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Immunology

Interleukin 1 receptor antagonist is a member of the interleukin 1 gene family: Evolution of a cytokine control mechanism

(gene organization/gene duplication/mutation rates/protein structure/secretion)

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ABSTRACT **Interleukin 1 receptor antagonist (IL-1ra) is a protein that binds to the IL-1 receptor and blocks the binding of both IL-1α and -β without inducing a signal of its own. Human IL-1ra has some sequence identity to human IL-1β, but the evolutionary relationship between these proteins has been unclear. We show that the genes for human, mouse, and rat IL-1ra are similar to the genes for IL-1α and IL-1β in intron–exon organization, indicating that gene duplication events were important in the creation of this gene family. Furthermore, an analysis of sequence comparisons and mutation rates for IL-1α, IL-1β, and IL-1ra suggests that the duplication giving rise to the IL-1ra gene was an early event in the evolution of the gene family. Comparisons between the mature sequences for IL-1ra, IL-1α, and IL-1β suggest that IL-1ra has a β-stranded structure like to IL-1α and IL-1β, consistent with the three proteins being related. The N-terminal sequences of IL-1ra appear to be derived from a region of the genome different than those of IL-1α and IL-1β, thus explaining their different modes of biosynthesis and suggesting an explanation for their different biological activities.**

Interleukin 1 (IL-1) is believed to be important in mediating inflammatory and immune responses (1, 2). IL-1 proteins have been cloned from several species, and in each case two proteins have been identified (3). These two proteins, IL-1α and IL-1β, have the same biological activities and bind to the same cell-surface receptors (4–6).

Inhibitors of IL-1 have been reported in the literature over the past several years (7, 8). We recently reported the isolation, cloning, and characterization of a cDNA for a protein from human monocytes, IL-1ra, that acts as a receptor antagonist on the 80-kDa IL-1 receptor (9, 10). Subsequently, Carter *et al.* (11) isolated and cloned an IL-1 inhibitor from the human monocyte-like cell line U937 and showed on the basis of its sequence that this molecule is IL-1ra. IL-1ra appears to be related to the IL-1 family based on its homology and similar hydropathy profile to IL-1β, but unlike the IL-1 proteins, IL-1ra has a classical secretory leader peptide and is glycosylated at a consensus N-linked glycosylation site (9, 10).

To determine whether IL-1 and IL-1ra evolved from a common precursor or whether their similarities are the result of convergent evolution of a structure that can bind to the IL-1 receptor, we isolated genomic clones for IL-1ra from human, mouse, and rat and compared their coding sequences with those of IL-1α and IL-1β from the same and other species.* Our findings support the view that these three proteins have a common ancestor and that the gene leading to IL-1ra diverged early in the evolutionary history of the IL-1 family. The results also argue for an important role of the sequence around the N terminus of IL-1ra in the unique properties of this protein.

MATERIALS AND METHODS

Isolation of Genomic Clones. The clones for human, mouse, and rat IL-1ra were isolated from genomic libraries [human, see ref. 12; mouse (DBA/2J) and rat (Sprague–Dawley) Clontech] propagated on the recBC host *Escherichia coli* strain CES200 (13). The human library was screened (14) with a 566-base-pair (bp) *Pst* I fragment from the cloned cDNA (10). Phage DNA was prepared from purified positive plaques (10), and an 8-kbp *Bam*HI fragment having all coding regions of the gene was transferred to pUC18 (Pharmacia) for sequencing.

A partial clone for mouse IL-1ra was isolated by screening the mouse library with the 566-bp *Pst* I fragment (see above) at reduced stringency (40% formamide instead of 50%). *Bam*HI-digested DNA isolated from purified positive plaques was subjected to Southern blot analysis (15). Probing with four oligonucleotides, 33–39 residues long (16), representing the four exons of the human clone, we found that exon 2 is on a 1.6-kbp fragment and exons 3 and 4 are on a 4.4-kbp fragment. Exon 1 was not on any of these clones. The two *Bam*HI fragments were transferred to pUC13 for sequencing.

