTGAATGCCAAAAAAGAAAAAAGAAAAAAGCCGAATCCCGCCGGAATTC 1740

FIG. 2 Sequence of the IL-1ra cDNA. **a**, Design of oligonucleotide probes. Five degenerate antisense probes were synthesized based on peptide sequences (single-letter amino-acid code)¹² as indicated. Oligonucleotides were synthesized on an Applied Biosystems Model 380A DNA synthesizer and labelled with [γ -³²P]ATP and polynucleotide kinase. In the nucleic-acid sequences shown, R represents A+G, Y represents C+T (or C+U), and N represents all four bases. **b**, Analysis of cDNA clone IL-1ra-2a by Southern blot. RNA was prepared from human monocytes induced for 17 h as described³⁴. Poly(A)⁺ RNA was prepared by standard techniques using oligo(dT) cellulose³⁵ from Bethesda Research Laboratories, and a λ gt10 library was constructed³⁶ using a cDNA synthesis kit (Boehringer)³⁷ and a λ gt10 cloning kit (Stratagene). *Eco*R1 linkers were purchased from New England Biolabs. The resulting library contained 1.4×10^7 recombinant phage as determined by their ability to form plaques on the *E. coli* strain C600 Hfl. Screening with oligonucleotide probes was performed as described³⁸. The λ gt10 DNA from cDNA clone IL-1ra-2a (see text) was prepared using Lambdasorb (Promega), digested with *Eco*R1, divided into five aliquots, loaded into 5 separate wells and electrophoresed on a 1% agarose gel. The digested DNA was resolved into the two λ arms (32.7 kb and 10.6 kb) and the insert (1.8 kb). The DNA on the gel was blotted onto nitrocellulose³⁹, which was

cut lengthwise into five strips, each representing a different lane of the gel. Each of the strips was hybridized to a different oligonucleotide probe (Fig. 2a, above). Probes are indicated on the top of each strip. After hybridization and washing³⁸, the strips were aligned next to each other and exposed to X-ray film. DNA size markers (in kb) are shown on the right. **c**, Sequence of the cDNA for IL-1ra. The DNA sequence and the predicted protein sequence of the large ORF are shown. Both strands of the cDNA were sequenced at least once, and in the coding region, both strands were sequenced at least three times^{40,41}. The cDNA sequence and the exon sequences of a genomic clone described elsewhere (S.P.E. *et al.*, manuscript in preparation) are identical except for nucleotide residue 100 (underlined), which is a C in the cDNA and a G in the genomic clone. We believe that the correct residue is a G, because the codon CGA codes for arginine, which agrees with the known N-terminal sequence of the IL-1 antagonist¹². The C residue is apparently due to an error made during the reverse transcription of the IL-1ra mRNA. The sequence of the mature protein, after removal of the 25 amino-acid signal sequence, is shown in bold. Cysteine residues are underlined and the consensus N-linked glucosylation site is indicated by the asterisk. The *Eco*R1 sites and flanking linker sequences are in italics.

times after plating the cells on IgG. We reasoned that the level of active mRNA should be directly related to the rate of synthesis of the protein, which could be measured by determining the amount of radioactive IL-1ra produced during a pulse with [³⁵S]-methionine. Because the protein is secreted, we used a cold methionine 'chase' so that the pulse-labelled protein could accumulate in the medium (Fig. 1a). We assessed the amount of radioactive IL-1ra made by induced monocytes during the pulse either by ion-exchange chromatography¹² or by estimating the amount of radioactivity in a protein of relative molecular mass 22,000 (*M_r* 22K) by SDS-PAGE. The results of the initial chromatographic analysis indicated that the level of IL-1ra mRNA is elevated about 20 h after induction (Fig. 1b). Further experiments indicated that the mRNA level is maximal at 15–17 h (data not shown). By contrast, the fluorogram of the gel (Fig. 1c) indicated that the mRNA level increases rapidly to a maximum 3–5 h after induction and remains at this level for the next 14 h. Despite indicating different kinetics of mRNA accumulation, both methods showed that the mRNA was at a maximum at 17 h. We therefore used RNA from 17 h cells to establish a cDNA library in λgt10.

