Cloning and Characterization of IL-1HY2, a Novel Interleukin-1 Family Member

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SUMMARY

The interleukin-1 (IL-1) family members play an important role in the process of inflammation and host defense. We describe here the identification and characterization of a novel member of the IL-1 family, IL-1HY2. The human IL-1HY2 protein shares significant amino acid sequence similarity (37%) with IL-1 receptor antagonist (IL-1ra) and has similar predicted three-dimensional structure to IL-1ra. The IL-1HY2 gene is located in close proximity to other IL-1 family genes on human chromosome 2 and the genomic organization of the IL-1HY2 gene is highly conserved with other IL-1 family members. IL-1HY2 protein is secreted from mammalian cells and the purified recombinant IL-1HY2 protein binds soluble IL-1 receptor type I (sIL-1RI). IL-1HY2 is expressed in human skin, spleen, and tonsil. Immunohistochemical analysis showed that the IL-1HY2 protein is expressed in the basal epithelia of skin and in proliferating B cells of the tonsil. These data suggest that IL-1HY2 is a novel IL-1 family member and it may participate in a network of IL-1 family members to regulate adapted and innate immune responses.
INTRODUCTION

Interleukin (IL)\(^1\)-\(\alpha\), IL-1\(\beta\), IL-1 receptor antagonist (IL-1ra), and IL-18 belong to the IL-1 family of cytokines. IL-1 cytokines have been implicated in a wide range of pathological conditions such as rheumatoid arthritis, inflammatory bowel disease, Alzheimers disease, diabetes, and susceptibility to infections (1,2). The balanced expression of the IL-1 family members has been postulated to be important in regulating inflammation and host defense responses. IL-1\(\alpha\) and IL-1\(\beta\) signal through the IL-1 type I receptor, while IL-1ra functions as an antagonist by competitively binding to the same receptor without eliciting receptor signaling (1,3). IL-18 signals through the IL-18 receptor, inducing the production of \(\gamma\)-interferon from T cells (4). Recently, five additional IL-1 family members, including IL-1HY1 (5,6), FIL1\(\epsilon\), FIL1\(\eta\), FIL1\(\zeta\) (7), and IL-1H1 (8), as well as some of their isoforms (8,9), have been reported.

Cytokines are often expressed at low levels owing to their potent effects. In order to identify novel cytokine genes expressed at low levels, a unique high-throughput cDNA screening approach (10,11) was employed. This approach utilizes a set of oligonucleotide probes to hybridize successively to arrays of human cDNAs from various tissues. Hybridization patterns allow the cDNA clones to be clustered into groups of similar or identical sequences. The number of clones in a particular cluster represents the relative expression level of a specific gene. Representative clones from clusters with low numbers of members were selected for sequencing. Novel cytokine family members were identified using various sequence analysis algorithms. The \(IL-1HYI\) gene was recently identified using this approach (5).
In the current report, we describe the discovery of a novel IL-1 family member, referred to as *IL-1HY2*. The characteristics of *IL-1HY2* indicate that it shares a common ancestral precursor gene with other IL-1 family members. We demonstrate that IL-1HY2 is secreted, binds the soluble IL-1 receptor, and is expressed in human skin, spleen, and tonsil, suggesting that it may play a role as a secreted ligand in host defense and other immune responses.
EXPERIMENTAL PROCEDURES

Isolation of the IL-1HY2 cDNA and sequence analysis. A cDNA library was constructed from human fetal skin mRNA (Invitrogen) in the pSport1 vector (Gibco). Inserts of the library were amplified by PCR using primers specific to pSport1 vector sequences that flank the inserts. These samples were then analyzed using the screening by hybridization approach as described previously (10,11). Briefly, the PCR products were spotted onto nylon membranes and hybridized with oligonucleotide probes to give sequence signatures. The clones were clustered into groups of similar or identical sequences, and single representative clones for rarely expressed transcripts were selected for gel sequencing. The 5’ sequence of the amplified inserts was then deduced using the reverse M13 sequencing primer by ABD Big Dye terminator cycle sequencing (Perkin Elmer). The cDNA sequence database was analyzed using the BLAST and motif search algorithms. A novel cDNA sequence encoding a polypeptide homologous to the IL-1ra protein was identified. The sequence was confirmed in part by sequencing PCR products generated from 5’ rapid amplification of cDNA ends (RACE) analysis using a Marathon cDNA amplification kit (Clontech). This novel gene sequence was designated IL-1HY2.

