Four New Members Expand the Interleukin-1 Superfamily*

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We report here the cloning and characterization of four new members of the interleukin-1 (IL-1) family (FIL1z, FIL1e, FIL1c, and FIL1α, with FIL1 standing for "Family of IL-1"). The novel genes demonstrate significant sequence similarity to IL-1α, IL-1β, IL-1ra, and IL-18, and in addition maintain a conserved exon-intron arrangement that is shared with the previously known members of the family. Protein structure modeling also suggests that the FIL1 genes are related to IL-1β and IL-1ra. The novel genes form a cluster with the IL-1s on the long arm of human chromosome 2.

The cytokine interleukin-1 (IL-1) elicits a wide array of biological activities that initiate and promote the host response to injury or infection, including fever, sleep, loss of appetite, acute phase protein synthesis, chemokine production, adhesion molecule up-regulation, vasodilatation, the pro-coagulant state, increased hematopoiesis, and production and release of matrix metalloproteinases and growth factors (1). It does so by activating a set of transcription factors that includes NFκB and AP-1, which in turn promote production of effectors of the inflammatory response, such as the inducible forms of cyclooxygenase and nitric oxide synthase (2, 3). Interleukin 1 activity actually resides in each of two molecules, IL-1α and IL-1β, which act by binding to a common receptor composed of a ligand binding chain, the type I IL-1 receptor, and a required signaling component, the IL-1R accessory protein (AcP) (4–7). A third member of the family, the IL-1 receptor antagonist (IL-1ra), also binds to the type I IL-1 receptor but fails to bring about the subsequent interaction with AcP, thus not only not signaling itself but also, by blocking the receptor, preventing the action of the agonist IL-1s (8, 9). Additional regulation is provided by the type II, or decoy, IL-1 receptor, which binds and sequesters the agonist IL-1s (especially IL-1β) without inducing any signaling response of its own (10–13). The two agonist IL-1s (IL-1α and IL-1β) are synthesized as larger precursors which undergo proteolytic removal of their pro-domains to generate the mature cytokines (14). At least for IL-1β, this processing is coupled to secretion (15, 16). IL-1ra, in contrast, contains a signal peptide and is secreted by the more traditional route through the endoplasmic reticulum (9).

Recently, another cytokine, interleukin 18 (17, 18) was recognized to be related to the interleukin-1 family based on the similarity of its amino acid sequence and predicted tertiary structure (19). IL-18 induces the production of γ-interferon from T cells, especially in combination with IL-12, and stimulates the killing activity of cytotoxic T lymphocytes and NK cells by up-regulating Fas ligand (20). Like the agonist IL-1s, IL-18 contains a prodomain that is removed by the same protease, caspase-1, that processes IL-1 (21, 22). Consistent with its being related to the IL-1s, IL-18 binds a receptor which is homologous to the IL-1 receptor. The ligand-binding chain IL-1Rrp1 (or IL-18Rα) (23, 24) was cloned initially on the basis of its homology to the IL-1R (25). The signaling subunit (IL-18Rβ) was originally named AcPL (AcP-like) for its similarity to the IL-1R signaling subunit (26). The IL-18 receptor subunits are encoded in the same gene cluster on chromosome 2 as are the type I and II IL-1 receptors (25–27).

We have searched for novel members of the IL-1 family. We report here the sequences and some of the characteristics of four genes that appear to have descended from the same common ancestor as did IL-1α, IL-1β, IL-1ra, and IL-18. We propose that these novel molecules be designated FIL1z, -e, -c, and -α, with FIL1 being an acronym for Family of IL-1.

EXPERIMENTAL PROCEDURES
Cloning of Novel Human IL-1 Family Members

The following details supplement the general descriptions given under "Results" for the cloning of the individual IL-1 family members.