We constructed the cDNA library by standard methods and screened it with a 64-fold degenerate 15-residue (15 mer) oligonucleotide (probe 1) based on the peptide sequence Lys-Met-Gln-Ala-Phe (KMQAF in single-letter (Fig. 2a)). We re-screened several positive plaques with probe 1 and a second oligonucleotide (a 128-fold degenerate 20mer) based on the amino-acid sequence Lys-Phe-Tyr-Phe-Gln-Glu-Asp (KFYFQED). One clone, IL-1ra-2a, hybridized to both probes.

Digestion of DNA isolated from this clone with *Eco*RI released a 1.8-kilobase (kb) insert that hybridized on a Southern blot to the two probes used in the screening and to three new oligonucleotide probes derived from additional peptide sequence (Fig. 2b). We cloned the 1.8-kb fragment into the *Eco*RI site of M13mp19¹³ and determined its nucleotide sequence.

The cDNA (Fig. 2c) has a single open reading frame (ORF) coding for 177 amino acids that is preceded by a relatively short 5' untranslated region (UTR) of 14 nucleotides (excluding the *Eco*RI linker sequence) and is followed by a long 3' UTR of 1,133 nucleotides excluding the poly(A) tail. The clone is close in length to that of the IL-1ra mRNA (~1.8 kb) estimated by northern-blot analysis (Fig. 3). On the basis of primer extension analysis, the 5' end of the cDNA is, at most, five nucleotides from the 5' end of the transcript (data not shown).

The ORF of the clone starts at an ATG codon flanked by

TABLE 1 Inhibition of IL-1-induced PGE₂ synthesis by IL-1ra

Lysate	PGE ₂ (ng ml ⁻¹)		
	*1:1	*1:10 ²	*1:10 ⁴
pT5T	23 (±3)	>63	>63
pRJ2	8 (±2)	5 (±1)	23 (±4)

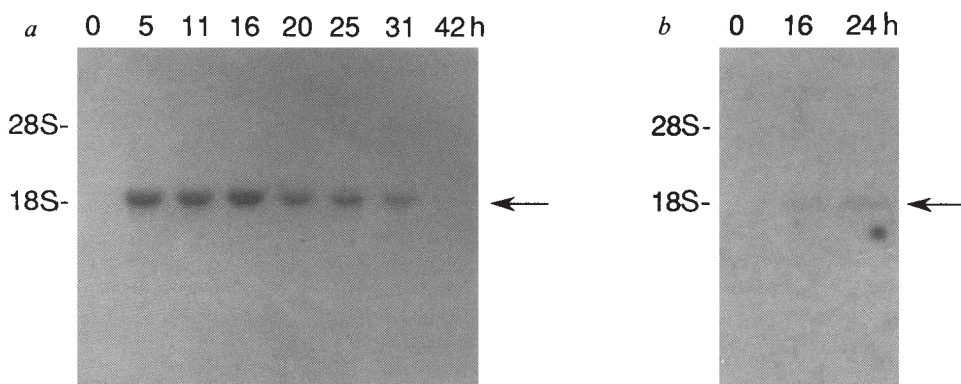
IL-1-induced PGE₂ synthesis in fibroblasts is inhibited by an *E. coli* lysate containing human IL-1ra. Human foreskin fibroblast (FF) cells (obtained from Clonetics Corp.) were seeded at 1×10^5 cells per well of a 96-well microtitre plate in RPMI-1640 medium (Mediatech) + 10% fetal bovine serum (FF medium). After 16–20 h, the cells were washed and 100 μl lysate or lysate diluted in FF medium was added. Lysate dilutions are indicated by the asterisks. IL-1β (100 μl; Genzyme) at 1 U ml⁻¹ in FF medium were then added to each well. After 6 h of incubation at 37 °C, the culture supernatant was removed and assayed for PGE₂ by a competitive enzyme-linked immunosorbent assay (ELISA) as described³². PGE₂ values (±s.d.) are given in ng ml⁻¹. Estimates of error are based on at least two determinations. *E. coli* strains and methods for preparing lysates are described in Fig. 4b. IL-1β alone induces >63 ng ml⁻¹ PGE₂. Untreated cells produce <0.3 ng ml⁻¹ PGE₂.

