Abstract: A therapeutic composition is described that can be used for treating or prevention of diseases association with modulation of activity of human IL-1β. In certain aspects, the disclosed invention is based, on engineering of a heterodimeric protein assembly that is capable of binding to human IL-1β and attenuating its function. The heterodimeric protein assembly comprises an extracellular portions of human IL-1R1 and of human IL-RAcP, or their functional fragments. Each, the IL-1R1 portion and the IL-RAcP portion, is fused to a distinct mutant of Fc portion of the human Ig Gamma-1. The two distinct Fc mutants in the heterodimeric protein assembly are engineered as to favor the heterodimeric dimer formation between the two Fc mutants over any homomeric assembly. DNA expression vectors and expression systems for overproducing the polypeptides in mammalian cells are also provided for.

**FIGURE 1**

[Diagram showing IL-1β and IL-1R1 with Fc domains]

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IL-ip INHIBITOR COMPOSITION AND USE THEREOF

FIELD OF THE INVENTION

Generally, the invention relates to the field of biological pharmaceuticals as well as their use in conditions associated with inflammatory disorders (e.g. rheumatoid arthritis, Crohn's disease, etc.), diabetes, cardiovascular disease and gout. More specifically, the invention relates to a heterodimeric IL-1R1/IL-1RAcP-derived composition that is capable of inhibiting IL-1β cytokine.

BACKGROUND

The interleukin-1 (IL-1) family of cytokines comprises 11 proteins (IL-1F1 to IL-1F11) encoded by 11 distinct genes in humans and mice. IL-1-type cytokines are major mediators of innate immune reactions, and blockade of the founding members IL-1 or IL-1β by the interleukin-1 receptor antagonist (IL-1RA) has demonstrated a central role of IL-1 in a number of human autoinflammatory diseases. IL-1 or IL-1β rapidly increase messenger RNA expression of hundreds of genes in multiple different cell types. The potent proinflammatory activities of IL-1 and IL-1β are restricted at three major levels: (i) synthesis and release, (ii) membrane receptors, and (iii) intracellular signal transduction. This pathway summarizes extracellular and intracellular signaling of IL-1 or IL-1β, including positive- and negative-feedback mechanisms that amplify or terminate the IL-1 response. In response to ligand binding of the receptor, a complex sequence of combinatorial phosphorylation and ubiquitination events results in activation of nuclear factor kappa-B signaling and the TNK and p38 mitogen-activated protein kinase pathways, which, cooperatively, induce the expression of canonical IL-1 target genes (such as IL-6, IL-8, MCP-1, COX-2, IL-1, IL-1β, MKP-1) by transcriptional and posttranscriptional mechanisms. Of note, most intracellular components that participate in the cellular response to IL-1 also mediate responses to other cytokines (IL-18 and IL-33), Toll-like-receptors (TLRs), and many forms of cytotoxic stresses (see Weber A, et al, Sci Signal, 2010 Jan 19;3(105), the entire teachings of which are incorporated by reference herein).

IL-1 and IL-1β independently bind the type I IL-1 receptor (IL-1R1), which is ubiquitously expressed. A third specific ligand, the IL-1 receptor antagonist (IL-1RA), binds the IL-1RI with similar specificity and affinity but does not activate the receptor and trigger downstream signaling. The IL-1 receptor accessory protein (IL-1RAcP) serves as a co-receptor that is required for signal
transduction of IL-1/IL-1RI complexes, and this co-receptor is also necessary for activation of IL-1R1 by other IL-1 family members, in particular IL-18 and IL-33. The type II IL-1 receptor (IL-1R2) binds IL-1 and IL-1β but lacks a signaling-competent cytosolic part and thus serves as a decoy receptor. The IL-IRA, the plasma membrane-anchored IL-1R2, and the naturally occurring "shed" domains of each of the extracellular IL-1 receptor chains (termed sIL-1RI, sIL-1RII, and sIL-IRA, where "s" stands for soluble) provide inducible negative regulators of IL-1 signaling in the extracellular space whose abundance, which is regulated by a combination of increased transcription and controlled release, can limit or terminate IL-1 effects.

The initial step in IL-1 signal transduction is a ligand-induced conformational change in the first extracellular domain of the IL-1RI that facilitates recruitment of IL-1RacP. Through conserved cytosolic regions called Toll- and IL-IR-like (TIR) domains, the trimeric complex rapidly assembles two intracellular signaling proteins, myeloid differentiation primary response gene 88 (MYD88) and interleukin-1 receptor-activated protein kinase (IRAK) 4. Mice lacking MYD88 or IRAK4 show severe defects in IL-1 signaling. Similarly, humans with mutations in the IRAK4 gene have defects in IL-1RI and Toll-like receptor (TLR) signaling. IL-1, IL-1RI, IL-RAcP, MYD88, and IRAK4 form a stable IL-1-induced first signaling module. This is paralleled by the (auto)phosphorylation of IRAK4, which subsequently phosphorylates IRAKI and IRAK2, and then this is followed by the recruitment and oligomerization of tumor necrosis factor-associated factor (TRAF) 6. IRAKI and 2 function as both adaptors and protein kinases to transmit downstream signals. Complexes of IRAKI, IRAK2, and TRAF6 dissociate from the initial receptor complex, and cells lacking these proteins have impaired activation of the transcription factors nuclear factor kappa-B (NF-kappa-B) and activator protein 1 (AP-1).

Overproduction of IL-1 is the cause of many inflammatory disorders. For example, IL-1 has been linked to the pathology of diabetes, cardiovascular disease, gout and certain types of arthritis (e.g. rheumatoid arthritis (RA)).

Rilonacept is an IL-1 antagonist which includes an IL-1-specific fusion protein which comprises an IL-1 binding portion of the extracellular domain of human ILI-RAcP, an IL-1 binding portion of the extracellular domain of human IL-IRI, and a multimerizing component. This IL-1-specific fusion protein is described in U.S. Pat. No. 6,472,179, U.S. patent publication No. 2003/0143697, published 31 Jul. 2003, U.S. Pat. No. 7,361,350, and U.S. patent publication No. 2005/0197293, published 8 Sep. 2005 (all of which are incorporated by reference herein in
their entirety). Rilonacept under the trade name ARCALYST was approved by U.S. Food and Drug Administration (FDA) for the treatment of Cryopyrin-Associated Periodic Syndromes (CAPS), including Familial Cold Auto-inflammatory Syndrome (FCAS) and Muckle-Wells Syndrome (MWS) in adults and children 12 and older. Further clinical trials of rilonacept are currently under way, i.e. for gout.

**SUMMARY OF THE INVENTION**

This summary is provided to introduce a selection of concepts in a simplified form that are further described below in the Detailed Description. This summary is not intended to identify key features or essential features of the claimed subject matter, nor is it intended to be used as an aid in determining the scope of the claimed subject matter.

In certain aspects, the present invention provides for a heterodimeric protein composition capable of binding human IL-1β (GenBank: AAH08678.1). The protein composition comprises a first polypeptide which includes a first amino acid sequence which contains amino acids 18 through 333 of human IL1-R1 (GenBank: AAM88423.1), and a second amino acid sequence which contains a first mutant of a Fc portion of human immunoglobulin gamma-1 Fc (GenBank: J00228.1). The protein composition also comprises a second polypeptide which includes another first amino acid sequence containing amino acids 21 through 358 of human IL1-RACP (GenBank: BAA25421.1), and another second amino acid sequence which contains a second mutant of the Fc portion of human immunoglobulin gamma-1 Fc. In the protein composition, the first and second mutants are selected as to favor heterodimeric assembly between the first and second mutants over any homodimeric assembly. The protein composition may be capable of exhibiting human IL-1β binding activity in an ELISA assay with an EC50 of about 50 ng/ml. The first polypeptide of the protein composition may contain amino acid sequence of SEQ ID NO. 1, while the second polypeptide may contain amino acid sequence of SEQ ID NO. 2.