Exon 1 of the mouse IL-1ra gene in EL4 cells (ATCC) was isolated by AmpliTaq DNA polymerase (Perkin–Elmer/Cetus)-catalyzed PCR amplification (17) of genomic DNA (18). One PCR primer (a 30-mer) had the antisense sequence of a segment of mouse exon 2, and the other primer had the sequence coding for the first 10 amino acids of the rat IL-1ra signal peptide (see below). The 2-kbp fragment generated by this reaction was cloned into pUC13 and sequenced.

Also, the mouse genomic library was rescreened with an oligonucleotide having the coding sequence for mature rat IL-1ra amino acids 8–14. Phage DNA from positive plaques was the substrate for PCR amplification. One of the PCR primers had the sequence encoding the N-terminal six amino acids of the rat IL-1ra signal peptide and the other primer, 5′-ATATCTCCTATTCCTGC-3′, was derived from within the sequence of intron 1 obtained from the cloned 2-kbp fragment of EL4 cell DNA. An ≈230-bp fragment was generated from this PCR.

The rat library was screened by using three oligonucleotide probes (16) based on the first exon of the human gene and the second and fourth exons of the mouse gene. DNA from positive purified plaques was analyzed by Southern blotting. A 7-kbp *Hin*dIII fragment that hybridized to the same three probes was transferred to pUC13 for sequencing.

Sequencing the Coding Regions of IL-1ra Genes. Sequencing of exon coding regions and flanking sequences was

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Abbreviations: IL-1, interleukin 1; IL-1ra, IL-1 receptor antagonist; cns, consensus; Myr, million years.

*The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M63099–M63101).

Table 1. Percentage amino acid identities between IL-1α, IL-1β, and IL-1ra

	IL-1α			IL-1β			IL-1ra		
	H	M	R	H	M	R	H	M	R
IL-1α									
H	100								
M	54	100							
R	58	79	100						
IL-1β									
H	22			100					
M		17		78	100				
R			19	78	91	100			
IL-1ra									
H	18			26			100		
M		16			30		77	100	
R			15			30	75	90	100

Values are based on pairwise sequence comparisons as described in the text. H, human; M, mouse; R, rat.

performed (19) with a Sequenase kit from United States Biochemical. Primers for sequencing the 8-kbp *Bam*HI fragment from the human clone were derived from the known sequence of the human cDNA. The 4.4-kbp and the 1.6-kbp *Bam*HI fragments from the mouse clone were sequenced with oligonucleotides used for probing the Southern blot and other primers as needed. The 230-bp PCR product containing mouse exon 1 coding sequences was purified by gel electrophoresis (20) and sequenced directly by using the PCR primers. Sequencing of the rat clone was done with oligonucleotides used in screening the library and other primers as needed. Intron–exon junctions identified by comparing genomic sequences with the sequence of human IL-1ra cDNA (10) all correspond to consensus sequences for splice sites (21).

Mutation Rate and Duplication Time Calculations. Mutation rates for IL-1α, IL-1β, and IL-1ra were calculated based on percentage identities from pairwise comparisons of protein sequences (Table 1) applied to the following equations (22):

$$K_{I-II} = -\ln(1 - d/n), \quad [1]$$

and

$$k = K_{I-II}/2T. \quad [2]$$

Combining Eqs. 1 and 2 gives

$$k = -\ln(1 - d/n)/2T, \quad [3]$$

where d is the number of differences (from Table 1), n is the number of residues being compared, T is the time since divergence in years (23, 24), and k is the mutation rate in changes per year. K_{I-II} is the difference coefficient.

Assuming constant rates of mutation, one can estimate times for gene duplication events based on percentage identities from comparisons of the different IL-1-related proteins (i.e., IL-1α vs. IL-1β, IL-1β vs. IL-1ra, IL-1α vs. IL-1ra; Table 1) applied to a rearranged form of Eq. 3:

$$T = -\ln(1 - d/n)/2k, \quad [4]$$

where k is the average mutation rate for the two proteins being compared.

