A Single Amino Acid Difference between Human and Monkey Interleukin (IL)-1β Dictates Effective Binding to Soluble Type II IL-1 Receptor*

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Soluble type II interleukin (IL)-1 receptor (sIL1R-II) binds human IL-1β with high affinity and neutralizes its activity. Recombinant sIL1R-II is considered a potentially useful anti-IL-1 therapeutic, and preclinical studies have been undertaken with this molecule in primates. To better understand the cytokine-receptor interactions occurring in this nonhuman context, monkey IL-1 and IL1R-II were cloned, and their binding abilities were examined in vitro. IL-1β from cynomolgus monkey was capable of binding and activating the human type I IL-1 receptor. However, unlike human IL-1β, it was unable to effectively bind and become neutralized by sIL1R-II. Human and cynomolgus IL-1β proteins are 96% identical, differing by only six amino acids. Structural and mutational analysis revealed that the unique sIL1R-II binding ability of human IL-1β is due to a single amino acid difference compared with monkey IL-1β.

Interleukin (IL)-11 is a multi-functional proinflammatory cytokine that mediates innate and adaptive immune responses in multiple cell types. It is believed to play a role in numerous diseases including arthritis, asthma/allergy, osteoporosis, and stroke (see Ref. 1 for review). The IL-1 family actually consists of two proteins with similar biological activity, IL-1α and IL-1β, as well as a nonsignaling ligand termed the IL-1 receptor antagonist (IL-1ra) (see Ref. 2 for review). All three proteins exhibit a similar tertiary structure comprised of 12 β strands that make up a barrel-shaped β-trefoil with pseudo-3-fold symmetry (3). IL-1 is thought to be the primary circulating cytokine that mediates the systemic effects of IL-1.

IL-1 exerts its biological action by binding and activating the membrane-associated IL1R-I (4). A second receptor, termed the IL-1R accessory protein (AcP), is not involved in direct ligand binding but is required for IL-1 signal transduction by complexing with IL-1 and the IL1R-I (5). ILIR-I and AcP both contain extracellular portions with three Ig-like domains and cytoplasmic portions containing conserved signaling motifs (6). A third IL-1 receptor exists termed the type II IL-1R (IL1R-II) that has an extracellular structure similar to that of IL1R-I and AcP but that contains a truncated cytoplasmic tail incapable of signaling. This receptor acts as a decoy by binding IL-1 with high affinity and neutralizing its activity (7). IL1R-II can also be proteolytically cleaved, which releases the extracellular domain from the cell surface. This creates a soluble form of the receptor (sIL1R-II) that possesses high affinity for IL-1β, but only low affinity for IL-1α, and virtually no affinity for IL-1ra.

For this reason, sIL1R-II is considered an ideal antagonist of the IL-1 system (8).

Animal models and ex vivo studies with human cells have demonstrated that sIL1R-II may be useful as a therapeutic agent (9, 10), and recombinant human sIL1R-II is being developed as a therapeutic for inflammatory disease. Preclinical studies have been undertaken to evaluate the toxicologic and pharmacokinetic effects of this receptor in nonhuman primates. Among these have been the examination of the interaction between cynomolgus and rhesus monkey IL-1 and human sIL1R-II. Surprisingly, we found that monkey IL-1β lacks the sIL1R-II binding ability of human IL-1β. A structural and mutational analysis revealed this to be due to a single amino acid difference between the two cytokines.

**EXPERIMENTAL PROCEDURES**

Cloning of Monkey Genes—IL-1α and IL-1β genes were cloned from cynomolgus monkey by reverse transcription-PCR using sequences previously deposited into GenBank™ (accession numbers AB000553 and D63353, respectively). Primers were designed to amplify a cDNA corresponding to the predicted (based on analogy to the human sequence) mature form of each IL-1. The products were amplified from cDNA from cynomolgous primary cells isolated from whole blood (kindly provided by Shin Nippon Biomedical Laboratories, Everett, Washington). Briefly, PBMCs were isolated from heparinized blood by density centrifugation. The cells were cultured in RPMI with 10% fetal bovine serum and 1% bromide (AET)-treated sheep erythrocytes and further density centrifugation. The cells were cultured in RPMI with 10% fetal bovine serum and were incubated for 3.5 h in lipopolysaccharide and human CD40L (both at 1 μg/ml), and total RNA was extracted and used for cDNA synthesis. The sequence of rhesus monkey IL-1β was also present in GenBank™ (accession number M71M9845). The predicted mature protein sequences for cynomolgus and rhesus IL-1β are identical except for the presence of an additional serine at the C terminus of rhesus IL-1β. The IL1R-II gene was amplified by PCR from either activated cynomolgus PBMC cDNA (described above) or cDNA made from total RNA extracted from a rhesus monkey tonsil biopsy (tissue kindly provided by Tom McVittie of the University of Maryland). PCR primers were derived from the publicly available IL1R-II sequence from the African green monkey (accession number U64092). For all cDNAs, multiple
PCR products or subclones were sequenced from each species to obtain consensus sequences free of PCR-introduced errors.