In certain aspects, the present invention provides for a therapeutic composition. The therapeutic composition comprises a heterodimeric protein composition capable of binding human IL-1β. The protein composition comprises a first polypeptide which includes a first amino acid sequence which contains amino acids 18 through 333 of human IL1-R1, and a second amino acid sequence...
which contains a first mutant of the Fc portion of human immunoglobulin gamma-1 Fc. The protein composition also contains a second polypeptide which includes another first amino acid sequence containing amino acids 21 through 358 of human IL1-RacP, and another second amino acid sequence which contains a second mutant of the Fc portion of human immunoglobulin gamma-1 Fc. In the protein composition, the first and second mutants are selected as to favor heterodimeric assembly between the first and second mutants over any homodimeric assembly. The therapeutic composition may exhibit a half-life of the heterodimeric protein composition in systemic circulation in mice after a subcutaneous administration at a dose of 5 mg/kg of at least about 88 hours, as assayed by human Fc ELISA. The therapeutic composition may comprise a heterodimeric protein comprised of a first polypeptide containing amino acid sequence of SEQ ID NO. 1 and a second polypeptide containing amino acid sequence of SEQ ID NO. 2.

In certain aspects, the present invention provides for an isolated nucleic acid encoding a polypeptide comprising amino acid sequence of SEQ ID NO. 3. The codon usage of the nucleic acid may be optimized for high expression of the polypeptide in a mammalian cell. The nucleic acid may contain the sequence of SEQ ID NO. 5. The nucleic acid may comprise an expression vector.

In certain aspects, the present invention provides for an isolated nucleic acid encoding a polypeptide comprising amino acid sequence of SEQ ID NO. 4. The codon usage of the nucleic acid may be optimized for high expression of the polypeptide in a mammalian cell. The nucleic acid may contain the sequence of SEQ ID NO. 6. The nucleic acid may comprise an expression vector.

In certain aspects, the present invention provides for an heterologous expression system. The expression system harbors an expression vector comprising a nucleic acid sequence encoding a first polypeptide containing amino acid sequence of SEQ ID NO. 3 and another nucleic acid sequence encoding a second polypeptide containing amino acid sequence of SEQ ID NO. 4. The expression vector of the expression system may be harbored in a mammalian cell. The mammalian cell may be a CHO cell. The expression system may be capable of expressing a heterodimeric protein comprising a first polypeptide containing amino acid sequence of SEQ ID NO. 1 and a second polypeptide containing amino acid sequence of SEQ ID NO. 2. The level of expression of the heterodimeric protein may be at least 300 mg per liter of cell culture.
In certain aspects, the present invention provides for use of a substance for manufacture of a medicament for the treatment or prevention of a disease associated with modulation of activity of human IL-1β. The substance comprises a heterodimeric protein comprised of a first polypeptide containing amino acid sequence of SEQ ID NO. 1 and a second polypeptide containing amino acid sequence of SEQ ID NO. 2. The disease associated with modulation of activity of human IL-1β may be an arthritis, a gout, a rheumatoid arthritis, a Cryopyrin-Associated Periodic Syndromes (CAPS), a scleroderma, a diabetes, a atherosclerosis, a dry eye disease, an ocular allergy, or an uveitis.

In certain aspects, the present invention provides for a method of treating or preventing a disease or condition associated with modulation of activity of human IL-1β. The method comprising administering to a patient in need for treating or preventing a disease associated with modulation of activity of human IL-1β a therapeutically effective amount of a pharmaceutical composition. The pharmaceutical composition comprising a heterodimeric protein comprised of a first polypeptide containing amino acid sequence of SEQ ID NO. 1 and a second polypeptide containing amino acid sequence of SEQ ID NO. 2. The disease associated with modulation of activity of human IL-1β may be an arthritis, a gout, a rheumatoid arthritis, a Cryopyrin-Associated Periodic Syndromes (CAPS), a scleroderma, a diabetes, a atherosclerosis, a dry eye disease, an ocular allergy, or an uveitis.

**BRIEF DESCRIPTION OF THE DRAWINGS**

The following drawings and descriptions are provided to aid in the understanding of the invention:

**Figure 1** illustratively shows a heterodimeric protein assembly of the present teachings comprising an extracellular portion of IL1-R1 fused with an IgG-Fc domain (Fc-II) via a flexible linker and an extracellular portion of IL1-RAcP fused with another IgG-Fc domain (Fc-V) via another flexible linker;

**Figure 2** schematically shows the map of PKN012 plasmid and annotated sequence used in the cloning of the polypeptides of the present invention;

**Figure 3** shows representative transfection growth curve obtained in the process of generating stable cell lines for expressing the polypeptides of the present invention;
**Figure 4** shown a size-exclusion HPLC analytical chromatogram of the sample containing heterodimers comprising polypeptide of SEQ ID NO. 1 and polypeptide of SEQ ID NO. 2 after the anion exchange chromatography purification step;

**Figure 5** shown a SDS-PAGE analysis of the sample containing heterodimers comprising polypeptide of SEQ ID NO. 1 and polypeptide of SEQ ID NO. 2 after the anion exchange chromatography purification step; and

**Figure 6** shows a typical binding curve of a purified sample containing heterodimers comprising polypeptide of SEQ ID NO. 1 and polypeptide of SEQ ID NO. 2 in an ELISA assay using commercially available human IL-1β.

**DETAILED DESCRIPTION OF THE INVENTION**

The teachings disclosed herein are based, in part, upon engineering of a heterodimeric protein assembly that is capable of binding to human IL-1β and attenuating its function. The heterodimeric protein assembly of the present teachings comprises an extracellular portions of IL1-R1 (GenBank: AAM88423.1) and of IL-1RaCP (GenBank: BAA25421.1), or functional fragments thereof. Each, the IL1-R1 portion and the IL-1RaCP portion, is fused to a distinct mutant of Fc portion of the human Ig Gamma-1 (GenBank: J00228.1). The two distinct Fc mutants in the heterodimeric protein assembly are engineered as to favor the heteromeric dimer formation between the two Fc mutants over any homomeric assembly. To enable recombinant production of the heterodimeric protein assembly of the present teachings, a DNA expression vector has been constructed for overproducing the heterodimeric protein assembly in a heterologous protein expression system, and mammalian cells have been prepared stably expressing the heterodimeric protein assembly to a high expression level. A protein purification procedure has been devised allowing obtaining a physiologically relevant substantially pure preparation of the heterodimeric protein assembly of the present teachings. Thus purified protein molecule demonstrates a high degree of specific activity in an *in vitro* Enzyme-Linked Immunosorbent Assay (ELISA) using human IL-1β (GenBank: AAH08678.1). Unexpectedly, the protein molecule exhibits an acceptable pharmacokinetics profile upon subcutaneous animal administration, while not resulting in any body weight loss or adverse clinical events.
The terms used in this specification generally have their ordinary meanings in the art, within the context of this invention and in the specific context where each term is used. Certain terms are discussed below or elsewhere in the specification, to provide additional guidance to the practitioner in describing the compositions and methods of the invention and how to make and use them. The scope or meaning of any use of a term will be apparent from the specific context in which the term is used. "About" and "approximately" shall generally mean an acceptable degree of error for the quantity measured given the nature or precision of the measurements. Typically, exemplary degrees of error are within 20 percent (%), preferably within 10%, and more preferably within 5% of a given value or range of values. Alternatively, and particularly in biological systems, the terms "about" and "approximately" may mean values that are within an order of magnitude, preferably within 5-fold and more preferably within 2-fold of a given value.