RESULTS

Isolation and Sequencing of Genomic Clones for Human, Mouse, and Rat IL-1ra. Human, mouse, and rat genomic clones encoding IL-1ra were isolated from λ genomic libraries using either the human cDNA or exon-specific oligonucleotides as probes. Insert sequences were determined for the protein coding regions and intron–exon junctions. The human, mouse, and rat IL-1ra genes have the same intron–exon organization (Fig. 1). In each case, the first intron lies between the second and third base of the 14th codon of the mature protein, the second intron is between the first and second base of the 44th codon, and the third intron is located between the 81st and 82nd codon.

FIG. 1. Genomic clones for human, mouse, and rat IL-1ra. The complete protein coding portions of the genes for human, mouse, and rat IL-1ra are shown aligned based on homology. Underlined sequences correspond to oligonucleotides used as probes for library screening or primers for sequencing and PCR amplification. Dots at the start of the mouse sequence indicate that the sequence has not been determined directly, but it must be similar to the rat sequence because PCR amplification of mouse DNA was primed with an oligonucleotide based on the rat sequence (underlined) in that region. Arrows indicate the codon for the N terminus of mature IL-1ra, determined directly for the human protein (9) and presumed to be the same for the mouse and rat protein, and the locations of the three introns, determined by comparison with the sequence of the human IL-1ra cDNA (10).

FIG. 2. Alignment of IL-1 sequences and generation of consensus sequences. For IL-1ra (*a*), the human, mouse, and rat precursor protein sequences are compared. Amino acids are represented by the single letter code. Numbering begins at the N terminus of the mature protein. Residues in boldface type are identical in the three species. Symbols in the cns sequence for mature IL-1ra are defined as follows: uppercase symbols, two or three of the three sequences have the same amino acid and all three sequences have related amino acids at that position; lowercase symbols, two of the three sequences have the same amino acid and the third sequence has an unrelated amino acid; x, no obvious preferred residue. Related amino acids are grouped similar to the method of Dayhoff *et al.* (25). The five groups are as follows: 1, I, L, M, V; 2, F, W, Y; 3, R, H, K; 4, D, N, E, Q; 5, A, S, T. Dots are as in Fig. 1. For IL-1α (*b*) and IL-1β (*c*), the (mature) human, mouse, rat, bovine, and rabbit sequences are compared. Residues in boldface type are identical among these five species. Symbols in cns sequences for IL-1α and IL-1β are defined as follows: uppercase symbols, four or five of five sequences have the identical residue, or three of five sequences have the identical residue and the remaining two sequences have a related amino acid; lowercase symbols, three of five sequences have the same residue, or (in the absence of a majority amino acid), all five sequences have amino acids in the same group; x, no preferred amino acid at that position.

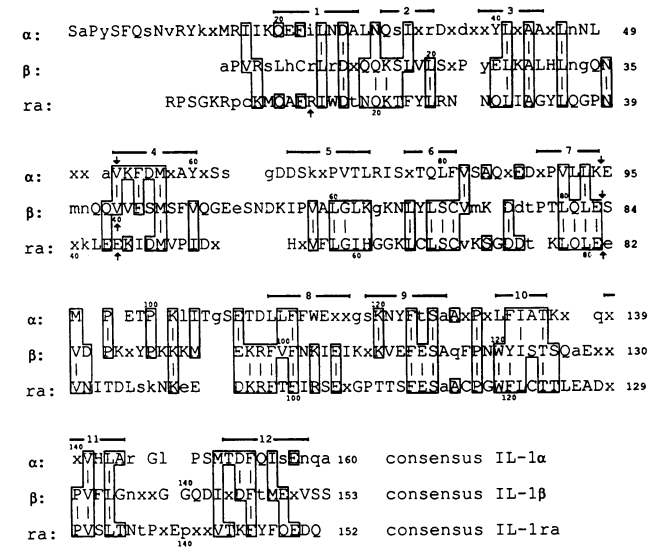

FIG. 3. Alignment of cns sequences for IL-1α, IL-1β, and IL-1ra. cns amino acid residues (uppercase symbols) that are identical between either IL-1α and IL-1β or IL-1β and IL-1ra are indicated by a vertical bar. Related residues (see Fig. 2) are enclosed in boxes. Common motifs within β-strands (26) are indicated by horizontal bars (numbered 1–12) above the IL-1α sequence. Residues are numbered based on cns sequences of the proteins. Arrows show the locations of intron–exon junctions.

