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Cloning and characterization of the cDNAs for human and rabbit interleukin-1 precursor

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Abstract

DNA sequence complementary to the mRNA for rabbit interleukin-1 precursor (preIL-1) has been cloned from the cDNA library constructed using partially purified poly(A)$^+$RNA from induced rabbit alveolar macrophages by mRNA hybridization-translation assay. By using this cDNA as a probe, human IL-1 cDNA was isolated from the cDNA library prepared using poly(A)$^+$RNA from induced HL-60 cells, a human monocyte-like cell line. The amino acid sequences of the human and rabbit preIL-1 deduced from the cDNA sequences reveal their primary structures which consists of 271 and 267 amino acid residues, respectively. The amino acid sequence is 64 % conserved between human and rabbit. The difference in number of amino acid residues results from the carboxy-terminal extention of 4 amino acid residues in human preIL-1. Expression of the cloned human cDNA in E. coli yielded biologically active IL-1.

INTRODUCTION

In 1972, Gery and his co-workers demonstrated the presence of a soluble factor, called lymphocyte activating factor (LAF), in the culture medium of lipopolysaccharide (LPS)- or phytohemagglutinin (PHA)- stimulated human and mouse peripheral blood adherent leukocytes that augmented thymocyte proliferation (1). LAF was later shown to be a macrophage-derived immune mediator acting on T and B lymphocyte and was designated as interleukin-1 (IL-1) (2). Many recent studies have revealed that IL-1 or closely related substances are produced by a variety of cell types and have effects on various non-lymphoid cells as well as lymphoid cells (3-5). The molecular basis for the diversity of biological function of IL-1 remains unclear. It is important to investigate the molecular structure of IL-1 for understanding the molecular mechanism involved.

To elucidate the molecular structure of IL-1, we isolated rabbit IL-1 cDNA by mRNA hybridization-selection assay, which was then used as a probe to isolate human IL-1 cDNA. Here we report the cloning and sequence analysis of cDNAs for human and rabbit IL-1 precursor (preIL-1). The deduced amino acid

sequences of human and rabbit preIL-1 show the primary structures of human and rabbit preIL-1, which is composed of 271 and 267 amino acid residues, respectively. We also report the expression of cloned human IL-1 cDNA in Escherichia coli, yielding biologically active IL-1. Recently, two groups have reported the cloning of mouse (6) and human (7) IL-1 cDNA. However, no homology was found in the nucleotide sequence between them. The nucleotide sequences of human and rabbit preIL-1 cDNA in this report are homologous to that of mouse preIL-1, but not to human's reported. Therefore, this is the first report of the human and rabbit nucleotide sequences corresponding to that reported for mouse IL-1.

MATERIALS AND METHODS

Plasmids and bacteria

The plasmid pCT-1 (8) was provided by Prof. K. Matsubara, Institute for Molecular and Cellular Biology, Osaka University, and pBR322 was purchased from Bethesda Research Laboratories (Gaitherburg, USA). E. coli χ1776 was supplied by Dr. M. Inoue, Laboratory of Drug Resistance in Bacteria, Gumma University School of Medicine, and E. coli HB101 by Prof. S. Numa, Department of Medical Chemistry, Faculty of Medicine, Kyoto University.