**FIL1z—**A 469-base pair single-stranded 32P-labeled PCR product spanning the entire mouse FIL1z coding region (found in GenBank™ W08205) was used to probe a human placenta cDNA library (in λUni-ZAP XR; Stratagene number 937225) (hybridization in 40% formamide at 42 °C; wash in 0.3 M NaCl at 55 °C). Several clones were isolated, all of which appeared to lack the full open reading frame by comparison with mouse FIL1z. Vector-anchored PCR on DNA from the same library was used to isolate the remaining coding sequence.

**FIL1e—**A human genomic library (Stratagene catalog number 946205; in AFixII) was screened using a 32P-labeled single-strand DNA probe corresponding to the entire IL-1-like coding sequence present in GenBank™ EST AA030324 (hybridization in 45% formamide at 42 °C; wash in 0.3 M NaCl at 63 °C). The insert from one positive plaque was mapped to locate the hybridizing region, sequencing of which then revealed the 3′-most exon of the human FIL1e gene. 5.3 kilobases of human genomic DNA to the 5′ side of this exon was isolated using the CLONTECH Human GenomeWalker kit (catalog number K1803-1). Sequencing of this DNA allowed identification of the remaining coding exons. The structure of the gene was confirmed by isolation of a PCR product in which the predicted exons were indeed spliced, using as template first-strand cDNA from the cell lines HL60 and THP1, and from human thymic tissue. Interestingly, while the original genomic DNA sequence coded for glutamine at amino acid 12, cDNA clones from all three sources contain arginine at amino acid 12.

**FIL1α—**The FIL1α open reading frame was identified in a cDNA library made from the pancreatic tumor cell line HPT-4. FIL1α—A human genomic DNA sequence was obtained using the CLONTECH Human GenomeWalker kit (catalog number K1803-1).

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† The nucleotide sequences reported in this paper for FIL1z, FIL1e, FIL1c, and FIL1α have been deposited in GenBank™ with accession numbers AF201830, AF201831, AF201832, and AF201833.

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1 The abbreviations used are: IL, interleukin; AcP, accessory protein; IL-1ra, interleukin 1 receptor antagonist; PCR, polymerase chain reaction; PDF, probability density function; LPS, lipopolysaccharide.
Structure Modeling

Table I

| Molecule | Primary or nested | Sense/anti | Sequence | No. cycles | Anneal °C |
|----------|------------------|------------|----------|------------|-----------|
| FIL1α    | Primary          | Sense      | GGAGGCTACACCCCTGGAGCTCAA | 30         | 58        |
| FIL1β    | Primary          | Sense      | CGTGGAAGTAGAAGTCTGTAAGG  | 30         | 58        |
| FIL1δ    | Nested           | Sense      | GACACCTGGAGAGGGAGGACCCAGG | 40         | 60        |
| FIL1ε    | Primary          | Sense      | AACAGCATATGTTAAACCCAGTA/GT | 40         | 60        |
| FIL1γ    | Primary          | Sense      | TGGATCATCTATGCGGTCGTATAGG | 35         | 60        |
| FIL1η    | Primary          | Sense      | CCATAAATCAGTCATACATTGTTCGAC | 35         | 58        |

Expression Analysis

First-strand cDNAs present in CLONTECH (Palo Alto, CA) Human Multiple Tissue cDNA Panels I (catalog number K1420-1) and II (catalog number K1421-1) and the Human Immune Panel (catalog number K1426-1) were screened by PCR amplification using primers given in Table I. The primers were designed to span introns so that products arising from genomic DNA and cDNA could be distinguished. In some cases, nested primers were used in a second PCR reaction. The presence of an amplification product for each gene/tissue combination was determined by analysis on agarose gels stained with ethidium bromide.