Chromosomal localization and genomic organization of the IL1HY2 gene. PCR primers specific to IL-1HY2 (5’-CCGCACCAAGGTCCCCATTTTC-3’ and 5’-GAGCCCAAGGATAACCCAGG-3’) were used to screen the NIGMS human/rodent somatic cell hybrid mapping panel #2 (12) and the Stanford G3 Human/Hamster Radiation Hybrid panel (13) (Research Genetics, Huntsville, AL). A PCR product of 824 bp was amplified using the following conditions: 94 °C for 3 min, 30 cycles of (94 °C for 30 sec, 58 °C for 30 sec,
72 °C for 1 min), and 72 °C for 10 min. Linkage analysis and subsequent chromosomal localization were obtained using the Stanford Human Genome Center RH server (http://www-shgc.stanford.edu/RH/). To determine the genomic structure of the IL-1HY2 gene, human BAC clones (Research Genetics) were screened by PCR with primers specific to the IL-1HY2 cDNA. The BAC393I6 containing the IL-1HY2 gene was partially digested by Sau3A I enzyme and size-selected restriction fragments were inserted into the BamH I site of pUC18 (Pharmacia) to generate a library for sequencing. Sequencing was performed using M13 forward and reverse primers flanking the inserts. Direct BAC DNA sequencing was also performed using the primers specific to IL-1HY2 cDNA to confirm the exon/intron organization.

cDNA expression analysis. Plasmid DNA (20 ng) of human cDNA libraries derived from a range of tissues were used as templates in a PCR reaction. Primers specific to IL-1HY2 (5-AGGACCAGACACCACTGATTG-3 and 5-TGGGGGCCACAAGGCTAAAAC-3) and β-actin (5-CCTAAGGCCAACCGTGAAAAG-3 and 5-TCTTCATGTTGGCTAGGAGCCA-3) were used. PCR was performed using the following conditions: 94 °C for 3 min, 30 cycles of (94 °C for 30 sec., 58 °C for 30 sec, 72 °C for 1 min), 72 °C for 10 min.

Transfection of Chinese Hamster Ovary (CHO) cells and protein analysis by Western blot analysis: The coding region of the IL-1HY2 cDNA was cloned into the pcDNA3.1/V5-His TOPO vector (Invitrogen) using PCR primers (5'-GAGCCGCCCATGTGTTCCCTCCCCCATGGCAAG-3' and 5'-GCTACCAGCTCTGGTTCAAGAGTAAAAC-3'). CHO cells obtained from the American Type Culture Collection were cultured in F12K supplemented with 10% FBS and 100 units/ml
penicillin G and 100 µg/ml streptomycin sulfate at 37 °C in 5% CO₂. CHO cells were transfected with the IL-1HY2 expression vector or a control empty vector using Fugene-6 per manufacturers instruction (Roche). After the transfection, medium was replaced with serum-free F12K medium, and the cells were incubated for an additional 24 h prior to harvesting. Conditioned medium was centrifuged and then filtered through a 0.2 µ filter (Pall Gelman Laboratory) to remove cell debris. The conditioned medium was subsequently concentrated by 10-fold using the Microcon YM-10 per manufacturer’s instruction (Amicon). Cell lysates and conditioned media were analyzed by Western blot analysis. After SDS-polyacrylamide gel (15%) electrophoresis (PAGE), protein samples were electrophoblotted onto an Immobilon-P membrane (Millipore) and probed with the anti-IL-1HY2 antibodies. Immunocomplexes were visualized by SuperSignal West Pico chemiluminescence detection reagents with goat anti-rabbit IgG conjugated with horseradish peroxidase as a secondary antibody (Pierce).