Enzymes and reagents

Restriction endonucleases were purchased from Takara Shuzo (Kyoto, Japan), Toyobo (Osaka, Japan), New England Biolabs (Beverly, USA) and Bethesda Research Laboratories. Avian myeloblastosis virus reverse transcriptase was obtained from Life Science (St. Petersberg, USA), E. coli DNA polymerase I from New England Biolabs, the large fragment of E. coli DNA polymerase I (Klenow fragment) from Boeringer (Mannheim, FRG), calf thymus terminal deoxynucleotidyl transferase, E. coli alkaline phosphatase and T4 DNA ligase from Takara Shuzo, and endoribonuclease H, E. coli DNA ligase from P-L Biochemicals (Milwaukee, USA). The enzymes were used according to the supplier's instructions. Phorbol-12-myristate-13-acetate (PMA) and actinomycin D was obtained from P-L Biochemicals, oligo(dT)-cellulose type 2 from Collaborative Research (Lexinton, USA), Eagle's minimum essential medium, RPMI-1640 medium and fetal bovine serum (FBS) from Flow Laboratories (McLean, USA), E. coli lipopolysaccharide (LPS) and phytohemagglutinin (PHA) from Difco (Detroit, USA), and cycloheximide and retinoic acid from Sigma (St. Louis, USA). Radioisotopes were obtained from Amersham International (Amersham, UK). Ultrapure human IL-1 was obtained from Genzyme Corp. (Boston, USA). All the other reagents used were analytical grade.

Interleukin-1 activity assay

IL-1 activity was measured essentially as described (9). Mouse thymocytes (1 x 10^6 cells) obtained from 7-week-old C3H/He were incubated with 50 μl of the diluted samples in 200 μl of RPMI-1640 supplemented with 5 % FBS and 12.5 μg/ml of PHA. After incubation at 37°C for 54 h, cultures were pulse-labelled for 18 h with 1 μCi/well ^{3}H-thymidine. The authentic human IL-1 (100 units/ml) was used as standard.

Preparation of RNA

Rabbit alveolar macrophages were collected (10-12) and cultured in the presence of LPS (10 μg/ml), PMA (500 ng/ml) and cycloheximide(1 μg/ml). Human monocyte-like cell line, HL-60, was differentiated with PMA (500 ng/ml) and retinoic acid (500 ng/ml), and induced as above. Total RNA was extracted (13) from induced rabbit alveolar macrophages or induced HL-60 cells, and poly(A)$^+$RNA was isolated by oligo(dT)-cellulose chromatography (14). Poly(A)$^+$RNA from uninduced rabbit alveolar macrophages or uninduced HL-60 cells was isolated by the same procedure but without induction. Poly(A)$^+$RNA from induced rabbit alveolar macrophages was further fractionated by electrophoresis through acid-urea agarose gels (15,16). RNA extracted from each gel fraction was injected into Xenopus leavis oocytes (17). After incubation, an extract of oocytes was prepared and assayed for IL-1 activity. Poly(A)$^+$RNA extracted from the most active fraction was used for the following cloning experiments.

Construction and screening of cDNA libraries

For the isolation of rabbit preIL-1 cDNA, double-stranded cDNA was prepared by the method of Gubler and Hoffman (18) using 4.0 μg of gel-fractionated poly(A)$^+$RNA from induced rabbit alveolar macrophages and inserted into the PstI site of pBR322 by homopolymer tailing (19). The library was screened by the differential colony hybridization (16). Two replicas of the colony library were grown on nitrocellulose filters and lysed, and DNA from each colony was fixed to the filters. ^{32}P-labelled single-stranded cDNA probes were prepared using 1.0 μg of gel-fractionated poly(A)$^+$RNA from induced rabbit alveolar macrophages or 1.0 μg of the poly(A)$^+$RNA from uninduced rabbit alveolar macrophages. Two sets of filters were hybridized to induced and uninduced ^{32}P-labelled single-stranded cDNA probes respectively. Clones hybridized only with the induced probe were further screened by mRNA hybridization-selection assay (20). Plasmid DNAs were isolated (21) from groups of 10 clones, and 100 μg of plasmid DNA from each group was cleaved with EcoRI, denatured and fixed to a 24-mm diameter

nitrocellulose filter. Filters were then hybridized with 2.6 mg of poly(A)$^+$RNA from induced rabbit alveolar macrophages at 50°C for 5 h. The filters were then washed and hybridized RNA was recovered by heating from each filter and precipitated by adding ethanol. The RNA was dissolved in 10 μl of H_2O and injected into 10 oocytes of X. laevis at 50 nl per oocyte. IL-1 activity in the extract of oocytes was assayed. The plasmid DNAs were isolated from the individual clones of the positive group and examined essentially in the same manner as above except that 15 μg of plasmid DNA from each clone, 8-mm square nitrocellulose filters and 700 μg of poly(A)$^+$RNA from induced rabbit alveolar macrophages were used.

