

IL-1 β 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
5 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-1 β cytokine.

BACKGROUND

10 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-1 β 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-1 β rapidly increase messenger RNA expression of hundreds
15 of genes in multiple different cell types. The potent proinflammatory activities of IL-1 and IL-1 β
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-1 β , 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
20 combinatorial phosphorylation and ubiquitination events results in activation of nuclear factor
kappa-B signaling and the JNK 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, IB, IL-1, IL-1 β , MKP-1) by transcriptional and posttranscriptional mechanisms. Of note,
most intracellular components that participate in the cellular response to IL-1 also mediate
25 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-1 β 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
30 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-1 β but lacks a signaling-competent cytosolic part and thus serves as a decoy receptor. The IL-1RA, the plasma membrane-anchored IL-1R2, and the naturally occurring
5 "shed" domains of each of the extracellular IL-1 receptor chains (termed sIL-1RI, sIL-1RII, and sIL-1RAcP, 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
10 extracellular domain of the IL-1RI that facilitates recruitment of IL-1RAcP. Through conserved cytosolic regions called Toll- and IL-1R-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
15 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 IRAK1 and IRAK2, and then this is followed by the recruitment and oligomerization of tumor necrosis factor-associated factor (TRAF) 6. IRAK1 and 2 function as both adaptors and protein kinases to transmit
20 downstream signals. Complexes of IRAK1, 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
25 (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 IL-1RAcP, an IL-1 binding portion of the extracellular domain of human IL-1RI, and a multimerizing component. This IL-1-specific fusion protein is described in U.S. Pat. No. 6,472,179, U.S. patent publication
30 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-1 β (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-1 β 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-1 β . 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 comprises 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 isolated nucleic acid of SEQ ID NO. 7.

In certain aspects, the present invention provides for a 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-1 β . 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
5 sequence of SEQ ID NO. 2. The disease associated with modulation of activity of human IL-1 β 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
10 disease or condition associated with modulation of activity of human IL-1 β . The method comprising administering to a patient in need for treating or preventing a disease associated with modulation of activity of human IL-1 β a therapeutically effective amount of a pharmaceutical composition. The pharmaceutical composition comprising a heterodimeric protein comprised of a
15 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-1 β 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 heterodimeric protein composition
20 capable of binding human IL-1 β . The protein composition is capable of inhibiting human IL-6 production in human lung fibroblasts in response to treating the fibroblasts with human IL-1 β . The inhibiting is characterized by an IC₅₀ value of about 2.3 pM.

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
30 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;

5 **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;

10 **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;

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
15 using commercially available human IL-1 β ;

Figure 7 shows the calibration curve obtained in the IL-1 antibody mediated inhibition of IL-6 production assay experiment;

Figure 8 shows the results of IL-1 β -mediated IL-6 production and IL-6 recovery from the culture medium where MRC5 cells were incubated for 24 hours with IL-1 β at a final concentration
20 of 50 pg/ml (IL-1 alone) or left untreated (Cells Ctrl) while culture medium not exposed to the cells was spiked with IL-6 at a final concentration of 20 pg/ml to estimate IL-6 recovery;

Figure 9 shows the results of the measurements of IL-1 β -mediated IL-6 production and IL-6 recovery from the culture medium where MRC5 cells were incubated for 24 hours with IL-1 β at a final concentration of 50 pg/ml (IL-1 alone) or left untreated (Cells Ctrl). Culture medium not
25 exposed to the cells was spiked with IL-6 at a final concentration of 20 pg/ml to estimate IL-6 recovery;

Figure 10 shows the calibration curve obtained in the inhibition of IL-6 production assay conducted with IL1R-FcV-RAcP-FcII heterodimer of the present teachings;

Figure 11 shows the results of inhibition measurements of IL-1 β -mediated IL-6 production
30 with IL1R-FcV-RAcP-FcII heterodimer and IL-6 recovery from the culture medium where MRC5 cells were incubated for 24 hours with IL-1 β at a final concentration of 50 pg/ml (IL-1 alone) or

left untreated (Cells Ctrl) while culture medium not exposed to the cells was spiked with IL-6 at a final concentration of 30 pg/ml to estimate IL-6 recovery (RPH-10 + 30 pg/ml IL6); to ensure consistency between two cell culture plates used for this experiment, the effects of IL-1 on IL-6 production from both plates were compared (IL-1 alone and IL1 alone Plate 2, for plates 1 and 2 respectively); the effect of the highest concentration (20 µg/ml) of IL1R-FcV-RAcP-FcII heterodimer on IL-6 production was also tested (RPH-10 alone); IL1R-FcV-RAcP-FcII heterodimer dilution corresponding to a final concentration of 204.8 ng/ml was also included to demonstrate uniformity of the data between the two plates (RPH10 Plate 2); and

Figure 12 shows the results of the titration curve measurements for L1R-FcV-RAcP-FcII heterodimer experiment where MRC5 cells were incubated with IL-1β or IL-1β pre-mixed with various concentrations of L1R-FcV-RAcP-FcII heterodimer; IL-6 production was measured by Quantakine ELISA and the data was analyzed using 4-parameter fit algorithm; coefficient C at the resulting equation is numerically equal to an IC50 value of 0.3 ng/ml or about 2.4 pM.

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-1 β (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.

5 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
10 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
15 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
20 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
25 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.