sequences that are close to the consensus sequence for translation initiation¹⁴. The N-terminal 25 amino acids closely resemble a classical signal sequence, with a charged residue very near the N terminus, followed by a long stretch of hydrophobic amino acids and ending with an amino acid having a small side chain¹⁵. Cleavage by leader peptidase between the 25th and 26th amino acid encoded by the ORF would result in a mature protein of 152 amino acids (*M_r* 17,115) with a predicted isoelectric point of 5.2, and an N-terminal sequence corresponding to that of the monocyte-derived receptor antagonist¹². The IL-1ra protein sequence contains one N-linked glycosylation site, in accordance with the finding that two of the three forms of the antagonist produced by monocytes are glycosylated. The sequence shows limited homology to those of both IL-1 alpha (α) and IL-1 beta (β) (see below).

Evidence that the clone codes for the IL-1ra

The above data show a close correspondence between the predicted properties of the protein encoded by the cDNA and those of the monocyte-derived IL-1ra. To confirm that the cDNA codes for the antagonist, we expressed the cDNA in *Escherichia coli* and characterized the *M_r* and activity of the recombinant protein. The vector that we used to express the protein is derived from the phage T7 promoter-polymerase expression system

FIG. 3 Northern-blot analysis of RNA from induced monocytes and U937 cells. *a*, Monocytes were induced for various times, as indicated at the top of each lane, by plating on IgG-coated culture dishes as previously described^{11,12}. RNA was prepared as described³⁴. Each sample of total RNA (20 μg) was electrophoresed on a 1.2% formaldehyde-agarose gel⁴². The gel was stained with ethidium bromide, allowing the 18S and 28S rRNA bands to be seen. The RNA was blotted onto nitrocellulose and probed with 2 pmol [³²P]-labelled oligonucleotide, 5'-GATTCTGAAGGCTTGATCTT-3', at 55 °C in 10 ml 6×NET (1×NET is 0.15 M NaCl, 1 mM EDTA, 15 mM Tris HCl, pH 8.3) 0.1% SDS, 5×Denhardt's solution, 100 μg ml⁻¹ *E. coli* transfer RNA³⁸. Overnight hybridization was followed by four washes at 30 °C and two washes at 55 °C in 6×SSC, 0.1% SDS. The blot was then exposed overnight to X-ray film. The positions of the 18S and 28S RNA bands on the blot are indicated. The arrow indicates



the IL-1ra mRNA band. *b*, U937 cells obtained from ATCC were induced with PMA (10 μg ml⁻¹) and incubated for various times as indicated at the top of each lane of the gel. RNA was prepared, electrophoresed through agarose, and analysed by northern blotting as described in *a*, except that 10 μg total RNA were loaded on the gel, and the blot was exposed to X-ray film for 120 h.