For the isolation of human preIL-1 cDNA, the cDNA library was constructed as above except that 5.0 μg of poly(A)$^+$RNA from induced HL-60 cells was used as a template. The cDNA library was screened by hybridization (22) with the ^{32}P-labelled PstI insert from cloned rabbit IL-1 cDNA at 55°C for 16 h.

DNA sequence analysis

The cDNA inserts to be analysed were subcloned into the M13 phage vectors and DNA sequencing was carried out by the chain termination method (23, 24).

Blot hybridization analyses

Northern blot hybridization was carried out according to the method of Thomas (25). Poly(A)$^+$RNA was electrophoresed through 1.1 % agarose gel and transferred to nitrocellulose filters. The blots were hybridized with the nick-translated (26) cDNA probes.

Southern blot hybridization (27) was conducted using human genomic DNA prepared from placental cells.

Expression of human IL-1 cDNA in E. coli

A new expression vector, pEP302, was developed (Fig. 5), the components of which are; HindIII to AatII (in clockwise direction), pBR322 fragment containing replication origin and tetracycline and ampicillin resistance gene with the deletion of the 641 base pairs (bp) AvaI-PvuII fragment (28); AatII to HpaI, pCT-1 (8) fragment containing the E. coli trp promoter-operator sequence (29); HpaI to ClaI, synthetic DNA with the sequence (5')AACTAGTACGC-AAGTTCACGTAAGGAGGTTAT(3') (underlined sequence is complementary to the 3'-terminal 9 nucleotide sequence of E. coli 16S ribosomal RNA (30); ClaI to HindIII, synthetic polylinker with the sequence (5')CGATTATGAATTCCCGGGC-AGATCTA(3'). The expression plasmid pHILP370 was constructed as follows. The 994-bp BalI-AhaIII fragment isolated from pHL4 was ligated with synthetic

adaptor containing an ATG initiation codon followed by the first nucleotide of the second codon of human preIL-1 sequence and inserted between the ClaI and SmaI sites of pEP302. To construct pHILP371, the ClaI-HindIII fragment of pHILP370 coding for amino-terminal 63 amino acid sequence was removed and ligated with synthetic adaptor containing codons for initiative methionine and serine at position 63 of human preIL-1. The oligodeoxyribonucleotides used were synthesized by the phosphotriester method (31). Expression plasmid constructed were used to transform (32) E. coli HB101. Ampicillin resistant transformants were selected and grown in 5 ml of the M9 medium (33) supplemented with 0.5 % casamino acids, 25 μg/ml of ampicillin and 20 μg/ml of 3-indoleacrylic acid at 37°C for 24 h. The E. coli cells were collected from 100 μl of the culture and lysed in 1 ml of the buffer as described (34). Twofold dilutions of the E. coli extracts were made and the IL-1 activity was measured. To estimate the molecular weight of the IL-1 activity produced in E. coli, whole cell lysates were analysed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) as described (35).