Numerical quantities given herein are approximate unless stated otherwise, meaning that the term "about" or "approximately" can be inferred when not expressly stated.

The methods of the invention may include steps of comparing sequences to each other, including wild-type sequence to one or more mutants (sequence variants). Such comparisons typically comprise alignments of polymer sequences, e.g., using sequence alignment programs and/or algorithms that are well known in the art (for example, BLAST, FASTA and MEGALIGN, to name a few). The skilled artisan can readily appreciate that, in such alignments, where a mutation contains a residue insertion or deletion, the sequence alignment will introduce a "gap" (typically represented by a dash, or "A") in the polymer sequence not containing the inserted or deleted residue.

The methods of the invention may include statistical calculations, e.g. determination of IC50 or EC50 values, etc. The skilled artisan can readily appreciate that such can be performed using a variety of commercially available software, e.g. PRISM (GraphPad Software Inc, La Jolla, CA, USA) or similar.

"Homologous," in all its grammatical forms and spelling variations, refers to the relationship between two proteins that possess a "common evolutionary origin," including proteins from superfamilies in the same species of organism, as well as homologous proteins from different species of organism. Such proteins (and their encoding nucleic acids) have sequence homology, as reflected by their sequence similarity, whether in terms of percent identity or by the presence of specific residues or motifs and conserved positions. However, in common usage and in the instant
application, the term "homologous," when modified with an adverb such as "highly," may refer to sequence similarity and may or may not relate to a common evolutionary origin.

The term "sequence similarity," in all its grammatical forms, refers to the degree of identity or correspondence between nucleic acid or amino acid sequences that may or may not share a common evolutionary origin.

The terms "protein" and "polypeptide" are used interchangeably. The polypeptides described herein may be comprised of more than one contiguous amino acid chain, thus forming dimers or other oligomeric formations. In general, the polypeptides of the present teachings for use in mammals are expressed in mammalian cells that allow for proper post-translational modifications, such as CHO or HEK293 cell lines, although other mammalian expression cell lines are expected to be useful as well. It is therefore anticipated that the polypeptides of the present teachings may be post-translationally modified without substantially effecting its biological function.

In certain aspects, functional variants of the heterodimeric protein assemblies of the present teachings include fusion proteins having at least a biologically active portion of the human IL1-R1 or IL-IRAcP or a functional fragment thereof, and one or more fusion domains. Well known examples of such fusion domains include, but are not limited to, polyhistidine, Glu-Glu, glutathione S transferase (GST), thioredoxin, protein A, protein G, an immunoglobulin heavy chain constant region (e.g., an Fc), maltose binding protein (MBP), or human serum albumin. A fusion domain may be selected so as to confer a desired property. For example, the IL1-R1 or IL-IRAcP polypeptide portions may be fused with a domain that stabilizes the IL1-R1 or IL-IRAcP polypeptides in vivo (a "stabilizer" domain), optionally via a suitable peptide linker. The term "stabilizing" means anything that increases the half life of a polypeptide in systemic circulation, regardless of whether this is because of decreased destruction, decreased clearance, or other pharmacokinetic effect. Fusions with the Fc portion of an immunoglobulin are known to confer desirable pharmacokinetic properties on certain proteins. Likewise, fusions to human serum albumin can confer desirable properties. Other types of fusion domains that may be selected include multimerizing (e.g., dimerizing, tetramerizing) domains and functional domains that confer an additional biological function, e.g. promoting accumulation at the targeted site of action in vivo.

In certain aspects, the heterodimeric protein assemblies of the present teachings comprise an extracellular portion of IL1-R1, or a functional fragment thereof, fused with a IgG-Fc domain, and
an extracellular portion IL-1RAcP, or a functional fragment thereof, fused with another IgG-Fc
domain. The IgG-Fc domain and the other IgG-Fc domain are chosen as to favor a heterodimeric
protein assembly over any homodimeric protein assembly. The extracellular portion of IL1-R1
may be fused with the IgG-Fc domain via a flexible linker, while IL-1RAcP, or a functional
fragment thereof, may be fused with the other IgG-Fc domain via the flexible linker of the same
amino acid sequence or via another flexible linker.

In an example embodiment, illustratively shown in Figure 1, the extracellular portion of IL1-R1
fused with IgG-Fc domain (Fc-II) via a flexible linker may comprise the amino acid sequence of
SEQ. ID NO. 1, while IL-1RAcP fused with another IgG-Fc domain (Fc-V) via a flexible linker
may comprise the amino acid sequence of SEQ. ID NO. 2.

hIL1-Rl-hlgGl-Fc polypeptide (SEQ ID NO. 1)

LEADKCKERE EKIILVSSAN EIDVRPCPLN PNEHKGTITW YKDDSKPVS TEQASRIHQH 60
KEKLWFPVAK VEDSGHYCYV VRNSSYCLRI KISAKFVENE PNLCYNAQAI FQKQLPVAGD 120
GGLVCPLYMEF FKNNENELPK LQWYKDCKPL LLNIIHFSGV KDLRIVMNV AEKHGRNYTCH 180
ASYTYLGBKQY PITRVIEFIT LEENKTPTRPV IVSPANETME VDLGSQIQLI CNVTGQLSDI 240
AYWKNNGSVI DEDDPVLGED YSVENPANK RRRSTLTVLN ISEIESFRFYK HPFTCFAKNT 300
HGIDAAYIQL IYPVTNGSSG GDKHTHCPPC PAPELLGGS VFLFPKPFD TLMISRTPEV 360
TCWVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRWSVLTVL HQDWLNGKEY 420
KCKVSNKAP APIEKTKSA KGPQREPQVC TLPPSRDELT KNQVLSLC AVGFYPSDIAV 480
EWESNGQPEN NYKTPPVLD SDGSFFLVSK LTVSKSRWQQ GNVFSCSVMH EALHNHYTKQ 540
SLSLPGK 548

hIL-1RAcP-hlgGl-Fc polypeptide (SEQ ID NO. 2)

SERCDDWGLD TMRQIQVFED EPARIKCLPF EHFLKFNYST AHSAGLTLIW YWTRQDRDLE 60
EPINFRLPEN RISKEKDVLW FPRTLLENDTG NYTCMLRNTT YCSKVAFFLE VQQKDCSFNS 120
PMKLPVHKEY IYEYIQRTCN PNVDGYFPSS VKPTITWYM CYKIQNFNNV IPEGMNLSFL 180
IALISNNNGY TCVVTPYPEN RTFHLTRILT VKVGVSPKNA VPPVIIHSND HWYKEKPE 240
ELLIPCTYVF SPLMDSRNEV WTTIDGKPPD DITIDVTNE SISHIRTEDE TRQTILSIKK 300
VTSEDLKRSY VCHARSKAGE VAKAACKVQK VPAPRYTVGS GGGDKTHTCPC PAPELLGGS 360
PSVFLFPKP KDTLMISRTDP EVTCWVDVS HEDPEVKFNW YVDGVEVHNA KTPREEQYN 420
In certain aspects, the present teachings provides for a recombinant DNA molecule having an open reading frame coding for a polypeptide comprising the leading 333 amino acids of the human IL1-R1 fused with IgG-Fc domain (Fc-II) via a flexible linker, and for another recombinant DNA molecule having an open reading frame coding for another polypeptide comprising the leading 358 amino acids of the human IL-1RAcP fused with another IgG-Fc domain (Fc-V) via a flexible linker.