**Mammalian Expression and Purification—**Cynomolgus and rhesus IL-1β proteins were expressed from cDNAs corresponding to the predicted mature portion of the cytokines (amino acids 117–268 and 117–269, respectively). A construct for expression of cynomolgus IL-1β was also made using the predicted mature portion of this cytokine (amino acids 113–271). All of the IL-1 constructs were cloned in a mammalian expression vector (pPC409) (11) and were engineered with a heterologous initiating methionine to drive translation of the open reading frame. Cynomolgus and rhesus IL-1R-II sequences were found to be identical at the amino acid level. Soluble rhesus IL1R-II was expressed from a cDNA encoding amino acids 1–346 (C terminus indicated in Fig. 1). This was cloned in the 2aSib plasmid (12) for stable expression in Chinese hamster ovary cells.

Monkey IL-1s were expressed in COS1 cells (ATCC identification number CRL-1650) for 1 week (grown in Dulbecco’s modified Eagle’s medium with 5% fetal bovine serum) following transfection with DEAE-dextran. The IL-1s were purified from culture medium using a His Tag-covalently attached to Affi-Gel-10 (Bio-Rad). Eluted fractions were analyzed by SDS-PAGE, and pure fractions were pooled and dialyzed into PBS, and concentration was determined by amino acid analysis. Rhesus sIL1R-II was affinity-purified using human IL-1β covalently attached to Affi-Gel-10 (Bio-Rad). Purification elution fractions were pooled and dialyzed into PBS, and concentrations were determined by amino acid analysis.

**Escherichia coli Expression and Purification—**For expression in E. coli, cynomolgus or human mature IL-1β was cloned in pGEX4T-1 (Amersham Biosciences) with an N-terminal glutathione S-transferase fusion. A Factor Xa cleavage recognition sequence was integrated upstream of the first amino acid of IL-1β. Glutathione S-transferase proteins from E. coli lysates (strain DH10B) were bound to glutathione-Sepharose (Amersham Biosciences) and cleaved on-column with Factor Xa (Novagen, Madison, WI). Cleaved IL-1β was eluted with PBS, and the Factor Xa was removed with affinity agarose (Novagen). Purified IL-1 proteins were dialyzed against PBS and quantitated by the BCA method (Pierce).

**IL-1R-II Plate Binding Assay—**Human sIL1R-II (at 50 ng/ml) was bound to immobilized M25 antibody (a non-neutralizing anti-IL1R-II Ab) on Maxisorp 96-well enzyme-linked immunosorbent assay plates (Nalge Nunc International, Rochester, NY). Similarly, rhesus sIL1R-II protein (at 100 ng/ml) was captured on plates with immobilized M3 antibody (a non-neutralizing antibody that binds rhesus IL1R-II). Human IL-1β (R & D Systems) that had been bioinconjugated was added to the plate at 10 ng/ml in the presence or absence of unlabeled competitor IL-1 from different sources. Bound IL-1 was detected and quantitated using streptavidin-horseradish peroxidase conjugate (Jackson ImmunoResearch Laboratories, West Grove, PA) at 867 ng/ml followed by the addition of a peroxidase substrate/chromogen solution (Kirkegaard & Perry Laboratories). Plate fusions were arrested with phosphoric acid (1 M), and the optical density values were determined using a spectrophotometer plate reader. Human type II IL-1R protein was expressed in Chinese hamster ovary cells and purified as described for rhesus IL1R-II. M3 and M25 anti-human IL-1R-II antibodies were produced at Immunex. All of the samples were diluted in PBS with 0.05% Tween 20 with 0.1% bovine serum albumin.