Expression of Novel IL-1 Family Proteins for Receptor Binding

Novel IL-1 family members, as well as control IL-1β and IL-18 molecules, were generated by transfection of expression vector constructs into COS cells using DEAE-dextran (35). Expression vectors used were pDC409 (36) for FIL1α and FIL1ε, or pDC412, a close relative, for FIL1β and FIL1γ. The unmodified open reading frames were used for FIL1β, FIL1ε, and FIL1γ. For FIL1α, the sequence beginning with Lys27 (KNLN, . . .) was fused downstream of the human immunoglobulin κ light chain signal peptide. IL-1β, with an ATG codon added to the N terminus of the mature form (beginning with Ala117), was expressed in pDC409. Human IL-18 was expressed as the mature form fused to the IL-7 signal peptide in the expression vector pDC206 (37). For radiolabeling, 4 h after transfection cells were starved of cysteine and methionine for 60 min, then labeled with 70 μCi/ml of [35S]cysteine/methionine (Amersham; >1000 Ci/mmol) for 4–6 h. It is perhaps of interest that FIL1β, FIL1ε, and FIL1γ appear to be secreted from the COS cells despite the absence of either signal peptide or prodomain. C-terminal FLAG-tagged FIL1α and -ε were partially purified from the conditioned medium using the tags, and their N termini sequenced. The N-terminal amino acid of the secreted FIL1ε was methionine 1; it had been modified by N-terminal acetylation. The N-terminal amino acid of the secreted FIL1β is valine 2. Thus, there does not appear to have been
cleavage of an unrecognized signal peptide or prodomain in either molecule. There are a number of proteins which, when transfected into COS cells, do not later appear in the medium, so this is not a phenomenon attributable to leaky cells. However, the intracellular version of IL-1ra (icIL-1ra, a kind gift of William Arend, University of Colorado) also appears in the medium following transfection of COS cells. The significance of these findings is currently unknown.

**Receptor Binding Assays**

The novel IL-1 family members, present as [35S]-labeled proteins in conditioned medium from transfected COS cells, were tested for binding to Fc fusion proteins of the IL-1 receptor superfamily members (see Footnote 2 for general methods)² IL-1R type I, IL-1R AcP, IL-1Rrp1, IL-1Rrp2, IL-1R AcP, and T1/ST2 as follows: 0.5–1.0 ml of conditioned medium from transfected COS cells, were tested for binding to Fc fusion proteins of the IL-1 receptor superfamily members (see Footnote 2 for general methods)² IL-1R type I, IL-1R AcP, IL-1Rrp1, IL-1Rrp2, IL-1R AcP, and T1/ST2 as follows: 0.5–1.0 ml of conditioned medium was pre-cleared for 2 h at 4 °C with 50 μl of protein G-Sepharose (Amersham Pharmacia Biotech; 50% solution in phosphate-buffered saline) containing 1% Triton X-100, 0.02% NaN₃, and protease inhibitors (Roche Molecular Biochemicals catalog number 1 836 145). After a brief spin (3 min, 1000 rpm), the supernatant was transferred to a fresh tube and incubated overnight at 4 °C with 1 μg of receptor/Fc fusion protein plus another 50 μl of protein G-Sepharose. The mixture was centrifuged briefly, and the supernatant mixed with 0.5 ml of phosphate-buffered saline containing 5% glucose and protease inhibitors and spun again. The pellet was washed four times with 0.5 ml of a solution containing 0.4 M NaCl, 0.05% SDS, 1% Nonidet P-40, and protease inhibitors, and resuspended in 25 μl of 2 × reducing sample buffer (Zaxis, catalog number 220-211016). Samples were run on 4–20% Tris glycine gels (Novex) and autoradiographed.

**RESULTS**

The four previously known members of the IL-1 family (IL-1α, IL-1β, IL-1ra, and IL-18), while possessing a low overall fractional amino acid identity, share certain common amino acid sequence motifs, the most obvious of which can be summarized as F(X)₁₀₋₁₂/FXS/AVS(X)ₓ(P/E)(X)ₓ/FY/LL/CAS/STC) where X is any amino acid, and parentheses indicate that one of the included amino acids is present at that position. There are similarities in intron placement within the family as well. Relying on the sequence similarity, we searched public EST data bases and found sequences corresponding to three novel IL-1 family members, described below as FIL1α, FIL1ε, and FIL1ζ. A fourth novel family member, described below as FIL1η, was originally revealed in a published patent application. Examination of the sequence (called IL-16 by the inventors) in the patent application suggested that it was derived from an aberrantly spliced mRNA. We searched for and found an alternative form of mRNA that contains the conserved family sequence motif in the extreme 3’ exon. A brief description of the cloning and characteristics of each of the family members is given below. The sequences, and a comparison with the previously known IL-1 superfamily members, are given in Fig. 1.