The proteins encoded by the IL-1ra genes have very similar sequences (Fig. 2*a*, Table 1). There is a very high level of conservation between the mouse and rat sequences and a moderately high degree of conservation between the human sequence and the two rodent sequences. This conservation is similar to that seen among these three IL-1β sequences and is significantly greater than that seen among the same IL-1α sequences (Table 1).

Sequence Comparisons Between IL-1 and IL-1ra. To identify the evolutionary relationships between IL-1ra, IL-1α, and IL-1β, consensus (cns) sequences for each protein were derived (Fig. 2) and then aligned (Fig. 3). This alignment was used to compare the IL-1α, IL-1β, and IL-1ra sequences for each individual species in order to calculate the approximate time of duplication of these genes (see below). The cns IL-1ra was derived from the human, mouse, and rat sequences (Fig. 2*a*). cns IL-1α and cns IL-1β (Fig. 2 *b* and *c*) are based on the sequences for these proteins from human, mouse, rat, bovine, and rabbit (3, 27–33). Our analysis is not changed significantly when the comparisons are carried out with cns sequences for IL-1α and IL-1β derived only from human, mouse, and rat protein sequences (data not shown).

To align cns IL-1α and cns IL-1β, we compared the three-dimensional structures of human IL-1α and human IL-1β (26, 34) and aligned those residues that appear to be structurally equivalent (or near equivalent) in the two proteins (Fig. 3). The gaps introduced to preserve the alignment of the equivalent residues are all located in surface loops rather than in the β-stranded core, which is the major structural motif of both proteins. Because the three-dimensional structure of IL-1ra has not yet been determined, we were unable to use the same procedure for aligning cns IL-1ra with cns IL-1α or cns IL-1β. Nevertheless, this alignment was relatively easy because, aside from the N-terminal region of IL-1ra, the homology between IL-1ra and IL-1β leaves little room for ambiguity. In aligning IL-1α and IL-1ra separately with IL-1β we, of course, aligned these two proteins with each other; this IL-1α/IL-1ra alignment is very similar to that obtained in a comparison of these sequences by the Intelligenetics ALIGN program (data not shown).

Using the alignment in Fig. 3, we performed pairwise comparisons between the IL-1α, IL-1β, and IL-1ra sequences for human, mouse, and rat, and we determined the fraction of identical residues between these sequences (Table 1). These data indicate that IL-1ra and IL-1β are more homologous to each other than either of them is to IL-1α.

Comparison of Intron Locations. A comparison of the intron–exon organization of the genes for IL-1α, IL-1β, and IL-1ra is shown in Fig. 4. The similarity between the IL-1α and IL-1β genes has supported the view that gene duplication was an important event in the evolution of the two genes (35–37). This argument is easily extended to IL-1ra; when the positions of the second and third introns of the IL-1ra gene (Fig. 4) are mapped onto the cns sequence alignment (Fig. 3), their locations coincide precisely with those of the fifth and sixth introns of the two IL-1 genes. The location of the first intron in IL-1ra does not correspond to introns in either IL-1α or IL-1β, and this is a major difference between the IL-1ra gene and the IL-1α and IL-1β genes. However, we believe that the presence of this intron does not argue against the view that IL-1ra is evolutionarily related to IL-1α and IL-1β, but that the modern IL-1 and IL-1ra genes arose by a partial duplication of an ancestral IL-1 gene (see below).

DISCUSSION

IL-1ra Is a Member of the IL-1 Gene Family. We report here the cloning of the genes for human, mouse, and rat IL-1ra and the sequence of the coding regions of these genes. We have compared the deduced protein sequences and intron–exon organization of these genes to those of IL-1α and IL-1β. Several features of these comparisons indicate that the three IL-1 receptor ligands have a common evolutionary ancestor.