**Soluble Receptor Binding—**10 × 10^5 COS1 cells were transiently transfected with 1 μg of expression plasmid for human or cynomolgus mature IL-1β using DEAE-dextran. After 48 h the cells were placed in Cys- and Met-free medium for 45 min and then metabolically labeled for 45 min. For SDS-PAGE analysis of the binding reactions, 20 μl was loaded onto 4–20% gradient Tris-glycine gels (Invitrogen). After electrophoresis, the gels were fixed, treated with Amplify (Amersham Biosciences), and exposed to autoradiography film.

**A375 Antiproliferation Assay—**E. coli-produced human and cynomolgus IL-1β proteins were used for all assays. A375 human melanoma cells were obtained from ATCC (identification number CRL-1572) and grown in Dulbecco’s modified Eagle’s medium with 5% fetal bovine serum, non-essential amino acids, and sodium pyruvate (Invitrogen). The cells were added to 96-well tissue culture plates at 1 × 10^3 cells/well in the presence or absence of IL-1. The cells were grown for 72 h and then washed with PBS and stained for 4–6 h with Alamar Blue (a fluorescent metabolic indicator from BioSource International, Camarillo, CA). Fluorescence units were measured using a SPEC-2000 fluorescence plate reader (560 nm excitation, 575 nm emission) (Tecan, Research Triangle, NC). For the experiments designed to measure sIL1R-II inhibition of IL-1 bioactivity, human affinity-purified sIL1R-II was added to the cells immediately prior to IL-1 addition, and the culture was grown and analyzed as described.

**Structural Modeling—**The human type II IL-1 receptor model was built using Modeler (13) based on Protein Data Bank (14) entries 11th chain B (15) and 1ira chain Y (16). Multiple structures were calculated to cover conformational space. After removing outliers, six structures remained and were shown to represent conformational space. They formed a structural ensemble with a Ca RMSD of 6.87 Å. From this ensemble a representative structure was chosen with a 1.68 Å Ca RMSD from the mean ensemble. The model structure of IL-1β was modeled based on Protein Data Bank entries 1hbb (17), 1iob (18), 21bi, 31bi, and 41bi (19). Ten initial structures were built and evaluated for errors; all were found suitable for inclusion in the structure ensemble, with Ca RMSD of 0.25 Å. The representative structure chosen was 0.11 Å Ca RMSD from the mean structure. Human IL-1β was taken from the crystal structure 1itb chain A, which has a resolution of 2.5 Å.

Because no ILIR-IL-1β complex crystal structure existed, the human and monkey IL-1β structures were docked onto human ILIR-II using Protein Data Bank identification number 1lbh as a template (1lbh is a complex of type I IL-1 receptor with IL-1β). First, ILIR-II was aligned and superimposed with ILIR-II, after which the ILIR-I chain was deleted. This model of human ILIR-II was superimposed with human ILIR-II. Next, monkey IL-1β was aligned and superimposed to human IL-1β, after which the human ILIR-I chain was deleted. This produced a model of monkey IL-1β complexed with human ILIR-II. Surface electrostatic differences were then calculated between human and monkey IL-1β using the Molecular Operating Environment from the Chemical Computing Group (Montreal, Canada). The surface electrostatics were calculated on IL-1β only in the presence of ILIR-II using an interior dielectric of 5, an exterior dielectric of 80, and 0 salt concentration.

**IL-1 Mutagenesis—**Site-directed mutagenesis of human and monkey IL-1β was performed using the QuikChange XL system (Stratagene, La Jolla, CA). Human and cynomolgus wild type IL-1β glutathione S-transferase (GST) fusion proteins were purified by immobilized Glutathione-Sepharose beads (Pharmacia). The GST tag and any changes were introduced. A natural Homo sapiens site adjacent to amino acids 13 and 15 provided a means for rapidly screening the correct recombinants. Mutant IL-1β proteins were subsequently expressed and purified from E. coli as described above.