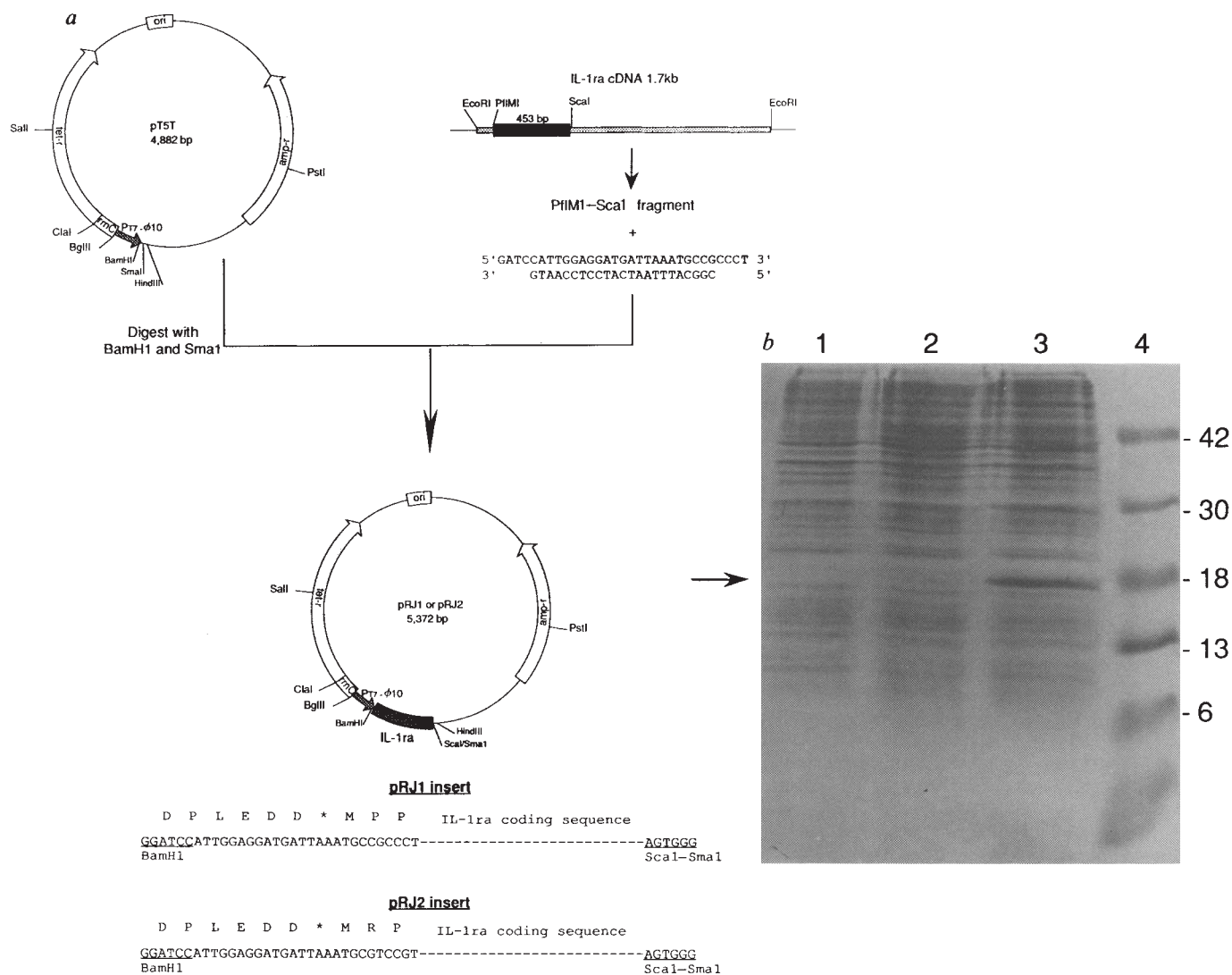


FIG. 4 Expression of recombinant IL-1ra in *E. coli*. **a**, The IL-1ra cDNA was inserted into the T7 expression vector pT5T, which is identical to plasmid pJU1003¹⁸ except for the insertion of the *E. coli* *rrnC* transcriptional terminator (*rrnC*, ref. 43) contained on the synthetic oligonucleotides