**FIL1α**

A search of GenBank TM revealed a murine EST, accession number W08205, that resembled the known IL-1αs but was not identical to any. The IMAGE clone corresponding to the EST was sequenced and found to contain the entire open reading frame of an IL-1-like molecule. Unlike the known family members, this novel polypeptide (called FIL1α) appeared to contain neither a signal peptide nor a prodomain at the N terminus. A human FIL1α cDNA was then isolated from a human placenta cDNA library, using mouse FIL1α as a probe. The human sequence predicted an open reading frame similar to that of mouse FIL1α. Multiple FIL1α cDNA clones from both species were subsequently isolated, and all had the same predicted open reading frame, with no evidence for isoforms containing either signal peptide or prodomain. Interestingly, among the cDNA clones from both species were found several different 5’-untranslated region sequences (data not shown). These different 5’ sequences appear to derive from separate exons, in that they can be found (separately) in genomic sequence up-stream of the FIL1α coding region, and have potential splice donor sites at their 3’ ends. Presumably the FIL1α gene is transcribed from at least two promoters.

**FIL1ε**

A later search of GenBank TM revealed a murine EST, accession number AA030324, that resembled a second novel IL-1 family member. Sequencing of the IMAGE clone corresponding to the EST showed an open reading frame of an IL-1-like molecule. The vertical lines within each sequence indicate intron positions. Some of these are taken from GenBank TM (accession number X08383; IL-1β, accession number X04500; IL-1ra, accession number X64523; IL-18, accession number E17138). The rest were determined for this paper. The sequences of FIL1α, FIL1ε, FIL1ζ, and FIL1η have been deposited in GenBank TM (accession numbers AF201830, AF201831, AF201832, and AF201833).

![Alignment of amino acid sequences for human members of the IL-1 superfamily.](Image)

**Fig. 1. Alignment of amino acid sequences for human members of the IL-1 superfamily.** The full-length predicted translation products are shown for FIL1α, -ε, -ζ, and -η, whereas the mature peptides, without prodomains or signal peptide, are given for IL-1α, IL-1β, IL-1ra, and IL-18. The alignment is based on that presented by Bazan et al. (19) with slight modifications. Bars above and below the sequence indicate regions of experimentally determined (IL-1α and IL-1ra) or proposed (IL-1β, IL-18) secondary structure. The vertical lines within each sequence indicate intron positions. The significance of these findings is currently unknown.

² Born, T. L., Morrison, L. A., Esteban, D. J., Vandenbos, T., Thebeau, L. G., Chen, N., Spriggs, M. K., Sims, J. E., and Buller, M. L. (2000) J. Immunol., in press.
spliced to form a single coding region, PCR primers were designed and used successfully to amplify the predicted product from several different human RNA sources. As for FIL1z, the predicted FIL1z reading frame contains neither a signal peptide nor a promdomain.

**FIL1z**

A third EST, accession number A1014548, was found in Gen-Bank™ that appeared to encode an IL-1-like molecule. However, further sequencing revealed that the corresponding (human) IMAGE clone contained a stop codon upstream of the open reading frame but no initiating methionine. Screening of two other cDNA libraries resulted in isolation of a second, distinct aberrant clone, as well as a clone that contained an open reading frame that did begin with a methionine and that extended for 192 amino acids. This last clone was named FIL1z. Sequence comparison with other family members suggests that FIL1z has a promdomain of some 15–30 amino acids.