In an example embodiment, the polypeptide comprising the leading 333 amino acids of the human IL1-R1 fused with IgG-Fc domain (Fc-II) via a flexible linker comprises the amino acid sequence of SEQ. ID NO. 3. The corresponding to it DNA molecule may comprise the nucleotide sequence of SEQ ID NO. 4. The another polypeptide comprises the leading 358 amino acids of the human IL-1RAcP fused with another IgG-Fc domain (Fc-V) via a flexible linker may comprise the amino acid sequence of SEQ. ID NO. 5. The corresponding to it DNA molecule may comprise the nucleotide sequence of SEQ ID NO. 6.

**hIL1-R1-hlgGl-Fc polypeptide (SEQ ID NO. 3)**

MKVLLRLI CF TALLI SSLLEA DKCKEREKKI I LVS SANE I D VRPC PLNPNE HKGT I TWYKD 60
DSKT PVSTEQ ASRI HQHKEK LWFVPAKVED SGHYCVVVRN S SYCLRIKI S AKFVENE PNL 120
CYNAQAI FKQ KLPVAGDGGL VC Pyme FFKN ENNELPKLQW YKDCPPLLLD NI HFS GV KDR 180
LIVMNVAEKH RGNYTCHASY T YLGKQY P I T RVI EFI TLEE NKPTRPVIVS PANETMEVDL 240
GSQI QL ICNV TGQLS DI AY W KWNGSVI DED DPVLGEDYYS VENPANKRNS TLI TVLNI SE 300
I ESRFYKHFP TCFAKNTHGI DAAYIQLI YP VTNGS GGGDK THTC PCCPAP ELLGGPSVFL 360
FPKPKDITLM T SRTEVTCV WDVSHEDE P VFNWVVDGV EVHNATKPR EEQYNSTYR V 420
V SVLTVLHHD W LNKEYKCK VSNKALPAP E KET I SKAKQ PREPVQCTLP PSRD ETLKNQ 480
V S LSCAVKGF YPS DIAVEWE SNGQPENNYK TTPVLDS DG SFLVSKLTV DKS RWQ QGNV 540
FSC SVMHEAL HNHYTQ KSL S LP G 565
hIL-RI-hlgGl-Fc  DNA (SEQ ID NO. 4)

ATGAAGGTCC TGTCAGGCT ATTCGCTGG TCATCAGCAG CCTGGAAGCC 60
GACAAGTGCA AGGAGAGGGA GGAGAAGATC ATCCTCGTCA GCTCCGCCAA 120
TGCTCAGGTT TTTCAAGCAA AAAGTCAAAGCCTGGAAGCC 180
GACAAGTGCA AGGAGAGGGA GGAGAAGATC ATCCTCGTCA GCTCCGCCAA 180
TGCTCAGGTT TTTCAAGCAA AAAGTCAAAGCCTGGAAGCC 180
GACAAGTGCA AGGAGAGGGA GGAGAAGATC ATCCTCGTCA GCTCCGCCAA 180

hIL-IRAcp-hlgGl-Fc polypeptide (SEQ ID NO. 5)

MTLLWCWSDL YFYGILQSDAA SERCDDWGLD TMRQIQVFED EPARIKCP LF EHLKFNYS 60
AHSAGLTLIW YWTRQDRDLE EPINFRLPEN RISKEKDVLW FRPTLLNDTGG NYTMLRNTT 120
YCSKVAFFLE VVQKSDCFSNS PMKLVHKLY IEYGIRITC PNVDGYFPSS VKPTITXMG 180
CYKIQNFNNV IPEGMNLSFL IALISNNGY TCVTYTPENG RTFLHTRTTLT VKVGSFKNA 240
VPPVHIHSPND HWYEEKEGPE ELLI PCTVFVF SFLMDSRNEV WWTI DKGKPD DITIDVTINE 300
SISHSRTDEE TRTQILSIK KVTSEDLKRSY VCHARSAKGE VAKAAVQPKP VPAPRYTVGS 360
GGGDKTHTCP PCPAPELGG PSVFLPPPKP KDTLMISRTP EVTCWVDVS HEDPEVKFNT 420
YVGDVEVHNA KTKPREEQYN STYRWSVLT VLHQDWLNGK EYKCKVSUKA LDPIEKTIS 480
KAKGQPREPQ VYTLPPCRDE LTKQNVSDSL LVKGHFPUSD AVEWESNGQPD ENYKTTFPPV 540
hIL-1RAcP-hlgGl-Fc DNA (SEQ ID NO. 6)

In certain aspects, the present invention provides for a recombinant mammalian expression plasmid for high expression of a polypeptide comprising the leading 333 amino acids of the human IL-1RI fused with IgG-Fc domain (Fc-II) via a flexible linker, and for another recombinant DNA molecule having an open reading frame coding for another polypeptide comprising the leading 358 amino acids of the human IL-1RAcP fused with another IgG-Fc domain (Fc-V) via a flexible linker. This plasmid comprises two cytomegalovirus (CMV) promoters to drive transcription of the two genes coding for said polypeptide and said another polypeptide, each followed by a transcription termination sequence and a polyadenylation sequence. The plasmid also contains an
origin of replication and a gene conferring ampicillin resistance, for supporting plasmid propagation and selection in bacteria. The plasmid further contains a gene for Glutamine synthetase, a selectable marker widely used for establishing stable CHOKI and NSO cell lines. The plasmid of the present invention is illustratively shown in Figure 2.

In an example embodiment, the mammalian expression plasmid of the present teachings comprises the nucleotide sequence of SEQ ID NO. 7.