5' ATGATA AGCTGTCAAA CATGAGAATT GAGTCCCCG GAGATCCTTA
3' GCTACTAT TCGACAGTTT GTACTCTTAA CTCGAGGGG CTCTAGGAAT

GCGAAAGCTA AGGATTTTTT TTA 3'
CGCTTTCGAT TCCTAAAAAA AATCTAG 5'

between the unique *Bgl*II site and the *Cla*I site at the 5' end of the tetracycline resistance (*tet*-r) gene. A PstI-M1-ScaI fragment derived from the IL-1ra cDNA (encoding amino acids 4–152) was inserted into *Bam*HI-SmaI-digested pT5T using a *Bam*HI-PstI oligonucleotide adaptor (shown) containing sequences for translational coupling and the first three amino acids. The derived plasmid, pRJ1, encodes a form of IL-1ra with an N-terminal sequence Met-Pro-Pro-Ser- rather than Arg-Pro-Ser-, which is the N-terminal sequence of the natural protein (see Fig. 2c, and ref. 12). The IL-1ra gene in plasmid pRJ1 was mutated to change the coding from Met-Pro-Pro-Ser- to Met-Arg-Pro-Ser- by cloning the *Bam*HI-PstI IL-1ra fragment of pRJ1

into M13mp19¹³ and performing oligonucleotide site-directed mutagenesis as described⁴⁴. The sequence of the mutagenic oligonucleotide was 5'-TGATTAATGCGTCCGTCTGGAG-3'. After mutagenesis, the *Bam*HI-PstI fragment was ligated into plasmid pT5T to yield the expression plasmid pRJ2. Abbreviations: amp-r, ampicillin resistance; ori, origin of plasmid replication; P_{T7}- ϕ 10, T7 promoter and start of the ϕ 10 gene¹⁷; bp, base pair. **b**, Expression of recombinant IL-1ra in *E. coli*. Either plasmid pRJ2 or plasmid pT5T was used to transform *E. coli* strain BL21 (DE3)¹⁶, which contains the phage T7 RNA polymerase gene under control of the inducible *lac* operon promoter. Expression of recombinant IL-1ra was achieved by growing the strain BL21 (DE3)-pRJ2 to mid-logarithmic phase, adding isopropyl β -D-thiogalactopyranoside (IPTG) to 1 mM, and incubating for 4 h. The control strain, BL21(DE3)-pT5T, was treated similarly. To prepare lysates, cells were collected by centrifugation, suspended in $\frac{1}{10}$ vol. of 50 mM Tris-HCl buffer, pH 7.0, and passaged twice through a french pressure cell at 20,000 lb in². Protein (5 μ g) from each lysate were electrophoresed on an 18% SDS-polyacrylamide gel³³, which was then stained with Coomassie brilliant blue. Lane 1, BL21(DE3)-pT5T with IPTG; lane 2, BL21(DE3)-pRJ2 without IPTG; lane 3, BL21(DE3)-pRJ2 with IPTG; lane 4, *M*_r markers. Arrow indicates the IL-1ra band. Abbreviation: bp, base pair.

originally developed by Studier and co-workers^{16,17}, and modified by Squires *et al.*¹⁸. The system links the expression of the mature antagonist (without the signal sequence) to that of the phage T7 ϕ 10 gene by a translational coupler (Fig. 4a). Figure 4b is a photograph of a Coomassie blue-stained gel to which we applied either control *E. coli* extract or extracts from *E. coli* in which the cDNA is expressed. There is a protein

band present in the latter extracts and not in control extracts that has a mobility equal to that of the nonglycosylated IL-1 antagonist (form x) produced by monocytes¹². We further analysed bacterial extracts for their ability to prevent IL-1-induced synthesis of prostaglandin E₂ (PGE₂) by foreskin fibroblasts (Table 1). Extracts from bacteria expressing the cDNA clearly inhibited the fibroblast response to IL-1 with a similar activity

to that of the natural IL-1ra¹², whereas control extracts had no inhibitory activity. This result shows that the IL-1ra cDNA clone codes for the IL-1 receptor antagonist.