Analysis of genomic DNA demonstrated that an intron lies between the nucleotides encoding the 23rd and 24th amino acids of the 192 amino acid open reading frame form (see Figs. 1 and 4). The stop codon-containing sequences found in the aberrant cDNA clones lie within this intron, and appear to be incorporated into mRNA by cryptic splicing events. Since we had found three different cDNA isoforms for FIL1z, only one of which appeared to contain a functional open reading frame, it was important to determine the relative levels of the different transcripts. This was done by designing PCR primers that would amplify and distinguish the three isoforms, and using them to examine expression in a panel of first-strand cDNAs. The (presumably functional) FIL1z transcript was found in lymph node, thymus, bone marrow stroma, lung, testis, and placenta (Table II). We could not detect the form of mRNA represented by the EST in any tissue, whereas that represented by the other form of "aberrant" mRNA was present in bone marrow stroma (from which we had originally isolated it), lung, and placenta but not in the other tissues (not shown). The mRNA encoding that form appeared to be much less abundant than the functional FIL1z mRNA.

**FIL1ς**

A cDNA clone containing part of the FIL1ς sequence was originally identified in an osteoclastoma library (38). The DNA sequence presented in this document appeared to encode the N-terminal half of an IL-1-like molecule, which then diverged in the C-terminal portion. Since the C-terminal regions of the different IL-1 family members contain the greatest sequence conservation, including the motif described above, and since the point of divergence lay exactly at the position of a conserved intron in the IL-1 family (see below), we searched for an alternative transcript that might encode a more typical member of the family.

PCR with first strand cDNA templates from various tissue sources, using primers lying entirely within the 5'-half of the osteoclastoma coding sequence (38), gave products from tonsil, bone marrow, heart, placenta, lung, testis, and colon. Only very faint bands were obtained, and only in tonsil and testis, when a 5' primer from the 5'-half and a 3' primer from the 3'-half of the osteoclastoma coding sequence were used, consistent with our interpretation. Human genomic DNA containing the 5'-half of the osteoclastoma sequence and extending further downstream was then isolated and sequenced. A putative exon was found 823 base pairs downstream of the point of divergence of the osteoclastoma sequence from other family members; this putative exon contained the expected sequence motifs for the C-terminal portion of an IL-1 family member, as well as a potential splice acceptor site at its 5' end. PCR primers designed to amplify a hypothetical cDNA formed by splicing of the 5' portion of the osteoclastoma sequence with this 3' exon did indeed give a product from human tonsil first strand cDNA, which when sequenced contained the predicted 157-amino acid open reading frame. The open reading frame, and the gene encoding it, were named FIL1ς. Like FIL1δ and FIL1ε, FIL1ς does not contain an apparent signal peptide or promdomain.
**Table III**  
**Sequence identity**  
The numbers represent percent sequence identity between the indicated IL-1 superfamily members, as determined by using the program "gap" (Wisconsin Package Version 10.0, Genetics Computer Group (GCG)). For IL-1α, IL-1β, IL-18, and IL-1ra, the mature peptide (lacking signal sequence or prodomain) was used for the comparison. For FIL1, the mature form was assumed to start with Lys27, based on primary sequence alignment and analysis of predicted eight-dimensional structure, and this sequence was used.

|    | IL-1α | IL-1β | IL-1ra | IL-18 | FIL1δ | FIL1ε | FIL1ζ | FIL1η |
|----|-------|-------|--------|-------|-------|-------|-------|-------|
| IL-1α | ...   | 24    | 20     | 21    | 20    | 23    | 21    | 26    |
| IL-1β | ...   | 31    | 17     | 32    | 27    | 24    | 32    |       |
| IL-1ra | ...  | 22    | 50     | 30    | 29    | 30    |       |       |
| IL-18 | ...   | 27    | 20     | 21    | 21    |       |       |       |
| FIL1δ | ...   | 31    | 35     | 37    |       |       |       |       |
| FIL1ε |       | 36    | 46     |       |       |       |       |       |
| FIL1ζ |       | ...   | 44     |       |       |       |       |       |
| FIL1η |       |       | ...    |       |       |       |       |       |

**Fig. 2. Dendrogram illustrating the relationship between members of the IL-1 superfamily.** The dendrogram was generated by the program Treeview (40) using the amino acid sequence alignment produced by the program Pileup (Wisconsin Package Version 10.0, Genetics Computer Group (GCG), Madison, WI).