RESULTS AND DISCUSSIONS

Isolation of cDNA clones

Because rabbit alveolar macrophage was shown to be a potent producer of IL-1 (10, 11), we attempted to clone rabbit IL-1 cDNA, and to use this cDNA as a hybridization probe for screening human cDNA library. As the first step, rabbit alveolar macrophages were collected and stimulated to produce IL-1 by adding LPS and PMA. Poly(A)$^+$RNA was prepared from total RNA isolated from induced rabbit alveolar macrophages by oligo(dT)-cellulose chromatography and subsequently fractionated by acid-urea agarose gel electrophoresis. RNA samples extracted from gel fractions were injected into oocytes of X. laevis, the homogenates of which were assayed for IL-1 activity. An IL-1 activity was found in the fraction migrated slightly slower than 18S ribosomal RNA, and we used poly(A)$^+$RNA extracted from this fraction in the following cloning experiments. A cDNA library was constructed using this poly(A)$^+$RNA fraction by the method of Gubler and Hoffman (18). As IL-1 is an inducible polypeptide, we screened the cDNA library for clones having cDNA sequences specific for induction by differential colony hybridization. Two replicas of colony library grown on nitrocellulose filters were hybridized to either ^{32}P-labelled single-stranded cDNA synthesized from gel-fractionated poly(A)$^+$RNA of induced cells, or to ^{32}P-labelled single-stranded cDNA synthesized from poly(A)$^+$RNA of

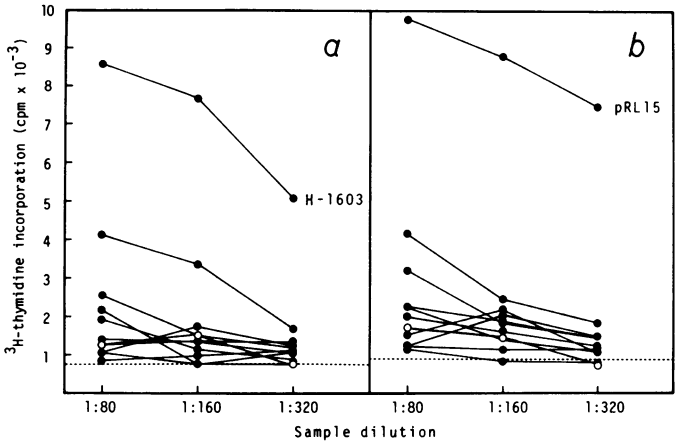

Fig. 1 Screening for rabbit IL-1 cDNA by mRNA hybridization-selection assay. (●), 10 groups of 10 clones selected by initial differential colony hybridization (a) or 10 individual clones from group H-1603 (b); (○), pBR322 as control. One positive group (H-1603) and one positive clone (pRL15) derived freom group H-1603 are indicated. The dotted line represents background incorporation.

uninduced cells. Out of 5,000 clones screened, 648 clones hybridized with the induced probe but not with the uninduced one. These clones were examined for the presence of IL-1 specific sequence by a mRNA hybridization-selection assay. Recombinant plasmid mixtures were isolated from groups of 10 bacterial clones, and plasmid DNA of each group was cleaved with EcoRI, denatured and fixed to a nitrocellulose filter. The DNAs on the filters were hybridized with poly(A)$^+$RNA from induced rabbit macrophages. Hybridized mRNA was recovered from each filter and translated in oocytes of X. laevis. Plasmid pBR322 was used as the negative control. One group (H-1603) out of 10 groups assayed, was clearly positive (Fig. 1a). The plasmid DNAs were isolated individually from each of 10 clones in group H-1603 and examined essentially in the same manner as above. Out of the 10 clones, one clone (designated as pRL15) gave a strongly positive result (Fig. 1b). The plasmid DNA was isolated from clone pRL15 and the cDNA insert was excised with PstI. This cDNA insert (about 1.1 kilobase pairs, kbp) was labelled with ^{32}P by nick-translation and hybridized to 648 clones selected in the initial differential colony hybridization experiment. Eight clones (other than clone pRL15) were strongly hybridized with this probe. The plasmid DNAs were isolated and characterized. Two clones (pRL15 and pRL26) were selected for nucleotide sequencing according to the strategy indicated in Fig. 2a.