**Affinity Measurements—**The affinities were determined by surface plasmon resonance using a Biacore 3000 instrument (Biacore AB, Uppsala, Sweden) at 25°C with a research grade CM5 sensor chip (Biacore). A capture system was employed with ~800 response units of goat anti-human IgG, Fcγ chain-specific antibody (Jackson ImmunoResearch Laboratories, West Grove, PA) immobilized using standard amine-coupling chemistry. Immobilized capture antibody alone was run on all the media containing IL-1R-II. After the capture receptors were expressed by transient transfection of COS cells and purified by chromatography on protein A-Sepharose. ILIR-II-Fc at 1 μg/ml or ILIR-II-Fc at 2 μg/ml in HBS-EP (10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 0.05% (w/v) surfactant P20, pH 7.4; Biacore) with 0.1 mg/ml bovine serum albumin was injected over the immobilized ILIR-II receptors coupled to 2 μl of ILIR-II-Fc at 1 000 response units. Recombinant IL-1β (Amgen) in HBS-EP with 0.1 mg/ml bovine serum albumin was injected over both the receptor and reference surfaces at concentrations ranging from 10 to 100 times the KD down to 0.1 times the KD at a flow rate of 50 μl/min. Duplicate injections of each sample and triplicate injections of a buffer blank were injected in random order with both IL-1β and IL-1β complexed with RII-Fc for 2–3 min, and IL-1β completely dissociated within 5 min. Complexes with slow off-rates associated for 5 min and dissociated for 30 min before regeneration with a single 30-s injection of 10 mM glycine HCl, pH 1.5, at a flow rate of 50 μl/min. The saturation maximum response
Acid 130 (indicated on Fig. 1). Cynomolgus IL-1/ H9252
other, at position 389, results in a Ser to Asn change at amino
constructs.

The cynomolgus IL-1/H9252 sequence (accession number D63353). One is a silent
nucleotide substitutions relative to the publicly available cyno-
obtained from activated PBMCs and was found to have two
sources of recombinant human IL-1β tested were able to bind in a comparable manner
either human or monkey sIL1R-II. Surprisingly, cynomolgus IL-1β bound poorly to both human and monkey sIL1R-II. This
was true when using purified protein (from E. coli) as well as unpurified protein in the form of COS supernatants. The same
result was obtained with the cynomolgus IL-1β that differs from
cynomolgus by only a single amino acid. These experiments
were performed across a wide range of immobilized receptor
and ligand concentrations with similar results (not shown).
Additionally, cynomolgus IL-1α was tested in the form of COS
supernatants at several dilutions and also found to be unable to
bind monkey or human sIL1R-II (not shown).

Soluble Receptor Binding—To address the possibility that
receptor immobilization could be affecting the results, we
performed a solution binding experiment. Radiolabeled IL-1αs
were incubated with recombinant soluble receptor proteins. Recep-
tor complexes were immunoprecipitated and analyzed by SDS-
PAGE. The presence of a correctly sized IL-1β band in lanes
representing precipitated receptor indicated a productive recep-
tor-ligand interaction. For these experiments cynomolgus
IL-1β was used because the single amino acid difference be-
tween the cynomolgus and rhesus proteins did not appear to
affect receptor binding in the plate binding assay. The results
of this experiment are shown in Fig. 3 and are consistent with
and support the initial observation that monkey IL-1 and monkey IL-1 appear to bind human IL1R-I. These results indicate that cynomolgus IL-1 and monkey IL-1 are capable of binding human IL1R-I. Human and cynomolgus IL-1β were also added at 50 ng/ml. Human, cynomolgus, and rhesus IL-1β from COS were added as 1:10 dilutions of conditioned medium from 5 day cultures of transfected COS1 cells. IL-1β concentrations in undiluted medium were estimated (by Western blot analysis) to be ~3 µg/ml (not shown). Competition for IL1R-II binding is expressed as a percentage of inhibition of the signal obtained in the absence of competitor.

**Fig. 2.** IL1R-II plate binding assay. Immobilized human or monkey sIL1R-II was bound with biotin-conjugated human IL-1β (at 10 ng/ml), which was detected using streptavidin-horseradish peroxidase and a color substrate. The ability of various forms of unconjugated IL-1β to compete for IL1R-II binding was tested. Human IL-1β (recombinant, R & D Systems) was added at 50 ng/ml. E. coli-produced human and cynomolgus IL-1β were also added at 50 ng/ml. Human, cynomolgus, and rhesus IL-1β from COS were added as 1:10 dilutions of conditioned medium from 5 day cultures of transfected COS1 cells. IL-1β concentrations in undiluted medium were estimated (by Western blot analysis) to be ~3 µg/ml (not shown). Competition for IL1R-II binding is expressed as a percentage of inhibition of the signal obtained in the absence of competitor.