In addition, the complete IL-1ra cDNA has been inserted into a mammalian cell expression vector downstream of the human cytomegalovirus early promoter (J. Vannice *et al.*, manuscript in preparation). After COS cell transfection, an IL-1 receptor antagonist activity is detected in the medium, indicating that the cDNA codes for the antagonist pre-protein, with a functional signal sequence.

IL-1ra mRNA induction in human cells

By northern-blot analysis, we found that the induction of IL-1ra mRNA in monocytes is rapid. It is detectable 1–2 h after plating cells on IgG (data not shown) and reaches a peak concentration 4–5 h after induction (Fig. 3a). This level is maintained for about 12 h and then gradually decreases. We predicted this time course from the SDS-PAGE analysis of the [³⁵S]methionine pulse-labelling experiment (see above). IL-1ra mRNA is also produced by the U937 cell line derived from transformed human monocytes. The mRNA is induced by phorbol myristate acetate (PMA; Fig. 3b), although the rate of induction and maximal level of this message both seem to be lower than observed in human IgG induced monocytes. IgG, lipopolysaccharide and zymosan A can also induce this message in U937 cells, although not as efficiently as PMA (data not shown).

Structural relationship to IL-1

How does IL-1ra inhibit the activity of IL-1? Hannum *et al.*¹² show that radiolabelled recombinant IL-1ra binds to the IL-1 receptor on EL-4 6.1 cells with an affinity close to that of IL-1α and IL-1β. It therefore seems reasonable to expect that IL-1

and IL-1ra have similar structures, at least in the region(s) recognized by the receptor. We compared the sequence of IL-1ra with that of human IL-1α and human IL-1β¹⁹ and found 26% homology between the antagonist and IL-1β, and 19% homology between the antagonist and IL-1α (Fig. 5a). Identical or similar residues between the three sequences are candidates for amino acids that are important for receptor binding. Those residues in common between IL-1α and IL-1β, but different in the IL-1ra sequence could be important for agonist activity.

Discussion

Control of IL-1 action could occur through regulation of its synthesis at the level of transcription, translation, protein processing or secretion, as previously proposed^{20–23}. But, there are circumstances in which it could be advantageous to control IL-1 effects post-synthetically by inhibiting its ability to interact with cellular targets. The isolation of a monocyte-derived IL-1 inhibitor that is a receptor antagonist¹² is experimental support for this mechanism. The physiological relevance of this mechanism of IL-1 regulation is supported by the finding that the antagonist is made by the same cells that make IL-1⁹, that its synthesis is induced *in vitro* by immune complexes which are a known pathological signal, and that levels of an inhibitor of IL-1 seem to be elevated in the urine of patients with pathologies including fever and myelomonocytic leukaemia (ref. 24, see below).

Cloning and expression of a cDNA for IL-1ra should aid the determination of the role of IL-1 in diseases because only disease processes involving IL-1 should be modified by this specific¹¹ antagonist. In addition, attempts to study IL-1-mediated diseases by correlating IL-1 levels with the disease state will be helped by simultaneous determinations of the levels of IL-1 and