**A Gene Family**

**Sequence Comparison**—The novel members of the IL-1 family are approximately as similar to one another in sequence as they are to the classical IL-1α; this level of identity is in turn similar to that shown by the classical IL-1s among themselves. Typically any given pair of family members shows 20–35% sequence identity (Table III). Those that stand out as being more similar to one another than average are FIL1δ/IL-1ra, FIL1ε/FIL1η, and FIL1ζ/FIL1η. These relationships can also be seen in the dendrogram presented in Fig. 2, in which it appears that IL-1α, IL-1β, and IL-18 form one sequence subfamily; FIL1ε, FIL1ζ, and FIL1η form a second subfamily, and IL-1ra and FIL1δ form a third. The novel members can easily be placed onto the structure-based sequence alignment presented by Bazan et al. (19) (Fig. 1).

**Three-dimensional Protein Structure Prediction**—The structures of FIL1δ, FIL1ε, and FIL1ζ have been modeled using as templates the experimentally determined structures of IL-1β and IL-1ra. The novel IL-1 superfamily member amino acid sequences could with minimal energy violations be folded into structures which superimpose well onto the IL-1β and IL-1ra crystal structures. In particular, the core 12-stranded, β-trefoil structure appears well conserved (see Fig. 3 for FIL1ε). The major points of difference between the FIL1δ, FIL1ε, and FIL1ζ models, and between them and the experimental structures, lie in the loops connecting the β strands, where IL-1β and IL-1ra also differ most from each other.

**Genomic Structure**—The known genes of the IL-1 family display a conserved pattern of intron placement and intron/exon junctions, clearly indicating their derivation from a common ancestor. The novel IL-1 family members presented here demonstrate the same pattern. The most C-terminal intron in the coding region always falls between codons, and lies immediately after the predicted β-strand 7. By analogy to the structure of the IL-1α and IL-1β genes, we have called this intron 5, even though the rest of the family has only three introns within the coding sequence (except IL-18, which has four). Intron 4 (the intron N-terminal to intron 5) falls between the first and second nucleotides of the codon, and lies just N-terminal to β-strand 4. Intron 3 is more variable in placement. In IL-1α, IL-1β, IL-18, and probably in FIL1ζ, it lies within the prodomain, not far upstream of the site of processing. In the other family members, it appears to lie not far downstream of the initiating methionine. It is also more variable in placement within the codon, falling after either the first (IL-1α, IL-1β, IL-18, FIL1ε, FIL1ζ, FIL1η) or second (IL-1ra, FIL1δ) nucleo-
Expression Pattern

We have analyzed the expression pattern of the novel IL-1 family members in several ways. Using a panel of first strand cDNAs derived from various tissues as templates for PCR, we find that the novel family members are all expressed in lymphoid organs, although the detailed pattern differs somewhat from cytokine to cytokine (Table II). RNA for each is also present in a small number of non-lymphoid tissues. Table II also summarizes expression data obtained from cDNA library screening, from searching EST data bases, and from PCR analysis of individual RNA samples. No easy generalization about expression patterns, either for the individual cytokines or for the family, is obvious.

Hematopoietic Subsets—We wanted to look specifically at expression of each of the novel IL-1 family members in individual cell types from peripheral blood. Cells were prepared from human blood and cultured for a short time in various conditions. RNA was made and analyzed by RT-PCR for the presence of the different novel ligands and with labeled with [35S]cys/Met, as well as from COS cells transfected with IL-18 as a positive control, were incubated with Fc fusions of the characterized IL-1R family members (IL-1R type I, IL-1R type II, IL-1Rrp1, IL-1Rrp2, AcPL, and T1/ST2), followed by precipitation of the Fc proteins using protein G. The precipitates were electrophoresed on SDS gels, and autoradiographed to see whether any of the ligands were able to bind to any of the receptors. While IL-18 was seen consistently to bind to IL-1Rrp1, no other complexes were observable using this technique.