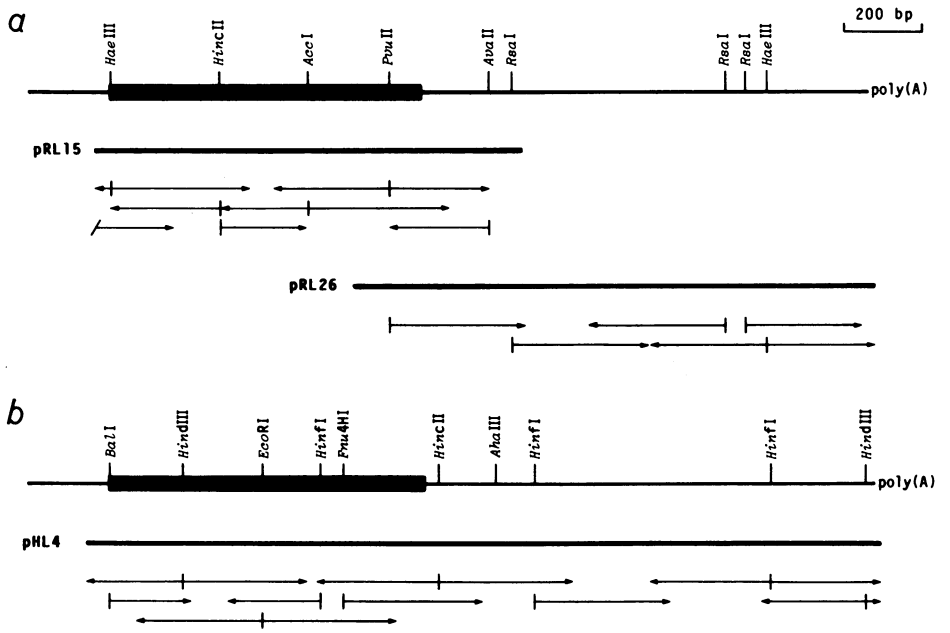

Fig. 2 Strategy for sequencing of cloned cDNAs encoding rabbit (a) and human (b) preIL-1. PreIL-1 mRNA is schematically displayed and the protein-coding region is shown as a shaded box. The cDNA clones are represented as a thick line below the mRNA, in which the poly(dG)/(dC) tails are not included. The direction and extent of sequence determination are shown by arrows. The PstI site located at left end of clone pRL15 is indicated as a slash mark.

Human monocyte-like cell line, HL-60, was used as a source for human IL-1 mRNA to construct a cDNA library, as we detected apparent IL-1 activity in the homogenates of X. laevis oocytes injected with poly(A)$^+$RNA from HL-60 cells induced with LPS and PMA, and cultured for 5 h. Poly(A)$^+$RNA was isolated from induced HL-60 cells and used to construct a cDNA library according to the procedure of Gubler and Hoffman (18). The cDNA insert of clone pRL15 was labelled with ^{32}P by nick-translation and used as a hybridization probe to screen the human cDNA library. Five clones out of 20,000 clones screened, were hybridized with this probe. The cDNA inserts from these clones were characterized by restriction endonuclease mapping and found to be related one another. One clone (designated as pHL4) having the longest cDNA insert of about 2.1 kbp was subjected to nucleotide sequencing according to the strategy indicated in Fig. 2b.