**Fig. 3.** Soluble receptor binding. COS1 cells were transiently transfected with expression plasmids encoding human (hu) or cynomolgus (cyno) IL-1β or empty vector. After 48 h, the cells were metabolically labeled with [35S]Met and [35S]Cys, and the harvested medium was precleared with protein G. Untagged soluble type II IL-1 receptors from human and monkey were added, along with a nonblocking anti-IL1R-II antibody, and the bound IL-1β was precipitated using protein G-agarose. Control precipitations were performed using human IL1R-I-Fc followed by protein G-agarose. The no-receptor control reactions were preincubated but incubated in the absence of any receptor, antibody, or protein G-agarose. The recovered receptor complexes were washed and analyzed by SDS-PAGE followed by autoradiography. 10 µl of the no-receptor control reactions were also run alongside to indicate bands corresponding to human and cynomolgus IL-1β. The mature human IL-1β runs as a doublet of ~17 and 19 kDa. Cynomolgus IL-1β is expressed as a single band that migrates at the predicted 17.5 kDa.

The plate binding results. No band is observed when the control supernatants are incubated with human sIL1R-II; however, human IL-1β but not cynomolgus IL-1β is detected in the immunoprecipitated human sIL1R-II reaction. Similarly, when using monkey sIL1R-II, it is only the human IL-1β that exhibits detectable binding to the precipitated receptor. Both human and monkey IL-1 appear to bind human IL1R-I. These results are independent of receptor immobilization or IL-1 purification and support the initial observation that monkey IL-1β does not bind well to sIL1R-II. It does appear, however, that monkey IL-1β is capable of binding human IL1R-I.

**A375 Functional Assay**

The results from the soluble receptor binding experiment indicated that cynomolgus IL-1β is capable of binding human IL1R-I. Human and cynomolgus IL-1β were used in an anti-proliferation cell assay to determine the biological activity of cynomolgus IL-1β as well as to establish a cell-based system for better understanding the interaction, or lack thereof, between sIL1R-II and monkey IL-1β. A375 human melanoma cell proliferation is inhibited in this assay by IL-1 through an IL-1R-dependent mechanism. The number of viable cells following culture in the presence of IL-1 correlates inversely with increasing amounts of IL-1 bioactivity.

Purified recombinant human and cynomolgus IL-1β were titrated in the assay to determine their relative level of bioactivity. As seen in Fig. 4, introduction of both human and monkey IL-1β at 2.5 and 5 ng/ml, respectively, resulted in a significant inhibition of A375 proliferation, indicating that both cytokines were active. Further dilution of the IL-1s resulted in a corresponding reduction in this activity that was nearly identical between the two cytokines. The calculated IC50 values (defined as the concentration of IL-1 at which approximately a 50% reduction in the number of viable cells occurs) are 0.068 and 0.063 ng/ml for the human and cynomolgus cytokines, respectively. This result indicates that the recombinant human and monkey IL-1βs are active and have nearly equivalent levels of activity through the human type I IL-1R.

**Structural Analysis**

The results presented so far suggest that because human but not monkey IL-1β is capable of binding human and monkey type II IL-1R, the difference must be accounted for by sequence differences in the IL-1β molecules. To understand this better, we generated a three-dimensional model (Fig. 5) of the interaction between IL-1β and IL1R-II based upon the published crystal structure of the IL-1β/IL1R-I interaction (15) (see “Experimental Procedures” for details). The six amino acid differences between the human and monkey IL-1β proteins (highlighted on the alignment in Fig. 1) were identified in the model and analyzed for potential receptor contacts. Four of the amino acids, which differ between the species (Asn7, Ser13, Gln15, and Met36; the reference was the human sequence), showed solvent accessibility differences and were predicted to make contact with the receptor. The Met/Leu change at position 36 is a conservative change because both amino acids are hydrophobic and differ little in size. The Asn to His change at position 7 does not appear to affect the surface electrostatics significantly. In contrast, the Ser to Ala change at position 13 and the Gln to Leu change at position 15 are both polar to nonpolar changes with partial charge differences that induce surface electrostatic differences. Serine 13 lies on the edge of the receptor-interact-
the potency of human IL-1