hIL-lRI-hlgGl-Fc-II/ IL-lRAcP- hlgGl-Fc-V expression plasmid (SEQ ID NO. 7)
TGGCCAATGC ATATCGATCT ATACATTGAA TCAATATTGG CCATTAGCCA TATTATTCAT
TGGTTATATA GCATAAATCA ATATTGGCTA TTGGCCATTG GCAGTCTAC GTGCATAATG CCAAGACAAA GCCGCGGGAG GAGCAGTACA
ACAGCACGTA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA CCAGGACTGG CTGAATGGCA
ACAGCACGTA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA CCAGGACTGG CTGAATGGCA
5
TGGCCAATGC ATATCGATCT ATACATTGAA TCAATATTGG CCATTAGCCA TATTATTCAT
TGGTTATATA GCATAAATCA ATATTGGCTA TTGGCCATTG GCAGTCTAC GTGCATAATG CCAAGACAAA GCCGCGGGAG GAGCAGTACA
ACAGCACGTA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA CCAGGACTGG CTGAATGGCA
ACAGCACGTA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA CCAGGACTGG CTGAATGGCA
10
TGGCCAATGC ATATCGATCT ATACATTGAA TCAATATTGG CCATTAGCCA TATTATTCAT
TGGTTATATA GCATAAATCA ATATTGGCTA TTGGCCATTG GCAGTCTAC GTGCATAATG CCAAGACAAA GCCGCGGGAG GAGCAGTACA
ACAGCACGTA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA CCAGGACTGG CTGAATGGCA
ACAGCACGTA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA CCAGGACTGG CTGAATGGCA
15
TGGCCAATGC ATATCGATCT ATACATTGAA TCAATATTGG CCATTAGCCA TATTATTCAT
TGGTTATATA GCATAAATCA ATATTGGCTA TTGGCCATTG GCAGTCTAC GTGCATAATG CCAAGACAAA GCCGCGGGAG GAGCAGTACA
ACAGCACGTA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA CCAGGACTGG CTGAATGGCA
ACAGCACGTA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA CCAGGACTGG CTGAATGGCA
20
TGGCCAATGC ATATCGATCT ATACATTGAA TCAATATTGG CCATTAGCCA TATTATTCAT
TGGTTATATA GCATAAATCA ATATTGGCTA TTGGCCATTG GCAGTCTAC GTGCATAATG CCAAGACAAA GCCGCGGGAG GAGCAGTACA
ACAGCACGTA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA CCAGGACTGG CTGAATGGCA
ACAGCACGTA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA CCAGGACTGG CTGAATGGCA
25
TGGCCAATGC ATATCGATCT ATACATTGAA TCAATATTGG CCATTAGCCA TATTATTCAT
TGGTTATATA GCATAAATCA ATATTGGCTA TTGGCCATTG GCAGTCTAC GTGCATAATG CCAAGACAAA GCCGCGGGAG GAGCAGTACA
ACAGCACGTA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA CCAGGACTGG CTGAATGGCA
ACAGCACGTA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA CCAGGACTGG CTGAATGGCA
30
TGGCCAATGC ATATCGATCT ATACATTGAA TCAATATTGG CCATTAGCCA TATTATTCAT
TGGTTATATA GCATAAATCA ATATTGGCTA TTGGCCATTG GCAGTCTAC GTGCATAATG CCAAGACAAA GCCGCGGGAG GAGCAGTACA
ACAGCACGTA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA CCAGGACTGG CTGAATGGCA
ACAGCACGTA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA CCAGGACTGG CTGAATGGCA
35
TGGCCAATGC ATATCGATCT ATACATTGAA TCAATATTGG CCATTAGCCA TATTATTCAT
TGGTTATATA GCATAAATCA ATATTGGCTA TTGGCCATTG GCAGTCTAC GTGCATAATG CCAAGACAAA GCCGCGGGAG GAGCAGTACA
ACAGCACGTA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA CCAGGACTGG CTGAATGGCA
ACAGCACGTA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA CCAGGACTGG CTGAATGGCA
40
TGGCCAATGC ATATCGATCT ATACATTGAA TCAATATTGG CCATTAGCCA TATTATTCAT
TGGTTATATA GCATAAATCA ATATTGGCTA TTGGCCATTG GCAGTCTAC GTGCATAATG CCAAGACAAA GCCGCGGGAG GAGCAGTACA
ACAGCACGTA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA CCAGGACTGG CTGAATGGCA
ACAGCACGTA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA CCAGGACTGG CTGAATGGCA
45
TGGCCAATGC ATATCGATCT ATACATTGAA TCAATATTGG CCATTAGCCA TATTATTCAT
TGGTTATATA GCATAAATCA ATATTGGCTA TTGGCCATTG GCAGTCTAC GTGCATAATG CCAAGACAAA GCCGCGGGAG GAGCAGTACA
ACAGCACGTA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA CCAGGACTGG CTGAATGGCA
ACAGCACGTA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA CCAGGACTGG CTGAATGGCA
50
TGGCCAATGC ATATCGATCT ATACATTGAA TCAATATTGG CCATTAGCCA TATTATTCAT
TGGTTATATA GCATAAATCA ATATTGGCTA TTGGCCATTG GCAGTCTAC GTGCATAATG CCAAGACAAA GCCGCGGGAG GAGCAGTACA
ACAGCACGTA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA CCAGGACTGG CTGAATGGCA
ACAGCACGTA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA CCAGGACTGG CTGAATGGCA

- 14 -
In certain aspects, the present teachings provide for a mammalian expression system for production of a heterodimeric protein assembly comprising a polypeptide comprising amino acid residues 18 through 333 of the human IL1-R1 fused with IgG-Fc domain (Fc-II) via a flexible linker, and another polypeptide comprising amino acid residues 21 through 358 of the human IL-1RAcP fused with another IgG-Fc domain (Fc-V) via a flexible linker.

In an example embodiment, the mammalian expression system of the present invention comprises Chinese hamster ovary cells (CHO-K1) harboring a plasmid comprising nucleotide sequence of SEQ ID NO. 7.

In certain aspects, the present teachings provide for a method of treatment of a mammal effected by the following disorders associated with IL-1β modulation: arthritis, gout, rheumatoid arthritis, Cryopyrin-Associated Periodic Syndromes (CAPS), scleroderma, diabetes, atherosclerosis, dry eye disease, ocular allergy, or uveitis.

**EXAMPLES**

The following Examples illustrate the forgoing aspects and other aspects of the present teachings. These non-limiting Examples are put forth so as to provide those of ordinary skill in the
art with illustrative embodiments as to how the compounds, compositions, articles, devices, and/or methods claimed herein are made and evaluated. The Examples are intended to be purely exemplary of the inventions disclosed herein and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperature, etc.) but some errors and deviations should be accounted for.

Example 1: Construction of plasmids for expression of polypeptides of the present invention.

Optimized gene sequences comprising sequences encoding residues amino acid residues 1 through 333 of IL-1RI or residues 1 through 358 of IL-lRAcP were chemically synthesized. The resulting fragments were cloned in-frame into the recipient intermediate vectors comprising sequences coding for Fc-V or Fc-II, via HindIII and BspEI restriction sites. Obtained positive clones were verified by DNA sequencing. The resulting constructions were termed PKN001 '-IL-lR-FcV and PKN001'-RAcP-FcII, respectively.

The gene encoding IL-lRI-Fc-V was then cloned into a second intermediate vector, termed PKN002, via HindIII and EcoRI restriction sites. Positive clones were screened by double digestion and the insertion sequence of the correct plasmids was verified by DNA sequencing. The resulting constructions were termed PKN002-IL-lR-FcV.

Finally, the expression cassette for RAcP-FcII from PKN001 '-RAcP-FcII was integrated into PKN002-IL-lR-FcV plasmid via NotI and Sail restriction sites and yielded a new recombinant construction that contained expression elements for both RAcP-FcII and IL-lR-FcV. The resulting clones were screened by NotI and Sail double digestion followed by DNA gel electrophoresis, the correct clones were exhibiting one band migrating approximately as an 8kb DNA fragment and another band migrating approximately as a 4kb DNA fragment. The final plasmid was termed PKN012-ILIR-FcV-RAcP-FcII.

Recombinant plasmid PKN012-ILIR-FcV-RAcP-FcII combines expression cassettes for both RAcP-FcII and IL-lR-FcV. The plasmid can be used for co-expressing RAcP-FcII and IL-lR-FcV proteins in a 1 to 1 ratio under the control of a CMV promoter. The majority of these two fusion proteins would then form ILIR-FcV/RAcP-FcII heterodimers after secretion. The plasmid also expresses Glutamine synthetase (GS) protein via a SV40 promoter, which can be used as a
selection marker to generate stable cell lines for ILIR-FcV/RAcP-FcII heterodimer production. The plasmid map for PKN012-ILIR-FcV-PvAcP-FcII is illustratively shown in Figure 2.

**Example 2:** Generation of stable cell lines for expressing polypeptides of the present invention.

A stable clone of CHO-K1 cells co-expressing hIL1-R1-hlgGl-Fc polypeptide of SEQ ID NO. 1 and hIL-1RAcP-hlgGl-Fc polypeptide of SEQ ID NO. 2 has been generated through standard cell biology protocols. The expression plasmid PKN012-ILIR-FcV-RAcP-FcII described in Example 1 was used for generating stable cell lines for high expression of said polypeptides. Expression levels of said polypeptides in a plurality of clones were about or over 100 mg/L in a 7-day batch culture. One clonal cell line showed expression levels of about or over 300 mg/L in shake flask batch culture.

**Materials**

Chinese hamster ovary cells (CHO-K1) were obtained as frozen stocks from ATCC (CCL-61™). The cells were adapted in house into a CD CHO media. Media and reagent were obtained from commercial source. Upon full adaptation, the cells were grown to high density for a few passages. The resulting cells were subcloned. One of the resulting clones with a doubling time under 20 hrs and good morphology was selected as parental cell line.