**Purification of recombinant IL-1HY2 protein and measurement of receptor-ligand binding affinities.** The coding region of IL-1HY2 cDNA was cloned into pCR II/TOPO vector (Invitrogen) using PCR primers (5’-GTCATATGGTGTCATGCCCATGCAAG-3’ and 5’-GAAGCTTGATCACTACCAGCTCTGTTCAAAGTAAAAC-3’). The sequence of the IL-1HY2 coding region was confirmed by sequencing. The Nde I/Hind III restriction fragment containing IL-1HY2 was isolated and inserted between the Nde I and Hind III sites of the bacterial expression vector pRSET B (Invitrogen). The resulting plasmid, pRSET IL-1HY2, was transformed into *Escherichia coli* strain BL21(DE3)pLysS (Invitrogen). IL-1HY2 protein expression was induced by IPTG and cells expressing IL-1HY2 were harvested by
centrifugation at 10,000 x g for 30 min. The cells were then lysed in 10 volumes of lysis buffer (20 mM Tris, pH 8.0, 1 mM EDTA) by passing through an Avestin homogenizer. IL-1HY2 present in the supernatant was purified by a combination of Q-Sepharose anion exchange, Phenyl Sepharose hydrophobic interaction, and DEAE-Sepharose anion exchange chromatography (AP Biotech). The IL-1HY2 protein was 95% pure as determined by SDS-PAGE analysis and Coomassie staining. The dissociation equilibrium constants (K_D) of IL-1HY2, IL-1RA, and IL-1β binding to soluble IL-1 receptor type I (sIL-1RI) were measured using the IAsys affinity sensor (Labsystems Affinity Sensors, Franklin, MA) according to the manufacturers instructions and as described (14,15). Purified recombinant IL-1HY2 protein, recombinant IL-1RA and IL-1β proteins (R&D Systems) were biotinylated using EZ-Link Sulfo-NHS-LC-Biotin (Pierce, Rockford, IL) per manufacturers instructions. The biotinylated proteins (1.2 μM) were immobilized onto the avidin layered biotin coated cuvette surface. After ligand immobilization, sIL-1RI (R&D Systems) at various concentrations (70 - 500 nM) was added to the cuvette. Protein binding kinetics was measured and K_D of each protein to the IL-1 receptor was obtained using the software Fastfit (Labsystems Affinity Sensors, Franklin, MA) (14,15).

**Generation of antibodies and Immunohistochemistry:** Anti-IL-1HY2 polyclonal antibodies were raised in rabbit against a synthetic peptide (CTLPNRGLDRTKVP) specific to the IL-1HY2 protein. The antibodies specific to the peptide were subsequently isolated by affinity-purification using standard procedures (16). The anti-IL-1HY2 antibodies did not cross-react with other IL-1 family members, including IL-1ra, IL-1β, and IL-1HY1, in a Western blot.
analysis (data not shown). The anti-CD20 monoclonal antibody was purchased from DAKO (Carpenteria, CA) and the anti-Ki67 was obtained from Coulter Immunotech (Miami, FL). Immunohistochemistry was performed at QualTek Molecular Systems, Inc. (Santa Barbara, CA) using a procedure described previously (17). Briefly, sections of adult human tonsil and skin were reacted with the primary anti-IL-1HY2 antibodies that were detected by a biotinylated secondary antibody followed by streptavidin-AP. Fast Red was used as the chromogen (red) and the slides were counter stained with hematoxylin (blue nuclear stain). In the negative control staining, slides were processed without adding the primary antibody. For the double labeling immunohistochemistry, the second primary antibody, anti-CD20 monoclonal antibody or anti-Ki67 monoclonal antibody, was detected using a biotinylated secondary antibody followed by streptavidin-HRP. DAB was used as the chromogen (brown).