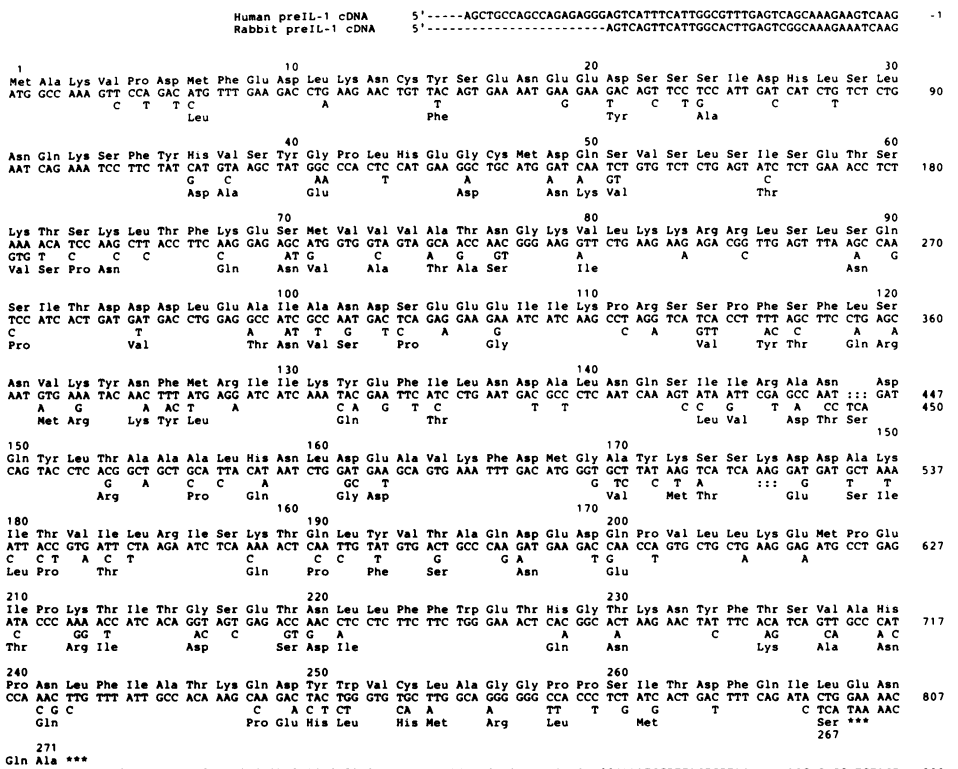

Fig. 3 Nucleotide sequence of the human (upper line) and rabbit (lower line) preIL-1 cDNA. The nucleotide sequence of the human sense strand, together with the deduced amino acid sequence, is shown. In the protein-coding region, only the rabbit nucleotide and amino acid sequences different from

the human sequence are shown; colons in either nucleotide sequene indicate a gap. The translational termination codons are indicated with asterisks, and the putative polyadenylation signals in the 3'-untranslated region are underlined. Both the 3'-terminal sequences presented are followed by a poly(A) tract.

DNA sequence analysis and deduced amino acid sequences

The human and rabbit cDNA sequences determined are shown in Fig. 3. There is a single large open reading frame in each cDNA sequence extending from ATG initiation codon (nucleotide residues 1-3) to a termination codon TAG or TAA (nucleotide residues 814-816 in human or 802-804 in rabbit). In 5'-untranslated region of both species, there is an in-frame termination codon TGA (nucleotide residues -19 to -21), 7 codons upstream from the ATG initiation codon. Each of the 3'-untranslated region of the human and rabbit cDNA sequences contains two polyadenylation signals (36), AATAAA (nucleotide residues 1657-1662 and 1944-1949 in human and 1646-1651 and 1927-1932 in rabbit), one of which is located 21 and 14 bp upstream from the poly(A) tract, respectively. Blot hybridization analysis of RNA from induced HL-60 cells and from induced rabbit macrophages exhibited a hybridization-positive band of about 2.3 kilobases (kb) (see Fig. 4), indicating that each of the human and rabbit cDNA sequences shown in Fig. 3 represents nearly full length copy of mRNA, in which only a part of the 5'-untranslated region (about 150 bp) is missed. In protein coding region, the nucleotide residues are highly conserved (77 %).

The nucleotide sequences of the human and rabbit cDNAs reveal the primary structures of human and rabbit preIL-1, which is composed of 271 and 267 amino acid residues having calculated molecular weights of 30,621 and 30,289, respectively. The difference in number of amino acid residues results from the carboxy-terminal structure of both sequences. In the rabbit sequence, translational termination codon TAA appears at immediately after codon for Ser residue at position 267, causing the lack of the carboxy-terminal 4 amino acid residues as compared with the human sequence. This implies that the carboxy-terminal 4 amino acid residues of the human preIL-1 are dispensable for IL-1 activity. In addition, one amino acid deletion (Ser between positions 148 and 149) and one amino acid addition (Lys at position 175) are observed in the human sequence. Because the molecular weight of IL-1 has been reported to be 12-16 kilodaltons (kD) (3-5, 10, 11), preIL-1 may be post-translationally processed (37) to a low-molecular-weight form of IL-1. Most of secretory proteins possess a signal peptide sequence at their