**Methods**

CHO-K1 cells at passage 4 were cultured in CD-CHO chemically defined media (Invitrogen) containing 6 mM L-Glutamine. The cells were maintained by 1:3 splits after reaching a cell density of 4x10^6 cells/ml. Cells were span down by centrifugation and resuspended into 1 ml of CD-CHO chemically defined media (Invitrogen).

The following electroporation protocol was utilized:

- 40 µg of plasmid DNA in 100 µl sterilized TE buffer was used for an electroporation; on the day of transfection the viability of cells was at least 95%;
- cells were centrifuged at 800rpm for 5 min; the super was removed and the cells were resuspended in 10 ml CD CHO media and centrifuged again;
the super was removed and the cells were resuspended in a small vol. of CD CHO media to 1.43x10⁷ cells/ml;
- 0.7 ml cells (10⁷ cells) were added to the DNA and mixed gently by pipetting, avoiding generating bubbles;
- cells were immediately electroporated by delivering a single pulse of 300 volts, 900 uF to each cuvette;
- 50 ml CD CHO (without L-glutamine) was immediately added to the electroporated cells and mixed gently;
- the cell suspension was distributed into ten 96 well plates at 50 ul/well; the following day, 150 ul of CD CHO containing 33.3 uM MSX was added to each well.

A number of transfections were carried out in CHO-K1 cells in the process of generation of potential ILIR-FcV-RAcP-FcII expression cell line; a representative transfection growth curve is shown in Figure 3 and the data is shown in Table 1. Protein expression levels were analyzed by SDS-PAGE. Cell lines with high levels of protein overexpression exhibit a strong band with an apparent molecular weight of about 180 kDa. Based on the preliminary analysis four clones were selected for further analysis, where expression levels were further assessed by SDS-PAGE and ELISA. Well expressing clones were inoculated into 125 ml shake flasks, the cells were expanded and frozen.

The chosen highest production cell line was selected and thawed into CD-CHO. Growth curves on the cell line were assessed, and samples were collected daily for cell count, cell viability and ILIR-FcV-RAcP-FcII productivity. Based on these studies, it was determined that selected highest productivity cell line was expressing ILIR-FcV-RAcP-FcII heterodimer in CD-CHO media in amounts necessary to support commercial production. The yield of the heterodimer after purification was at least about 300 mg/L (without any production optimization).
Table 1: A Representative Clonal Cell Line Growth curve

<table>
<thead>
<tr>
<th>Culture period (DAY)</th>
<th>Cell density (10^6 cells/ml)</th>
<th>Feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.09</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.93</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2.97</td>
<td>+10% feed B</td>
</tr>
<tr>
<td>5</td>
<td>4.13</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>4.55</td>
<td>+10% feed B</td>
</tr>
<tr>
<td>7</td>
<td>4.6</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>5.46</td>
<td>+10% feed B</td>
</tr>
<tr>
<td>9</td>
<td>4.86</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

**Example 3:** Purification of polypeptides of the present invention.

hIL-RI-hlgGl-Fc polypeptide of SEQ ID NO. 1 and hIL-IRAcP-hlgGl-Fc polypeptide of SEQ ID NO. 2 were co-expressed in CHO-K1 essentially as described in foregoing Example 2.

Cells were harvested and lysed utilizing well established protocols. After cell lysate clarification, the supernatant at protein concentration of about 0.4 mg/ml, containing expressed hIL-RI-hlgGl-Fc/ hIL-IRAcP-hlgGl-Fc polypeptides, was applied to a Protein A affinity column. The affinity purification step was carried out according to the procedure outlined in Table 2. The Protein A eluate containing hIL-RI-hlgGl-Fc/ hIL-IRAcP-hlgGl-Fc at pH of about 3.5 - 3.7 is incubated for 45-60 minutes to inactivate potentially existing in the contaminating materials.

After material incubation for about 45 minutes at pH 3.6 at room temperature its pH is adjusted to about 7.9-8.1 with 2 M Tris-HCl pH 9.5 (~ 3.5% v/v of the diluted Pro A eluate). The low pH treated Protein A eluate is pH-adjusted to pH of about 8.0 using 1 M Tris-HCl, pH 9.0. The conductivity is adjusted to about 5.5 mS/cm with deionized water (dH2O) if needed.

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In order to reduce the contents of DNA, HCP, endotoxin and potential viral contaminants, the pH adjusted Protein A column eluate was further purified by anion-exchange chromatography (AIEX) utilizing Q Sepharose resin. The AIEX step is operated according to a step-elution procedure outlined in Table 3. Pooled elution peak fractions are concentrated by microfiltration to a concentration of about 20 mg/ml, followed by addition of a 20% Sucrose stock solution to a final Sucrose concentration of about 1% (w/v) and freezing at -80°C.

A sample of thus purified protein was analyzed by size-exclusion HPLC (SEC-HPLC) and SDS-PAGE (reducing and non-reducing). The results of the analysis are presented in Figure 4 and Figure 5, respectively. In Figure 5, lanes 1, 3 show hIL1-RL-hlgGl-Fc/ hIL-IRAcP-hlgGl-Fc SDS-PAGE under non-reducing conditions, while lanes 4, 6 - under reducing conditions. Lanes 2, 5 show molecular weight markers. hIL1-RL-hlgGl-Fc/ hIL-IRAcP-hlgGl-Fc heterodimer has an apparent molecular weight of about 180 kDa, consisting of two di-sulfide linked monomers, each of an apparent molecular weight of about 90 kDa.

The SEC-HPLC operational procedure is outlined in Table 4. Loading sample is diluted with mobile phase to reach protein concentration of about 5 mg/ml. The biologically relevant form of hIL1-RL-hlgGl-Fc/ hIL-IRAcP-hlgGl-Fc heterodimer is represented by the major peak with retention time RT=14.979 min.

Table 2: The operational procedure of Protein A Affinity Chromatography

<table>
<thead>
<tr>
<th>Step</th>
<th>Buffer</th>
<th>Vol CV</th>
<th>Flow cm/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rinse Before-use</td>
<td>dH20</td>
<td>3</td>
<td>150</td>
</tr>
<tr>
<td>Equilibration</td>
<td>10mM NaPh, pH 6.0</td>
<td>5</td>
<td>150</td>
</tr>
<tr>
<td>Sample Load</td>
<td>Cell harvest</td>
<td>-</td>
<td>150</td>
</tr>
<tr>
<td>Wash 1</td>
<td>10mM NaPh, pH 6.0</td>
<td>3</td>
<td>150</td>
</tr>
<tr>
<td>Wash 2</td>
<td>25 mM NaPh, 0.5 M NaCl, 5% Isopropanol pH 7.0</td>
<td>5</td>
<td>150</td>
</tr>
<tr>
<td>Re-Equilibration</td>
<td>10mM NaPh, pH 6.0</td>
<td>3</td>
<td>150</td>
</tr>
<tr>
<td>Elution</td>
<td>20 mM Na-Citrate, pH 3.4</td>
<td>4</td>
<td>150</td>
</tr>
<tr>
<td>Re-Equilibration</td>
<td>10mM PB, pH 6.0</td>
<td>3</td>
<td>150</td>
</tr>
<tr>
<td>CIP</td>
<td>0.1 M NaOH (contact time 15 min), reversed flow, CIP every 5 cycles</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>Rinse with NaCl</td>
<td>1M NaCl</td>
<td>3</td>
<td>150</td>
</tr>
<tr>
<td>Rinse After-use</td>
<td>dH20</td>
<td>3</td>
<td>150</td>
</tr>
<tr>
<td>Storage</td>
<td>20% (v/v) Ethanol, 20 mM NaPh, pH 7.0</td>
<td>3</td>
<td>150</td>
</tr>
</tbody>
</table>
Table 3: The operational procedure of AIEX