**Three-Dimensional structure modeling:** The GeneAtlas™ software package (Molecular Simulations Inc., San Diego, CA 1999) was used to generate three-dimensional structural models for IL-1HY2. The three-dimensional structural models of the IL-1HY2 protein were predicted based on a search of 4250 non-redundant Protein Data Bank (http://www.rcsb.org/pdb) structures using multiple alignment sequence profile-based searching method, PSI-BL (18), an automated sequence and structure searching procedure, High Throughput Modeling (19), and a fold recognition method, SeqFold (20). The known structures of IL-1ra (21) and IL-1β (22) (1i1b for IL-1β and 1irp, 1ilr, and 1ilt for IL-1RA), which were the best fit structures, were used as templates.
RESULTS

To identify additional IL-1 family member genes, BLAST and motif algorithms were used to search an expressed human gene sequence database generated using a Screening by Hybridization technology (10,11). A cDNA sequence from a human fetal skin library was identified that encodes a polypeptide with homology to the IL-1ra protein. Sequencing of the cDNA clone and subsequent 5' RACE analysis led to the identification of a full-length coding sequence designated as IL-1HY2. The nucleotide and the deduced protein sequences are shown in Figure 1. Blast search analysis indicated that this nucleotide sequence had not been previously reported in any public databases. The IL-1HY2 cDNA contains an open reading frame corresponding to a 152 amino acid residue protein, with a predicted molecular weight of 17 kDa and a predicted isoelectric point of 4.7.

Sequence analysis indicated that the IL-1HY2 protein is highly homologous to IL-1HY1 (41% similarity) and IL-1ra (37% similarity) while it shares lower homology (14-30%) with IL-1β and other IL-1 family proteins (Fig. 2A and 2B). Based on amino acid similarities among the IL-1 family members, IL-1HY2, IL-1HY1 and IL-1ra may be grouped into a subfamily, and FIL1ε, FIL1η, FIL1ζ, and IL-1H1 belong to a second subfamily, while IL-1α, IL-1β, and IL-18 fall to a third subfamily (Fig. 2C).

The IL-1HY2 locus was mapped to human chromosome 2 using the NIGMS human/rodent somatic cell hybrid-mapping panel (12). The Stanford G3 Human/Hamster Radiation Hybrid panel (13) was used to further localize the IL-1HY2 gene to a region on human chromosome 2q14, with a distance of 7 cRs from the marker SHGC-7020 and a LOD score of 10.58. Interestingly, IL-1α (marker SHGC-10703), IL-1β (marker SHGC-11912),
IL-1ra (marker WI7030), IL-1HY1 (marker SHGC-7020), FIL1ε, FIL1η, and FIL1ζ were also mapped to the similar location on chromosome 2 (5.7-9.23,24).

The genomic organization of the IL-1HY2 gene was determined by sequencing of the human BAC clone containing the IL-1HY2 gene (see Experimental Procedures). Like the other IL-1 family members, the IL-1HY2 gene consists of four coding exons (Fig. 3). The structure of the IL-1HY2 gene is remarkably similar to that of the other IL-1 family genes, with a conserved pattern of intron placement (Fig. 2A).

To determine the expression pattern of the IL-1HY2 gene, a panel of cDNA libraries derived from various human tissues was analyzed by a semi-quantitative PCR using primers specific to IL-1HY2. The IL-1HY2 transcripts were expressed in fetal skin and at a lower level in spleen (Fig. 4A). To determine whether IL-1HY2 protein is secreted in mammalian cells, an IL-1HY2 expression construct was used to transfect CHO cells. The recombinant IL-1HY2 protein was detected in both the cell lysate and conditioned medium from transiently transfected CHO cells (Fig. 4B).