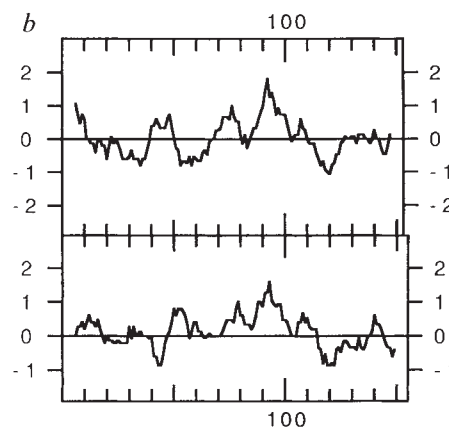
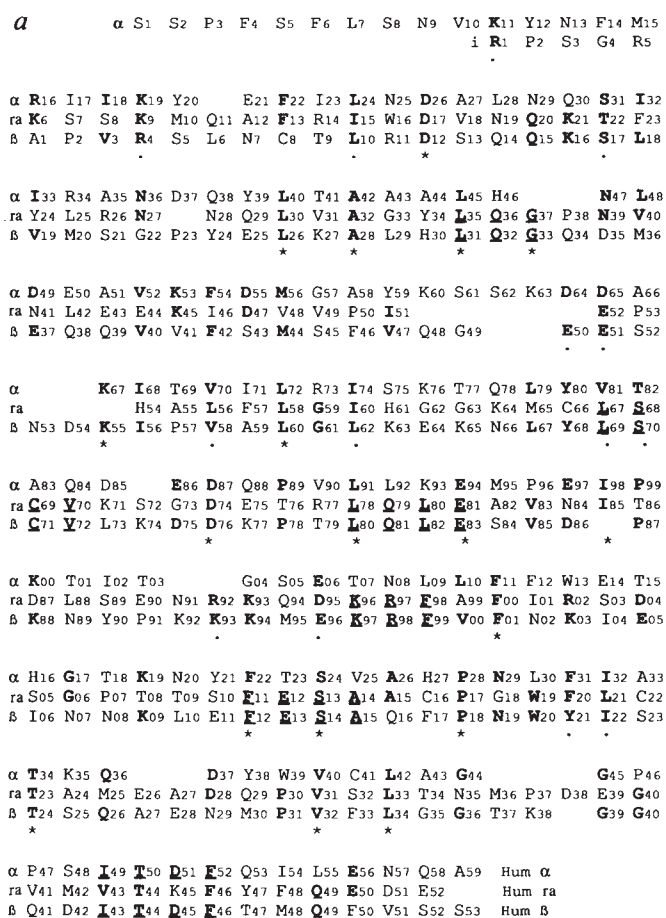


FIG. 5 Structural similarity between IL-1 and IL-1ra. **a**, Alignment of human IL-1α, IL-1β and IL-1ra. Alignment between the mature sequences of human IL-1α and IL-1β is based on the method of Young and Sylvester²⁵. IL-1ra is aligned to IL-1β according to the Beckmann Microgenie program using the normal protein homology parameters, except that gaps in the alignment are limited to turns between the beta strands of the IL-1β structure²⁶. Homologous or similar residues between at least two sequences are in bold. Homologous residues in all sequences are indicated by an asterisk. Similar residues in all sequences are indicated by a dot. Short stretches of homology (at least three consecutive homologous residues) between either IL-1β and IL-1ra, or IL-1α and IL-1β, are underlined. Hum, human. **b**, Comparison of hydrophilicity plots for IL-1β and IL-1ra. Hopp and Woods hydrophilicity plots⁴⁵ were drawn using the DNA Strider program written for the Macintosh computer⁴⁶. Positive values on the y-axis indicate hydrophilic regions. The x-axis refers to amino-acid residue number in the mature protein sequence. Upper panel, IL-1ra; lower panel, IL-1β.

its antagonist. Such attempts are otherwise fraught with difficulty because high levels of immunologically detectable IL-1 could be biologically ineffective owing to the presence of the antagonist and, conversely, low levels of IL-1 could be effective in the absence of the antagonist. These problems can now be corrected because it is possible to prepare reagents to make simultaneous measurements of agonist and antagonist levels in diseased tissue.

A global search of all sequences in the Protein Information Resource data base using the Intelligenetics FASTDB program showed that IL-1ra and IL-1 β are 30% homologous. The Young and Sylvester²⁵ alignment (Fig. 5a) finds slightly less homology (26%) because gaps are excluded from the beta strands of the three-dimensional (3-D) crystal structure of IL-1 β ²⁶. Many of the identical residues between IL-1ra and IL-1 β occur in short stretches of amino acids and are located in the C-terminal three-fifths of the two proteins. Hydrophilicity plots for the two proteins are remarkably similar in this same region (Fig. 5b). In this region, the two molecules could have similar 3-D structures and contain the residues that are necessary for receptor binding. In fact, when the homologous residues between IL-1ra and IL-1 β are mapped onto the 3-D structure of IL-1 β ²⁶, many of them cluster around one vertex of the pyramidal molecule. Other workers have attempted to align the sequences of IL-1 α and IL-1 β to identify homologous residues on the assumption that they would be involved in receptor binding. But many of these homologies are hydrophobic residues that could be important for maintaining the native structure of the protein². The addition of a third sequence, that of IL-1ra, to these sequence comparisons should help identify important residues.