<table>
<thead>
<tr>
<th>Step</th>
<th>Buffer</th>
<th>Vol CV</th>
<th>Flow cm/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rinse Before-use</td>
<td>dH20</td>
<td>3</td>
<td>150</td>
</tr>
<tr>
<td>Recharge</td>
<td>10 mM Tris-HCl, 1 M NaCl, pH 8</td>
<td>3</td>
<td>150</td>
</tr>
<tr>
<td>Equilibration</td>
<td>10 mM Tris-HCl, 50 mM NaCl, pH 8</td>
<td>3</td>
<td>150</td>
</tr>
<tr>
<td>Sample Load</td>
<td>Prepared Q Load</td>
<td>-</td>
<td>150</td>
</tr>
<tr>
<td>Wash</td>
<td>10 mM Tris-HCl, 50 mM NaCl, pH 8</td>
<td>3</td>
<td>150</td>
</tr>
<tr>
<td>Elution</td>
<td>10 mM Tris-HCl, 0.35 M NaCl, pH 8.0</td>
<td>3</td>
<td>150</td>
</tr>
<tr>
<td>CIP</td>
<td>1 M NaOH (contact time 1 hr), reversed-flow</td>
<td>3</td>
<td>40</td>
</tr>
<tr>
<td>Regeneration</td>
<td>10 mM Tris-HCl, 1 M NaCl, pH 8.0</td>
<td>3</td>
<td>150</td>
</tr>
<tr>
<td>Rinse After-use</td>
<td>dH20</td>
<td>3</td>
<td>150</td>
</tr>
<tr>
<td>Storage</td>
<td>20% (v/v) Ethanol</td>
<td>3</td>
<td>150</td>
</tr>
</tbody>
</table>

Table 4: The Operational Procedure of SEC-HPLC

<table>
<thead>
<tr>
<th>Mobile Phase:</th>
<th>20 mM phosphate, 300 mM NaCl, pH 7.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow Rate:</td>
<td>0.5 mL/min</td>
</tr>
<tr>
<td>Column:</td>
<td>G2000 SWx1, 7.8mmx300mm, TOSOH Bioscience</td>
</tr>
<tr>
<td>Guard column:</td>
<td>TSK Guard SWx1,6.0mmx40mm, TOSOH Bioscience</td>
</tr>
<tr>
<td>Column Temperature:</td>
<td>25 °C</td>
</tr>
<tr>
<td>Sampler temperature:</td>
<td>Rome temperature</td>
</tr>
<tr>
<td>Injection Volume:</td>
<td>10 µl</td>
</tr>
<tr>
<td>Detector Wavelength:</td>
<td>280 nm</td>
</tr>
<tr>
<td>Run Time:</td>
<td>30 min</td>
</tr>
</tbody>
</table>

The activity of thus purified sample, which was expressed and purified essentially as described in this example, was tested in a standard ELISA assay using commercially available human IL-1β (PrimGene, Shanghai, China; Cat#: 101-O1B). A typical binding curve obtained in...
the assay is shown in Figure 6. Based on the curve analysis, the calculated EC50 value is about 50 ng/ml.

**Example 4:** Pharmacokinetics (PK) of ILIR-FcV-RAcP-FcII heterodimer after subcutaneous administration in mice.

Polypeptides of ILIR-FcV-RAcP-FcII heterodimer (SEQ ID NO.1 and SEQ ID NO. 2) were co-expressed and purified essentially as described in the forgoing examples. For administration into animals, the polypeptides were formulated in the following buffer: 1% w/v Sucrose, 100mM Sodium Chloride, 20 mM L-Arginine Hydrochloride, 25 mM Sodium Bicarbonate, pH 6.3. The dosing stock concentration used was 0.5 mg/mL of the polypeptide.

Fourteen female Balb/c nu/nu mice were randomized based on body weight into seven groups of two animals on Day 0 of the study. A single treatment of the polypeptides (5 mg/kg) was administered subcutaneously (dorsal) on Day 0 to all groups except mice in Group 1, which were bled via cardiac puncture for plasma preparation on Day 0 of the study. Blood samples were collected from mice via the orbital sinus in the remaining groups at various times throughout the study for preparation of plasma.

Body weights were recorded for all animals on the treatment day (Day 0) and then three times per week, including the termination day of each group.

Groups of mice were culled at specific time points for plasma preparation. Body weight changes were not measured in groups culled for sample collection at 0 hours and within 36 hours of dose administration. All other mice gained body weight and no adverse clinical signs were reported during the study period.

Following the in-life phase of the study, plasma samples were analyzed by ELISA for Hu-Fc proteins. Quantification of Hu-Fc in mouse plasma samples by ELISA was used as a read-out for circulating levels of the polypeptides. The assay was performed on samples from all mice in the study.

The polypeptides were detected in the plasma of animals at 1 hour post-administration. One Phase Decay Model equation using Prism 5.0c (GraphPad Software Inc, La Jolla, CA, USA) was then used to determine pharmacokinetics of the polypeptides as detected by Hu-Fc ELISA. Peak circulating level of Hu-Fc (Cmax) was determined to be 1.65 µg/mL, and time to peak circulating...
levels (T_{max}) was 36 hours post treatment. The half-life (T_{1/2}) was 88.15 hours and the rate constant (K) was 0.0079 hr^{-1}. Hu-Fc levels were negligible in the plasma of the untreated Group 1 animals. The results of the study are summarized in Table 6.

**Table 6:** Mean Human-Fc Protein Concentration ± SEM (g/mL) at each Time Post-Administration

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Bleeding Schedule (time post-administration)</th>
<th>Mean Human-Fc Protein Concentration [g/mL]</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No treatment</td>
<td>0 hours</td>
<td>0.00</td>
<td>-*</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>30 minutes</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>1 hour</td>
<td>0.12</td>
<td>0.00</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>2 hours</td>
<td>0.24</td>
<td>0.09</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>4 hours</td>
<td>0.76</td>
<td>0.03</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>8 hours</td>
<td>1.11</td>
<td>0.10</td>
</tr>
<tr>
<td>7</td>
<td>polypeptide of SEQ ID NO. 1 (5 mg/kg, once only, s.c.)</td>
<td>10 hours</td>
<td>1.53</td>
<td>0.08</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>24 hours</td>
<td>1.47</td>
<td>0.14</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>36 hours</td>
<td>1.65</td>
<td>0.11</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>96 hours</td>
<td>1.27</td>
<td>0.01</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>7 days</td>
<td>0.43</td>
<td>0.01</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>14 days</td>
<td>0.13</td>
<td>0.07</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>21 days</td>
<td>0.06</td>
<td>0.04</td>
</tr>
</tbody>
</table>

*SEM unable to be calculated as level of Hu-Fc was below detectable limit of ELISA for one of samples in group.

The Human-Fc Protein Concentration was determined by Prism Software based on the mean absorbance of the triplicate samples.

^ Bleed via orbital sinus

^ Bleed via terminal cardiac puncture
All publications and patents mentioned herein are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference.

While specific embodiments of the subject matter have been discussed, the above specification is illustrative and not restrictive. Many variations will become apparent to those skilled in the art upon review of this specification and the claims below. The full scope of the invention should be determined by reference to the claims, along with their full scope of equivalents, and the specification, along with such variations.
What is claimed is:

1. A heterodimeric protein composition capable of binding human IL-1β, said protein composition comprising:
   a first polypeptide comprising
   a first amino acid sequence comprising amino acids 18 through 333 of human IL1-R1, and
   a second amino acid sequence comprising a first mutant of a Fc portion of human immunoglobulin gamma-1 Fc;
   a second polypeptide comprising
   another first amino acid sequence comprising amino acids 21 through 358 of human IL1-RAcP, and
   another second amino acid sequence comprising a second mutant of the Fc portion of human immunoglobulin gamma-1 Fc; and
   wherein said first and second mutants are selected as to favor heterodimeric assembly between said first and second mutants over any homodimeric assembly.