Since the IL-1HY2 protein, which belongs to the IL-1 family, is secreted in mammalian cells, the recombinant IL-1HY2 protein was tested for binding to the soluble IL-1 receptors type I (sIL-1RI) using the IAasys affinity sensor. Biotinylated recombinant IL-1HY2, IL-1ra, or IL-1β protein was immobilized on the avidin layered biotin coated cuvette surface and their dissociation equilibrium constants (K_D) with sIL-1RI were measured (see Experimental Procedures). The recombinant IL-1HY2 protein bound sIL-1RI although the binding affinity of IL-1HY2 was lower (higher K_D) than those of IL-1ra and IL-1β (Table I).

To analyze IL-1HY2 protein expression in the human skin, polyclonal antibodies raised
against a peptide specific to IL-1HY2 were used in the immunohistochemistry study (Fig. 5). IL-1HY2 protein was expressed mostly in the basal epithelia of the human skin (Fig. 5B). To identify the cell types in immune tissues that express the IL-1HY2 protein, immunohistochemistry was performed on tonsil (Fig. 6). From the staining data, the IL-1HY2 protein was expressed in the germinal center of the tonsil (Fig. 6B). Using double labeling immunostaining, the IL-1HY2 protein was detected in a subset of B cells (CD-20 positive) (Fig. 6C), most of which were proliferating (Ki67 positive) cells (Fig. 6D). Furthermore, the IL-1HY2-positive cells did not react with the anti-CD45RO (T cell marker) antibody or the anti-CD14 (monocyte marker) antibody (data not shown), suggesting that the IL-1HY2 protein was not expressed in T cells or monocytes in the tonsil.
DISCUSSION

Recently, five new IL-1 family members, including IL-1HY1 (5), FIL1ε, FIL1η, FIL1ζ (7), and IL-1H1 (8), were identified. In this report, we describe the discovery of a novel IL-1 family gene, IL-1HY2, expanding the IL-1 family to include a total of ten family members (IL-1α, IL-1β, IL-1ra, IL-18, IL-1HY1, FIL1ε, FIL1η, FIL1ζ, IL-1H1, and IL-1HY2). It would appear that IL-1HY2 was derived from a duplication event from an ancestral gene common to the other IL-1 family members. This notion is evidenced by significant amino acid sequence homologies (19 - 41%) that the IL-1HY2 protein shares with the other IL-1 family members (Fig. 2A, 2B) and by the high degree of conservation in the genomic organization of the IL-1HY2 gene with the other family member genes (Fig.1 and Fig. 3). Based on the amino acid sequence homologies, IL-1HY2, IL-1HY1, and IL-1ra appeared to form a subfamily that was evolved from a common ancestor (Fig. 2C).

The three-dimensional structural model of the IL-1HY2 protein was predicted based on a search of 4250 non-redundant Protein Data Bank structures. The known crystal structure of IL-1ra (21), which was one of the best fit structures, was used as a template (Fig. 7). The IL-1HY2 structural model displays a 12-β stranded trefoil structure and is highly similar to the IL-1ra crystal structure (Fig. 7). The structural model of IL-1HY2 predicted based on the IL-1β crystal structure (22) also shares similarity to the IL-1β structure (data not shown). The similarity between the IL-1HY2 structural models and the IL-1ra and IL-1β structures provides another evidence that IL-1HY2 belongs to the IL-1 family.

The recombinant IL-1HY2 proteins expressed in mammalian (CHO) cells had two forms, a major form at 25 kDa, and a minor form at 17 kDa (Fig. 4B), while the predicted
molecular weight based on the amino acid sequence of IL-1HY2 is 17 kDa. The major form of the IL-1HY2 may be a result of posttranslational modifications of the protein. However, the IL-1HY2 protein lacks any N-link glycosylation consensus sites. Neither N-link glycosylation nor O-link glycosylation on the recombinant IL-1HY2 protein expressed in CHO cells was detected using peptide N-glycosidase F (PNGase F) and O-glycosidase deglycosylation analysis (data not shown). Thus, the difference between the apparent molecular weight and the predicted molecular weight of IL-1HY2 may be due to other posttranslational modifications, such as phosphorylation.