First, the extensive sequence similarity between IL-1ra and IL-1β (26–30% identity; Table 1) is probably more than would be required for receptor binding alone. In fact, only ≈5% of the amino acid residues in human growth hormone or complement protein C5a (39, 40) are responsible for binding to their respective receptors. Thus, it appears that much of the IL-1ra/IL-1β sequence similarity is needed for the proteins to assume similar structures, consistent with the fact that most of the homology between cns IL-1ra and cns IL-1β (Fig. 3) is within the structurally important β-strands of IL-1β (34). In addition, the gaps introduced to align cns IL-1ra to cns IL-1α and cns IL-1β are all aligned with loops in the IL-1α and IL-1β structures, implying that there are β-strands in IL-1ra like those in IL-1α and IL-1β.

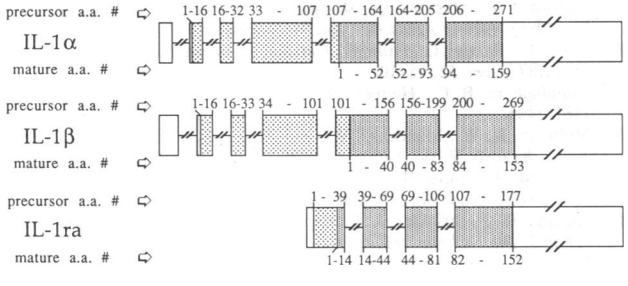

FIG. 4. Diagrammatic representation of the genes for IL-1α, IL-1β, and IL-1ra. The IL-1α and IL-1β genes are based on previously published reports (35–38), and the IL-1ra gene is based on the sequences of the cloned genes for human, mouse, and rat IL-1ra (Figs. 1 and 2*a*). Boxes correspond to exons, and broken horizontal bars are introns. The left end of the gene is the start of transcription. Empty boxes are untranslated regions, lightly stippled areas are translated sequences, and heavy stippling represents sequences encoding mature proteins. Amino acid (a.a.) numbering for the precursor proteins and the mature proteins is based on the human sequences. Mature a.a. 1 for IL-1α, IL-1β, and IL-1ra, corresponds to precursor a.a. 113, 117, and 26, respectively.

The alignment of consensus sequences also allows us to compare the positions of intron–exon junctions in the IL-1ra gene with those found in the genes for IL-1α and IL-1β. This comparison, showing that the positions of the second and third introns of the IL-1ra gene coincide precisely with the positions of the fifth and sixth introns in the two IL-1 genes (Figs. 3 and 4), indicates that the three genes were created by duplication of an ancestral IL-1 gene.

The N Terminus of IL-1 Proteins May Determine Their Different Properties. The first intron of the IL-1ra gene, which splits the 14th codon of the mature protein (Fig. 1), has no counterpart in either the IL-1α or IL-1β genes. Furthermore, the N-terminal sequence of cns IL-1ra through Trp-16 of the mature protein shows no significant homology to the same region of cns IL-1α or cns IL-1β. We believe that these features are due to an important event leading to the divergence of IL-1ra from IL-1—namely, a partial duplication of the ancestral IL-1 gene (Fig. 5; see below). Apparently, only sequences downstream from the codon for Trp-16 (of IL-1ra) were duplicated. Thus, the first exon and a small part of the second exon of the IL-1ra gene are presumably derived from some other segment of the genome than the same portions of the IL-1α and IL-1β genes.

The lack of homology between IL-1ra and IL-1α or IL-1β near the N terminus may provide an explanation for the biosynthetic and functional differences between IL-1ra and the two IL-1 proteins. One of the notable differences between IL-1ra and the two IL-1 proteins is in their mechanism for secretion from the cell. IL-1ra appears to be a typical secreted protein, whereas both IL-1α and IL-1β are directed to the cell surface and secreted through an uncharacterized pathway involving a nonleader peptidase-catalyzed proteolytic event (9, 10, 41–44). The secretion of IL-1ra is apparently mediated by a classical signal sequence present at the N terminus of the protein that is encoded in the first exon of the IL-1ra gene. Neither IL-1α nor IL-1β has such a sequence. Thus, the IL-1 gene family resembles the heparin binding (fibroblast) growth factor (HBGF) family of proteins, in that at least two HBGF proteins have a normal signal sequence and others, notably acidic and basic fibroblast growth factor, lack such a sequence (45).