The relationship between IL-1 and IL-1ra is reinforced by the fact that the organization of the IL-1ra mRNA is similar to that of both IL-1 α and IL-1 β ¹⁹. A large ORF encoding the protein is preceded by a relatively short 5' UTR and followed by a long 3' UTR.

Although there are sufficient similarities between IL-1ra and IL-1 β to support the assertion that the antagonist is a member of the IL-1 family of molecules, there are clearly significant differences between IL-1ra and either IL-1 α or β . First, IL-1ra is an antagonist of IL-1, biological activity and as such, cannot proceed through one or more of the steps in IL-1 signal transduction after binding to the receptor: these could include internalization and nuclear association²⁹, and activation of transcription factors leading to the induction of certain genes³⁰. The structural

elements leading to this difference are not yet known but comparison of the sequences of these molecules could provide a clue. Discovering this difference will be important because natural proteins that block the action of other proteins by competitively binding to their receptor without initiating a normal response have not been previously reported.

Second, IL-1ra differs from IL-1 α or IL-1 β in its mechanism of secretion. From the sequence or the cDNA it is predicted that IL-1ra is synthesized as a pre-protein consisting of a classical signal sequence fused to the mature active protein. IL-1 α and IL-1 β are also secreted proteins but they are synthesized as much larger precursors without obvious signal sequences, from which either 112 or 116 amino-acid residues are removed during processing^{2,23}. IL-1ra therefore apparently uses the classical pathway involving a signal sequence, signal recognition particle and leader peptidase, whereas IL-1 α and IL-1 β seem to be transported and processed by other so far unidentified mechanisms.

Third, the 3' UTR of the IL-1ra mRNA, although it is extremely A-U rich, lacks the sequence AUUUA. This pentanucleotide sequence has been implicated in regulating the half-life of mRNAs of many proteins that control the growth of cells, such as IL-1 α , IL-1 β , granulocyte macrophage colony-stimulating factor and tumour necrosis factor- α ²².

One other IL-1 inhibitor has been reported that has also been characterized as an IL-1 receptor antagonist. This is the urine-derived IL-1 inhibitor that has been partially purified and characterized by Seckinger *et al.*^{10,31}. There are good reasons to believe that this protein is closely related to the monocyte-derived receptor antagonist. The M_r s, isoelectric points and modes of action of the two proteins are similar, and we have recently identified the monocyte-derived antagonist in urine by western blotting with a polyclonal antibody against purified recombinant IL-1ra (data not shown).

We have cloned the cDNA for the human IL-1 receptor antagonist, IL-1ra, and expressed the protein in *E. coli*. Further study of this protein and its tissue location should be valuable in determining the role of IL-1 in human physiology. The protein should also be useful for studying the interactions between IL-1 and its receptor, and could also help elucidate the mechanism by which IL-1 triggers many cellular responses. Finally, the IL-1 antagonist is potentially useful as a therapeutic agent for the treatment of IL-1-mediated diseases in humans, in particular, inflammatory disorders such as rheumatoid arthritis. □

Received 1 December; accepted 19 December 1989.

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ACKNOWLEDGEMENTS. We thank David Hirsh and Anne Burkholder for critically reviewing the manuscript, Patricia Heimdal, Carol Wilcox, Bob Weaver and Fenneke Joslin for technical assistance, and Tim Klein for helping with figures.