Interestingly the Ser to Ala change at position 13 diminished
mine whether or not they still retained activity and to identify
assay to test for inhibition by sIL-1R-II. Initially, titrations of
The mutant IL-1s were first examined in the A375 biological
IL-1

Similarly, the Ala to Ser change at the same site in monkey
IL-1 \( \beta \) identified as being submaximal, to which increasing
amounts of soluble human IL1R-II was added. Forms of IL-1\( \beta \)
able to become neutralized by the type II receptor were appar-
ent as a reduction in bioactivity in the presence of soluble
receptor. The results are shown in Fig. 6. Whereas the wild
type and Ser to Ala mutant of human IL-1\( \beta \) were both still
inhibitable by soluble type II receptor, the single Gln to Leu
mutation and the double mutation both abolished this inhibi-
tion, even at the highest dose of soluble receptor. Conversely,
wild type monkey IL-1\( \beta \) or the Ala to Ser mutant were both still
recalcitrant to sIL1R-II inhibition. However, the single Leu to
Gln change or the double mutant containing this change both
bestowed the ability to become inhibited by sIL1R-II. The re-
results clearly demonstrate that the glutamine at position 15 in
mature human IL-1\( \beta \) is essential for neutralization by
sIL1R-II.

**Affinity Measurements**

To demonstrate that the effects of the position 15 IL-1\( \beta \)
mutations on sIL1R-II inhibition were related to binding affinity,
we performed Biacore-based affinity measurements. Wild
type human and monkey IL-1\( \beta \) as well as the Gln to Leu
mutants of each species were utilized for these experiments.
Each IL-1 was examined for binding to both human IL1R-I as
well as human IL1R-II, and binding affinities were derived.
The results are presented in Table I.

The affinities of human and monkey wild type IL-1\( \beta \) were
nearly identical for the human type I IL-1 receptor, and both \( K_D \)
values were in the nanomolar range. The effects of the Gln to Leu
mutations on IL1R-I binding were consistent with the results
observed in the A375 bioassay. The human Gln to Leu mutant
bound with a 20-fold lower affinity than wild type IL-1\( \beta \). Con-
versely, monkey IL-1\( \beta \) with the change from Leu to Gln actually
had a 10-fold higher affinity (\( K_D = 4.7 \times 10^{-10} \) m) than wild type
IL-1\( \beta \) from either species. The affinities obtained for IL-1\( \beta \)
binding to human IL1R-II also reflected the activities observed in
the bioassay. Wild type monkey IL-1\( \beta \)-bound IL1R-II with extremely
low affinity (\( K_D = 1.9 \times 10^{-6} \) m); however, the Leu to Gln mutant
bound with essentially the same high affinity (\( K_D = 5.9 \times 10^{-10} \)
m) as human IL-1\( \beta \), primarily because of a much slower dissoci-
ation rate (not shown). The effect on human IL-1\( \beta \) binding affinity
was just as profound, although in the opposite direction. The
dissociation constant of human IL-1\( \beta \) with the Gln to Leu change
dropped to \( 1.2 \times 10^{-6} \) m. These results confirm that the ability of
sIL1R-II to inhibit the different forms of human and monkey
IL-1\( \beta \) correlates with actual binding affinities. While performing
these binding measurements we also tested recombinant cymo-
nolus IL-1ra\(^2\) for binding to human IL1R-II (data not shown).
The results were as expected; the binding interaction was very
weak (\( K_D \) between \( 10^{-7} \) and \( 10^{-8} \) m), which revealed that at least
this aspect of the monkey IL-1 system paralleled the human
system.