Although IL-1HY2 lacks an obvious signal peptide, we have demonstrated that the recombinant IL-1HY2 protein is secreted from CHO cells (Fig. 3B), suggesting that IL-1HY2 may act as a secreted ligand. Other IL-1 family proteins, including IL-1β and IL-18, also lack a classical signal peptide and are still secreted. The secretion of IL-1β and IL-18 involves cleavage by the IL-1β converting enzyme (ICE) (25-27). The IL-1HY2 protein does not contain an apparent consensus site for ICE cleavage. Thus, secretion of the IL-1HY2 protein may involve an alternative secretory mechanism.

IL-1HY2 was shown to bind sIL-1RI, although it does not bind as avidly as IL-1β and IL-1ra to sIL-1RI (Table I). The KD values of IL-1ra and IL-1β binding to sIL-1RI presented here are higher than those reported by other groups (28,29). This may reflect the different methodology and instrumentation (IAsys affinity sensor) that we used. Thus, the binding affinities of IL-1HY2 can only be compared with those of IL-1ra and IL-1β determined using the same conditions. The affinity of IL-1HY2 binding to the IL-1 receptor was determined using the soluble form of the receptor. It has been shown that IL-1R accessory protein (AcP)
plays a role in enhancing IL-1 receptor-ligand binding affinities and signaling activities (30,31).

Thus, IL-1R AcP or another accessory protein may affect the binding affinity of IL-1HY2 to the IL-1 receptor. The fact that IL-1HY2 binds to sIL-1RI suggests that IL-1HY2 may play a role in regulating the IL-1 receptor function.

Data presented in the current report indicate that IL-1HY2 is expressed in skin and activated B cells of the human tonsil. The tissue-specific expression pattern of IL-1HY2 suggests that it may be involved in the regulation of normal immune responses and inflammatory pathophysiology.
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FOOTNOTES

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The IL-1HY2 cDNA and genomic sequences reported in this paper have been deposited in GenBank with accession numbers AF334755 and AF334756, respectively.

1 Abbreviations: IL, interleukin; IL-1ra, IL-1 receptor antagonist; sIL-1RI, soluble IL-1 receptor type I; IL-1R AcP, IL-1 receptor accessory protein.
FIGURE LEGEND

Fig. 1. cDNA and deduced amino acid sequences of human *IL-1HY2* (GenBank accession number AF334755). The putative polyadenylation signal is underlined.

Fig. 2. Sequence comparison of IL-1HY2 and other IL-1 family proteins.  
A. Amino acid sequence alignment of human IL-1HY2, intracellular IL-1ra, IL-1HY1, and IL-1β (mature form). Sequence alignment was obtained using the ClustalW algorithm of the Lasergene software (DNASTAR Inc., Madison, WI). The boxed area indicates matching residues. The vertical arrows indicate intron positions.  
B. Sequence pairwise comparison of IL-1 family members. Numbers represent percent sequence similarity determined by the J. Hein method with PAM250 residue weight table in the Lasergene software.  
C. Phylogenetic analysis of the IL-1 family. The inferred phylogenetic tree was generated based on degree of amino acid sequence similarity shown in B. Accession numbers in the public databases for proteins used in this figure are: IL-1α, #P01583 (mature protein, amino acid residues 113-271); IL-1β, #P01584 (mature protein, amino acid residues 117-269); intracellular IL-1ra, #AAB92268; IL-18, #BAA08706 (mature protein, amino acid residues 37-193); IL-1HY1, #AF186094; FIL-1ε, #AF201831; FIL-1η, #AF201833; FIL-1ζ, #AF201832; IL-1H1, #AF200492.

Fig. 3. Organization of the human *IL-1HY2* gene (GenBank accession number AF334756). Horizontal lines represent introns and boxes represent exons. Black areas of the boxes represent coding sequences. Sizes of exons and introns are indicated in nucleotide (nt).