2. The protein composition of claim 1, wherein said protein composition exhibits human IL-1β binding activity in an ELISA assay with an EC50 of about 50 ng/ml.

3. The protein composition of claim 1, said first polypeptide comprises amino acid sequence of SEQ ID NO. 1 and said second polypeptide comprises amino acid sequence of SEQ ID NO. 2.

4. A therapeutic composition, the composition comprising a heterodimeric protein composition capable of binding human IL-1β, said heterodimeric protein composition comprising:
   a first polypeptide comprising
   a first amino acid sequence comprising amino acids 18 through 333 of human IL1-R1, and
a second amino acid sequence comprising a first mutant of a Fc portion of human immunoglobulin gamma-1 Fc;
a second polypeptide comprising
another first amino acid sequence comprising amino acids 21 through 358 of human IL1-RAcP, and
another second amino acid sequence comprising a second mutant of the Fc portion of human immunoglobulin gamma-1 Fc; and
wherein said first and second mutants are selected as to favor heterodimeric assembly between said first and second mutants over any homodimeric assembly.

5. The therapeutic composition of claim 4, wherein half-life of said heterodimeric protein composition in systemic circulation in mice after a subcutaneous administration at a dose of 5 mg/kg is at least about 88 hours, as assayed by human Fc ELISA.

6. A therapeutic composition, the composition comprising a heterodimeric protein composition comprising a first polypeptide comprising amino acid sequence of SEQ ID NO. 1 and a second polypeptide comprising amino acid sequence of SEQ ID NO. 2.

7. An isolated nucleic acid encoding a polypeptide comprising amino acid sequence of SEQ ID NO. 3.

8. An isolated nucleic acid encoding a polypeptide comprising amino acid sequence of SEQ ID NO. 4.

9. The nucleic acid of claims 7 or 8, wherein the codon usage is optimized for high expression of said polypeptide in a mammalian cell.

10. The nucleic acid of claim 7, wherein the nucleic acid sequence comprises the sequence of SEQ ID NO. 5.

11. The nucleic acid of claim 8, wherein the nucleic acid sequence comprises the sequence of SEQ ID NO. 6.
12. The nucleic acid of claims 10 or 11, wherein said nucleic acid comprises an expression vector.


14. A heterologous expression system, the expression system harboring an expression vector comprising a nucleic acid sequence encoding a first polypeptide comprising amino acid sequence of SEQ ID NO. 3 and another nucleic acid sequence encoding a second polypeptide comprising amino acid sequence of SEQ ID NO. 4.

15. The expression system of claim 14, wherein said expression vector is harbored in a mammalian cell.

16. The expression system of claim 15, wherein said mammalian cell is a CHO cell.

17. The expression system of claim 15, wherein said expression system is capable of expressing a heterodimeric protein comprising a first polypeptide comprising amino acid sequence of SEQ ID NO. 1 and a second polypeptide comprising amino acid sequence of SEQ ID NO. 2, and wherein the level of expression of said heterodimeric protein is at least 300 mg per liter of cell culture.

18. Use of a substance for manufacture of a medicament for the treatment or prevention of a disease associated with modulation of activity of human IL-1β, the substance comprising a heterodimeric protein comprising a first polypeptide comprising amino acid sequence of SEQ ID NO. 1 and a second polypeptide comprising amino acid sequence of SEQ ID NO. 2.

19. The use according to claim 18, wherein said disease is an arthritis.

20. The use according to claim 18, wherein said disease is a gout.

21. The use according to claim 18, wherein said disease is a rheumatoid arthritis.

22. The use according to claim 18, wherein said disease is a Cryopyrin-Associated Periodic Syndromes (CAPS).
23. The use according to claim 18, wherein said disease is a scleroderma.

24. The use according to claim 18, wherein said disease is a diabetes.

25. The use according to claim 18, wherein said disease is atherosclerosis.

26. The use according to claim 18, wherein said disease is a dry eye disease.

27. The use according to claim 18, wherein said disease is an ocular allergy.

28. The use according to claim 18, wherein said disease is an uveitis.

29. A method of treating or preventing a disease or condition associated with modulation of activity of human IL-1β, the method comprising administering to a patient in need for treating or preventing a disease associated with modulation of activity of human IL-1β a therapeutically effective amount of a pharmaceutical composition comprising a heterodimeric protein comprising a first polypeptide comprising amino acid sequence of SEQ ID NO. 1 and a second polypeptide comprising amino acid sequence of SEQ ID NO. 2.

30. The method according to claim 29, wherein said disease is an arthritis.

31. The method according to claim 29, wherein said disease is a gout.

32. The method according to claim 29, wherein said disease is a rheumatoid arthritis.

33. The method according to claim 29, wherein said disease is a Cryopyrin-Associated Periodic Syndromes (CAPS).

34. The method according to claim 29, wherein said disease is a scleroderma.

35. The method according to claim 29, wherein said disease is a diabetes.

36. The method according to claim 29, wherein said disease is atherosclerosis.

37. The method according to claim 29, wherein said disease is a dry eye disease.
38. The method according to claim 29, wherein said disease is an ocular allergy.

39. The method according to claim 29, wherein said disease is an uveitis.
FIGURE 3
## FIGURE 4

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Figure 6

4-P Fit: \( y = (A - D)/(1 + (x/C)^B) + D \)

\[\begin{array}{cccccc}
\text{Plot#1 (STD3: conc. vs values)} & 0.0751 & 1.26 & 45.3 & 2.78 & 0.999 \\
\end{array}\]
### A. CLASSIFICATION OF SUBJECT MATTER

(see extra sheet)

According to International Patent Classification (IPC) or to both national classification and IPC

### B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K 14/715, 19/00, 16/18, C12N 15/62, 15/63, A61K 38/17, A6 IP 19/00, 9/10, 27/14

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PatSearch, EMBL, NCBI, PAJ, Espacenet, DWPI, PCT Online, USPTO DP, CIPO (Canada PO), SIPO DB

### C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>JP 20065 18985 A (REGENERON PHARMACEUTICALS INC.) 24.08.2006, claims, examples 5,6,9, 14,17-20, paragraphs [0003]-[0005], [0013]-[0039], [0043]-[0048],[005 1],[0066],[0106],[01 10],[0 111], [01 14],[01 18]-</td>
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<td>JP 2008501716 A (REGENERON PHARMACEUTICALS INC.) 24.01 .2008, claims, example 1, paragraphs [0008]-[002 1],[0026]-[0029], [0034]-[0036],[0039]-[0042],[0046]-[0048], sequences N DD669674, DD66968 1, DL0788 12.1, retrieved from Database &quot;EBI/FASTA&quot;</td>
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<td>RU 2450824 C2 (RIDZHENER FARMASJUTIKALZ, INK) 20.05 .2012, abstract, claims</td>
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<td>WO 207 047266 A1 (ABBOTT LABORATORIES et al) 2 1.04.20 11, claims</td>
<td>19-2 1,23-28,30-32,34-39</td>
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Further documents are listed in the continuation of Box C.

See patent family annex.

### Date of the actual completion of the international search

16 May 20 13 (16.05.20 13)

### Date of mailing of the international search report

30 May 20 13 (30.05.2013)

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Authorized officer

I.Goretova

Telephone No. 495 531 65 15

Form PCT/ISA/210 (second sheet) (July 2009)
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