**DISCUSSION**

We have examined the ability of monkey IL-1\( \beta \) to bind and
become neutralized by sIL1R-II. Although human IL-1\( \beta \) binds

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\(^2\) The cloning, expression, and purification of cymolagus IL-1ra is
described elsewhere (see footnote 3).
Receptor Binding Ability of Monkey IL-1β

TABLE I
Receptor binding affinities for human and monkey IL-1β mutants

| IL-1 version tested | hu IL1R-I | hu IL1R-II |
|--------------------|-----------|------------|
| Human IL-1β wild type | $3.8 \times 10^{-9}$ | $5.2 \times 10^{-10}$ |
| Human IL-1β (Gln → Leu) | $7.9 \times 10^{-6}$ | $1.2 \times 10^{-6}$ |
| Monkey IL-1β wild type | $5.8 \times 10^{-9}$ | $1.9 \times 10^{-6}$ |
| Monkey IL-1β (Leu → Gln) | $4.7 \times 10^{-10}$ | $5.9 \times 10^{-10}$ |

sIL1R-II with high affinity, the monkey cytokine is unable to do so because of a single IL-1β amino acid difference. It is striking that a single amino acid is able to confer such an essential quality with regards to receptor binding and neutralization. The glutamine at position 15 of human IL-1β lies within the beginning of a hairpin loop immediately following the first β strand. This is within a region on the side of the IL-1β barrel referred to as site A, which is present in IL-1β, IL-1α, and IL-1ra. This site forms a contact between the cytokine and the first and second Ig domains of the type I receptor. Previous mutational studies of human IL-1β have identified this region as being critical for IL1R-I interaction (15, 24). Evans et al. (25) found that when Gln was changed to Leu, the ligand lost 100% of its IL1R-I binding ability. Although we did in fact observe that the Gln to Leu change diminished human IL-1β activity in the A375 assay, biological activity was not completely abolished. It may be that a change to Leu, the side chain of which is similar in size to that of the original Gln, is less disruptive than a change to the smaller glycine. The same authors (25) found that when the Gln was changed to His, IL-1β exhibited greater activity than wild type, indicating that different substitutions can have different effects on type I IL-1 receptor binding. Specific amino acid substitutions may also confer different consequences with respect to type I versus type II receptor binding, depending on the nature of the interacting residues on each receptor surface.

The lack of effective sIL1R-II binding by monkey IL-1β was unexpected both because of the degree of homology with human IL-1β and because a previous study with rhesus monkeys suggested that human sIL1R-II is efficacious in a model of acute inflammation. We have recently discovered that significant levels of soluble AcP protein are present in the serum of humans and monkeys and that soluble AcP is able to enhance the binding affinity between monkey IL-1β and sIL1R-II. This provides a possible mechanism to explain how sIL1R-II may be capable of inhibiting monkey IL-1 in vivo.

A comparative study of multiple cytokine sequences in non-human primates noted that the level of sequence conservation with human orthologs varied considerably (21). Despite the high degree of sequence homology, IL-1α and IL-1β were among the most diverged cytokines between human and monkey. This suggests that different evolutionary patterns may be associated with different cytokines. The degree of intricate regulation inherent in the IL-1 system may affect its evolution differently among species. The subtle IL-1β sequence difference between humans and the Old World monkeys in this study confers upon the human IL-1 system an even tighter degree of regulation. This is due to a higher affinity for the soluble IL1R-II that allows neutralizing complexes to form through a 1:1 interaction. In monkeys, inhibition of circulating IL-1β may require higher levels of sIL1R-II as well as sufficient levels of soluble AcP.

Glutamnate at position 15 is not unique to human; it is also present in the IL-1β sequence from mouse, rabbit, sheep, dolphin, etc. (not shown). It is certainly possible, however, that its essentiality for high affinity receptor binding is only in the context of the entire IL-1β sequence, which will differ across species at multiple sites. It is tempting to speculate, however, that this difference between human and monkey IL-1β is a
fairly recent event in primate evolution, potentially linked to the particular evolution of the human immune system. Out of this curiosity we decided to clone and compare the IL-1β sequence from chimpanzee, the closest related primate to humans. The gene sequence was amplified from cDNA made from lipopolysaccharide-activated chimp PBMC RNA. The predicted mature portion of chimpanzee IL-1β was identical to human IL-1β (not shown); therefore, one would predict that the sIL1R-II binding capability of human IL-1β is not entirely unique to our species. Nonetheless, this study has revealed that there can be profound differences when examining cytokine-receptor interactions between humans and other primates. It also further strengthens the notion that sIL1R-II has become a very effective, naturally occurring antagonist of IL-1β in humans. Further development of this molecule as an anti-inflammatory therapeutic remains a promising prospect.

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