Fig. 4. IL-1HY2 expression in human tissues and secretion of the IL-1HY2 protein in transiently transfected CHO cells.  
A. IL-1HY2 expression analysis. Plasmid DNA from a panel of cDNA
libraries was analyzed by PCR using primers specific to IL-1HY2 and β-actin. The cDNAs were derived from the following human tissues: 1, heart; 2, brain; 3, liver; 4, lung; 5, spleen; 6, ovary; 7, testis; 8, kidney; 9, placenta; 10, fetal brain; 11, fetal skin; 12, fetal lung; and 13, fetal liver. B. Secretion of IL-1HY2 protein in transiently transfected CHO cells. CHO cells were transfected transiently with the IL-1HY2 expression construct. Proteins from the cell lysate and culture medium (10-fold concentrated) were analyzed by Western using polyclonal antibodies against the IL-1HY2 protein. A vector without the IL-1HY2 cDNA was used as a control in the transfection of CHO cells.

Fig. 5. Analysis of IL-1HY2 protein expression in human skin by immunohistochemistry. Sections of the human skin were processed without primary antibodies (A) or with affinity-purified polyclonal antibodies specific to the IL-1HY2 polypeptide (B). A biotinylated secondary antibody was used followed by streptavidin-AP. The anti-IL-1HY2 antibodies were detected using Fast Red as the chromogen (red). Slides were counter-stained with hematoxylin (blue nuclear stain). Pictures are shown at 150x magnification.

Fig. 6. Cell type-specific expression of the IL-1HY2 protein in the human tonsil. Sections of the human tonsil were reacted without primary antibodies (A) or with affinity-purified polyclonal antibodies specific to the IL-1HY2 polypeptide (B) as described in Fig. 5. For double labeling of the tissues, anti-CD20 antibody was used as a second primary antibody in addition to the anti-IL-1HY2 antibodies (C), or the anti-IL-1HY2 and anti-Ki67 antibodies (D) were used as primary antibodies. The second primary antibody (anti-CD20 antibody or anti-Ki67 antibody) was detected using a biotinylated secondary antibody followed by streptavidin-HRP. DAB was used as the chromogen (brown). Sections shown represent the edge of a
germinal center within a tonsil. Pictures are shown at 400x magnification.

**Fig. 7.** Comparison of the three-dimensional structural model of IL-1HY2 with the crystal structure of IL-1ra. The IL-1HY2 structural model was generated using the GeneAtlas™ software package (Molecular Simulations Inc., San Diego, CA 1999). **A** and **C.** The crystal structure of IL-1ra (blue) and the predicted 12-β stranded trefoil structural model of IL-1HY2 (yellow) based on the IL-1ra template, respectively. Both the crystal structure and the model are viewed down the β-barrel axis. **B.** The superimposed crystal structure and the IL-1HY2 structural model.
Table I. Dissociation equilibrium constants (KD) of IL-1HY2, IL-1ra, and IL-1β binding to sIL-1RI. The dissociation equilibrium constant (KD) was measured using the IAsys affinity sensor (Labsystems Affinity Sensors, Franklin, MA) as described (14,15). Data from a representative experiment are presented.