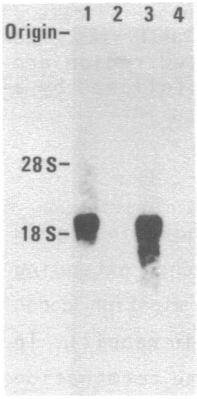

Fig. 4 Northern blot hybridization analysis of human and rabbit IL-1 mRNA. 10 μg of poly(A)$^+$RNA from induced (lane 1) and uninduced (lane 2) HL-60 cells and 6 μg of poly(A)$^+$RNA from induced (lane 3) and uninduced (lane 4) rabbit alveolar macrophages were analysed. Human and rabbit RNA were hybridized separately with their respective cDNA probes (see text).

amino-terminus which is eliminated during secretion (38), but preIL-1 shown here does not contain a typical signal peptide sequence, suggesting that preIL-1 is released from macrophages in a quite different manner from the ther secretory proteins. Another macrophage-derived monokine, tumor necrosis factor (TNF), also contains no typical signal peptide sequence, but a long hydrophobic segment is present in its amino-terminal region (39, 40). Each preIL-1 contains a tetrabasic sequence, Lys-Lys-Arg-Arg (positions 82-85), which may be the site of proteolytic processing (37). Human and rabbit preIL-1 contain three and two cysteine residues (positions 14, 47 and 253 in human and 14 and 47 in rabbit) which could form an intramolecular disulfide bond, and two and three possible sites of N-glycosidic linkage (41) (positions 102 and 141 in human and 64, 100 and 141 in rabbit), respectively. Comparison of the amino acid sequences of human and rabbit preIL-1 shows that 64 % of the amino acid residues are conserved.

Recently , two groups have reported the nucleotide sequences of mouse (6) and human (7) IL-1 cDNA, in which no homology was found between them. The nucleotide sequences of human and rabbit preIL-1 cDNA reported in this paper are homologous to that of mouse cDNA, but not to human's. These findings strongly support the idea that there may be two or more structurally distinct form(s) of IL-1 with identical or similar biological activity.

Northern and Southern blot hybridization analysis

Since IL-1 is known as an inducible polypeptide, we examined the expression and the size of human and rabbit IL-1 mRNA by northern blot hybridization analysis. Poly(A)$^+$RNA from induced or uninduced cells was separated by electrophoresis and transferred to nitrocellulose filters. The human and rabbit RNA were hybridized to the ~0.9-kbp PstI-HincII fragment

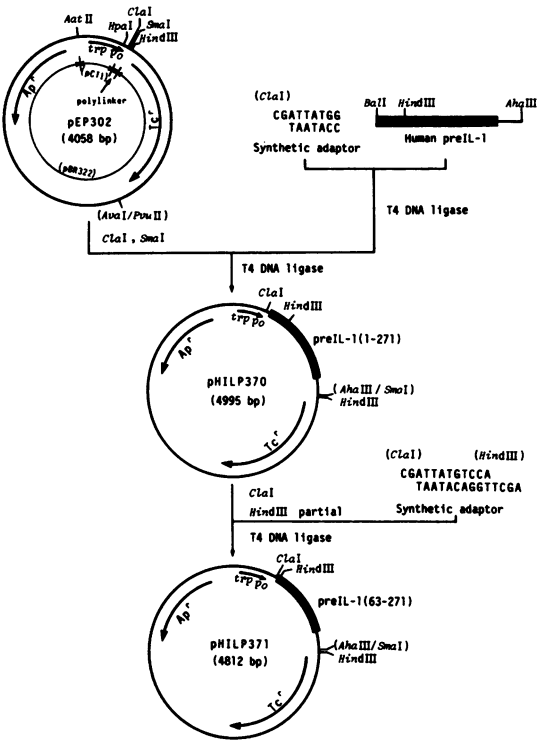

Fig. 5 Construction of human IL-1 expression plasmids. The construction is outlined in Materials and Methods.