**TABLE IV**

Expression of novel family members in hematopoietic cell subsets

Expression of IL-1 family members was determined by PCR analysis of RNA isolated from subsets of peripheral blood mononuclear cells, as obtained as described under "Experimental Procedures."

| Cell subset       | FIL1α | FIL1ε | FIL1ζ | FIL1η |
|-------------------|-------|-------|-------|-------|
| NK cell + IL-12   | ND    | ND    | ND    | ND    |
| T cell            |       |       |       |       |
| Monocyte          | +     | +     | ND    |       |
| Monocyte + LPS    | +     | +     | +     | +     |
| B cell            | +     | +     | +     | +     |
| B cell stimulated | +     | +     | +     | +     |
| Dendritic cell + LPS | +   | +     | ND    | ND    |

* ND, not done.

![Intron positions](image)

We describe here the discovery of novel genes that double the size of the IL-1 superfamily. Assessment of the FIL1α, FIL1ε, FIL1ζ, and FIL1η genes as paralogs of IL-1α, IL-1β, IL-1ra, and IL-18 is based on several factors. First, sequence alignment (Fig. 1) reveals certain conserved amino acid motifs. Not only is there easily recognizable conservation of primary structure, but the amino acid sequences readily allow modeling into a predicted three-dimensional structure that is conserved with the known IL-1α (Fig. 3). In addition, intron placement is highly conserved in these new genes and is similar to that found in the "traditional" IL-1α as well as IL-1β (Figs. 1, 4). This provides an independent measure of evolution from a common ancestor. Finally, consistent with evolution by gene duplication, the new IL-1 superfamily members are all clustered in the same region of human chromosome 2q that contains IL-1α, IL-1β, and IL-1ra. IL-18 is the only superfamily member that does not map to this location.

The novel IL-1 family members are expressed in a variety of hematopoietic and non-hematopoietic cell types. It is not easy to formulate generalizations about expression patterns, except to say that FIL1ε appears to be the only one of these putative cytokines routinely expressed in T cells, and (not unexpectedly) all of the family members are expressed in activated monocytes and B cells. From the infrequency of ESTs corresponding to these genes in GenBank™, as well as the number of PCR cycles required to detect an amplification product in positive RNA sources, it would appear that they are all expressed at relatively low abundance. Nevertheless, FIL1α, FIL1ε, and
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FIL1z can be regulated by LPS (and most likely other agents) in monocytes and spleen cells, and FIL1β appears to be transcribed from at least two different promoters, indicating that regulation of expression in this family is active.

It might be expected that the new IL-1 superfamily members would bind to members of the IL-1 receptor superfamily. However, we have been unable to demonstrate this in co-precipitation assays using Fc fusions of the known receptors and orphan receptor homologs. It could be that there are as yet undiscovered members of the IL-1R superfamily. Alternatively, unlike the case with the IL-1s and IL-18, high affinity binding detectable by co-precipitation may require two receptor subunits to be present. Finally, of course, it is possible that these cytokines bind to a different type of receptor. IL-18, for example, was recently shown to be capable of binding with high affinity to a soluble protein that has little similarity to other IL-1R family members (39).

The biological activity of the novel IL-1 family members remains to be characterized. IL-1α, IL-1β, and IL-18 are all capable of activating gene expression programs that enhance immune responses and promote inflammation. It is obvious to speculate that FIL1β, FIL1ε, FIL1γ, and FIL1δ might have similar actions. On the other hand, IL-1ra acts to block the biological activity of the IL-1 and IL-18, high affinity binding detectable by co-precipitation may require two receptor subunits to be present. Finally, of course, it is possible that these cytokines bind to a different type of receptor. IL-18, for example, was recently shown to be capable of binding with high affinity to a soluble protein that has little similarity to other IL-1R family members (39).

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