Another significant difference between IL-1ra and IL-1α or IL-1β is in their respective activities after binding to the 80-kDa receptor. Evidence that the activity of IL-1 might be

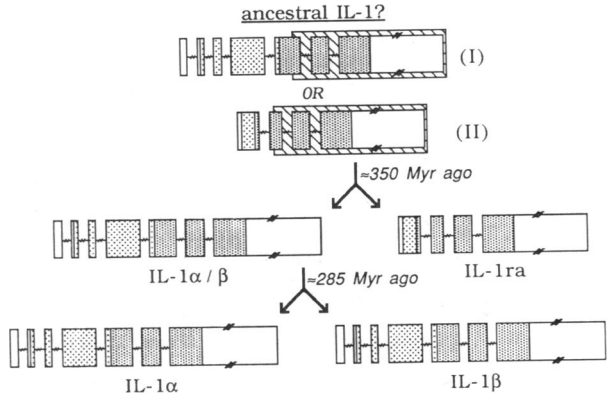

FIG. 5. Gene duplications during the creation of the IL-1 family. The structures of the modern IL-1α, IL-1β, and IL-1ra genes are shown (see Fig. 4), along with the presumed structure for the immediate ancestral gene to IL-1α and IL-1β (IL-1α/β). The structure of the "original" ancestral IL-1 gene is not known but may resemble either the modern IL-1α and IL-1β gene (I) or the modern IL-1ra gene (II). Forked arrows indicate gene duplication events. The time of each duplication is shown. The hatched area (in I and II) is the segment of the original ancestral gene that duplicated to give rise to the IL-1ra gene and the IL-1α/β gene.

encoded in its N-terminal area is provided by a recent paper (46) in which Arg-11 of mature human IL-1β is replaced by a glycine. This mutein was shown to be a much weaker agonist than wild-type IL-1β on mouse T cells, while retaining the ability to bind with high affinity to the IL-1 receptor on these cells.

Mutation Rates and Time of Divergence of the Three IL-1 Receptor Ligands. IL-1β and IL-1ra appear to be evolving at a slower rate than IL-1α. Based on the data in Table 1 and an estimated time of divergence for primates and rodents of 75 million years (Myr) (23), the calculated mutation rates (22) for IL-1α, IL-1β, and IL-1ra are $3.6–4.2 \times 10^{-9}$ yr^{-1}, $1.6–1.7 \times 10^{-9}$ yr^{-1}, and $1.7–1.9 \times 10^{-9}$ yr^{-1}, respectively. These rates are similar to those based on a divergence time for mouse and rat as 25–30 Myr ago (24). Applying these mutation rates to the percentage identities between IL-1α and IL-1β, IL-1β and IL-1ra, and IL-1α and IL-1ra (Table 1), we estimate that the IL-1/IL-1ra duplication occurred 320–400 Myr ago, roughly corresponding to the time of the appearance of amphibians (23), whereas the IL-1α/IL-1β separation apparently occurred more recently, approximately 270–300 Myr ago (Fig. 5), consistent with the estimate of Young and Sylvester (47). The time of the IL-1/IL-1ra duplication may require a slight correction (from an average of $\approx$360 Myr ago to $\approx$350 Myr ago) to account for the possibility that only part of the gene was duplicated. Our data therefore favor the hypothesis that the gene for IL-1ra was the first to diverge from the common ancestor of this family. An alternative possibility, that IL-1α diverged first, is favored by the observation that the sequence of IL-1α has diverged more from IL-1ra or IL-1β than these two proteins have from each other (Fig. 3, Table 1). However, our analysis of mutation rates shows that this simply reflects the fact that IL-1α is mutating faster than the other two proteins.

An analysis of the sequences of the IL-1 family of proteins and the structure of their genes indicates that they have evolved from a common ancestor. From their mutational rates, it appears that the divergence of an agonist and an antagonist was an early event in the history of IL-1, and was probably associated with the introduction of DNA encoding different sequences around the N terminus of these proteins.

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