derived from pHL4 and the ~1.1-kbp PstI insert of pRL15, respectively. As shown in Fig. 4, both human and rabbit RNA from induced cells represented a hybridization-positive band with a similar size estimated approximately 2.3 kb (lane 1 and 3), while no hybridization-positive band was detected in RNA from uninduced cells (lane 2 and 4). Thus, the expression of IL-1 mRNA closely correlated with IL-1 production.

Our preliminary experiments to study the human IL-1 gene structure by southern blot hybridization analysis suggest that there is only one gene for human IL-1 and no other closely related gene (data not shown). Further analysis is currently under way to elucidate the structure and organization of IL-1 gene.

Expression of human IL-1 cDNA in E. coli

To demonstrate that the isolated cDNA indeed encodes biologically active IL-1, the 994-bp BalI-AhaIII fragment of human preIL-1 cDNA containing the entire protein-coding sequence of 271 amino acid residues except for the ATG initiation codon and the first nucleotide of the second codon was isolated

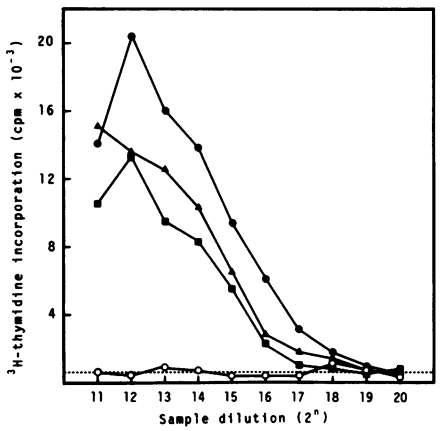

Fig. 6 Dose response curves for the IL-1 activity expressed in E. coli. (●), E. coli extract of HB101/pHILP370; (■), that of HB101/pHILP371; (○), that of HB101/pEP302; (▲), authentic human IL-1 (Genzyme, 100 units/ml). The dotted line represents background incorporation.

from clone pHL4, ligated with a short synthetic adaptor and inserted into the plasmid pEP302 (see Materials and Methods). The structure and the construction of this expression plasmid, pHILP370, is shown in Fig. 5. The E. coli HB101 strain transformed with this plasmid was grown and a cell extract was prepared to assay for IL-1 activity. As shown in Fig. 6, the extract from pHILP370-transformed HB101 cells exhibited considerable IL-1 activity, but no detectable activity was found in the extract from pEP302-transformed cells. The IL-1 activity detected showed the same titration curve as the authentic human IL-1 (Genzyme, 100 units/ml). We next constructed the expression plasmid, pHILP371 (Fig. 5), which contained the cDNA sequence corresponding to the carboxy-terminal 209 animo acid sequence of human preIL-1. E. coli cells transformed with pHILP371 also produced the biologically active IL-1 as shown in Fig. 6. These results suggest that at least 62 amino acid residues of amino-terminal sequence of preIL-1 are not essential for IL-1 activity.

The SDS-PAGE analysis of the E. coli whole cell lysates prepared from pHILP370- and pHILP371-transformed cells showed no difference in the protein profile as compared with that of pEP302-transformed cells (data not shown). However, in case of pHILP370-transformed cells, the IL-1 activity was found in the fractions migrated at 16~23-kD, when the eluates from successive gel slices were assayed for IL-1 activity (data not shown). This suggests that the precursor polypeptides produced in E. coli are degradated by some proteinase activity and generated an active low-molecular-weight form(s) of IL-1. The structure and biological and biochemical properties of this low-molecular-weight form(s) of IL-1 remains to be characterized. This may

provide a useful information for understanding the structure-function relationships of IL-1 and for comparing with natural IL-1.

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