|          | sIL-1RI |
|----------|---------|
| IL-1HY2  | 93 nM   |
| IL-1ra   | 38 nM   |
| IL-1β    | 21 nM   |
Fig. 1

```plaintext
ATACACAAGAGACGCGCCAGCTGGCTGGTAGACTCTGGTCAGACAACTGGTGTCAGAGAAGATCTGCAACTCTTCAA

YTRDGQLLVGDPVADNCCCAEKICTLP

ACAGAGGCTTGGACACCCCAAGGATCCCCATTTCTCGGGATCCAGGGGGAGGCCGCTGGCAGATGTGAGACGA

NRGLDRTKVPIFLGIGQGSSRCLACVET

GAAAGGGCCCTTCCTACAGCTGGAGGATGTAACATGGAGAACGTAGAAGCCACACGCCTCAC

EEGPSLQLEDVNYIEELYKGGEEAATRF

CTTCTTCCAGAGACGCTACGGCTCCGCTTACGCCGGCTTGGCAGCTGGCTTCGGCTTGTTCTGGTGAGGCCCGCAG

FFQSSSSGSAFRLAEAAAWPGWFLCGPA

AGCCCCAGCAGCAGCTACAGCTACCCAGAGGAGAGTGACGCTACGCCCCTAGAAGTTTACTTTGAACAGAGCTGGTAG

EPOQPVQLKESEPFTSYFQSW

GGAGACAGGGAACTGCGTTTACCCCGCTGCCCCAAAACCAAGCTCTACCTGGTCAGGGTTCTATGGTAACGAGAATAGT

TCCCCCGAATATGCTCCACATCTTAATCCCAAGATCTGTGCATATGTTACCATACATGTGCAAAAGAGTTTTGCACATGT

GATTATGTTAAGGATCTTGGAATGAGGAGAATCTCTGGTTATACCTCTGGTGAGCTCAGTTATATCCTCAAGAAGGAGGAGC

GAGGGGAGCTACAGGAGAGAATGGAAGATCCATGCTTTCTAAATTTGAGATGGAGTGAGGGCCTGGACAGCAACATAT

GCTTGTGTTTTTAGAGGAGAAAGAAGCAGGGATTCTCCTCTATAGCTCCGGAAGGACACAGCTCCTGGACAC

ATGGATTTCAGTCAGTGCACCCATTTCCAGACTTCTGGACCTCCAACACTTAAATATAACCTTGTTTTGTTAAGAC

CTCTAAAAAAAAAAA 1376
```
### B

|       | IL-1HY2 | IL-1α | IL-1β | IL-1ra | IL-18 | IL-1HY1 | FIL1ε | FIL1η | FIL1ζ | IL-1H1 |
|-------|---------|-------|-------|--------|-------|---------|-------|-------|-------|--------|
| IL-1HY2 | --      | 19    | 21    | 37     | 14    | 41      | 28    | 30    | 29    | 28     |
| IL-1α   | --      | 25    | 17    | 24     | 18    | 26      | 22    | 17    | 20    |        |
| IL-1β   | --      | 23    | 21    | 26     | 24    | 27      | 21    | 20    |       |        |
| IL-1ra  | --      | 13    | 52    | 30     | 31    | 27      | 31    |       |       |        |
| IL-18   | --      | 13    | 13    | 15     | 18    | 15      |       |       |       |        |
| IL-1HY1 | --      | 33    | 33    | 35     | 32    |         |       |       |       |        |
| FIL1ε   | --      | 46    | 36    | 57     |       |         |       |       |       |        |
| FIL1η   | --      | 32    | 45    |       |       |         |       |       |       |        |
| FIL1ζ   | --      |       | 36    |       |       |         |       |       |       |        |
| IL-1H1  |         |       |       |        |       |         |       |       |       |        |

### C

![Dendrogram diagram](http://www.jbc.org/Downloaded from)
Fig. 3

Exon:

I
(453 nt)

II
(86 nt)

III
(128 nt)

IV
(697 nt)

Intron:

I
(1562 nt)

II
(308 nt)

III
(301 nt)
Fig. 4

A

![Image of gel electrophoresis with bands labeled IL-1HY2 and β-actin.]

B

| Cell Lysate | Medium |
|-------------|--------|
| Control     | Control| kDa     |
| IL-1HY2     | IL-1HY2| 31.6    |
|             |        | 18      |
|             |        | 7.5     |
A. Negative Control

B. Anti-IL-1HY2

C. Anti-IL-1HY2 and Anti-CD20

D. Anti-IL-1HY2 and Anti-Ki67
Cloning and characterization of IL-1HY2, a novel interleukin-1 family member
Haishan Lin, Alice S. Ho, Dana Haley-Vicente, Jun Zhang, Juanita Bernal-Fussell, Ann M.
Pace, Derek Hansen, Kathi Schweighofer, Nancy K. Mize and John E. Ford

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