30 "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-1RAcP 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-1RAcP polypeptide portions may be fused with a domain that stabilizes the IL1-R1 or IL-1RAcP 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
 5 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 another 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
 10 fragment thereof, may be fused with the another 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
 15 may comprise the amino acid sequence of SEQ. ID NO. 2.

hIL1-R1-hIgG1-Fc polypeptide (SEQ ID NO. 1)

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  LEADKCKERE EKIILVSSAN EIDVRPCPLN PNEHKGTITW YKDDSKTPVS TEQASRIHQH 60
  20 KEKLWFVPAK VEDSGHYICV VRNSSYCLRI KISAKFVENE PNLCYNAQAI FKQKLPVAGD 120
  GGLVCPYMEF FKNENNELPK LQWYKDCCKPL LLDNIHFSGV KDRLIVMNVA EKHRGNYTCH 180
  ASYTYLGKQY PITRVIEFIT LEENKPTRPV IVSPANETME VDLGSQIQLI CNVTGQLSDI 240
  AYWKWNGSVI DEDDPVLGED YYSVENPANK RRSTLITVLN ISEIESRFYK HPFTCFAKNT 300
  HGIDAAYIQL IYPVTNGSGG GDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV 360
  25 TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY 420
  KCKVSNKALP APIEKTISKA KGQPREPQVC TLPPSRDELT KNQVSLSCAV KGFYPSDIAV 480
  EWESNGQPEN NYKTTTPVLD SDGSFKLVSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK 540
  SLSLSPGK 548
  
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hIL-1RAcP-hIgG1-Fc polypeptide (SEQ ID NO. 2)

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  SERCDDWGLD TMRQIQVFED EPARIKCPLF EHFLKFNYST AHSAGLTLIW YWTRQDRDLE 60
  EPINFRLPEN RISKEKDVLW FRPTLLNDTG NYTCMLRNTT YCSKVAFFLE VVQKDSCFNS 120
  
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PMKLPVHKLY IEYGIQRITC PNVDGYFPSS VKPTITWYMG CYKIQNFNNV IPEGMNL SFL 180
 IALISNNGNY TCVVTYPENG RTFHLTRTLT VKVVGSPKNA VPPVIHSPND HVVYEKEPGE 240
 ELLIPCTVYF SFLMDSRNEV WWTIDGKKPD DITIDVTINE SISHSRTEDE TRTQILSIKK 300
 VTSEDLKRSY VCHARSAKGE VAKAAKVKQK VPAPRYTVGS GGGDKTHTCP PCPAPELLGG 360
 5 PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN 420
 STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPCRDE 480
 LTKNQVSLWC LVKGFYPSDI AVEWESNGQP ENNYKTTTPV LDSDGSFFLY SALTVDKSRW 540
 QQGNVFSCSV MHEALHNHYT QKSLSLSPGK 570

10 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
 15 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
 20 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-hIgG1-Fc polypeptide (SEQ ID NO. 3)

25 MKVLLRLICF IALLISSLEA DKCKEREEKI ILVSSANEID VRPCPLNPNE HKGTITWYKD 60
 DSKTPVSTEQ ASRIHQHKEK LWFVPAKVED SGHYCVVRN SSYCLRIKIS AKFVENEPNL 120
 CYNQAIFKQ KLPVAGDGGL VCPYMEFFKN ENNELPKLQW YKDCKPLLLD NIHFSGVKDR 180
 LIVMNVAEKH RGNYTCHASY TYLGKQYPIT RVIEFITLEE NKPTRPVIIVS PANETMEVDL 240
 30 GSQIQLICNV TGQLSDIAYW KWNGSVIDED DPVLGEDYYS VENPANKRRS TLITVLNISE 300
 IESRFYKHPF TCFAKNTHGI DAAIYQLIYP VTNGSGGGDK THTCPPCPAP ELLGGPSVFL 360
 FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV 420
 VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVCTLP PSRDELTKNQ 480

VSLSCAVKGF YPSDIAVEWE SNGQPENNYK TTPVLDSDG SFKLVSKLTV DKSRWQQGNV 540
 FSCSVMHEAL HNHYTQKSLS LSPGK 565

5 hIL1-R1-hIgG1-Fc DNA (SEQ ID NO. 4)

ATGAAGGTCC TGCTCAGGCT GATCTGCTTC ATTGCCCTGC TCATCAGCAG CCTGGAAGCC 60
 GACAAGTGCA AGGAGAGGGA GGAGAAGATC ATCCTCGTCA GCTCCGCCAA CGAGATTGAT 120
 GTCAGGCCCT GCCCCCTCAA CCCCAATGAG CACAAGGGCA CAATCACCTG GTACAAGGAC 180
 10 GACAGCAAGA CCCCTGTCTC CACCGAGCAG GCCAGCAGAA TCCACCAGCA CAAAGAGAAG 240
 CTGTGGTTCG TGCCTGCCAA GGTGGAAGAC AGCGGCCACT ACTACTGTGT GGTGAGGAAC 300
 AGCTCCTACT GCCTCAGGAT CAAGATCTCC GCCAAGTTCG TGGAGAACGA GCCCAACCTC 360
 TGTTACAACG CTCAGGCTAT TTTCAAGCAA AAGCTCCCCG TGGCTGGAGA CGGAGGCCTG 420
 GTCTGTCCCT ACATGGAGTT CTTCAAGAAT GAGAATAATG AGCTCCCCAA GCTCCAGTGG 480
 15 TACAAGGACT GTAAGCCTCT GCTCCTGGAC AACATCCACT TCTCCGGCGT GAAGGACAGA 540
 CTGATCGTCA TGAACGTGGC CGAGAAGCAC AGGGGAAACT ACACCTGTCA CGCCTCCTAC 600
 ACCTACCTCG GCAAGCAATA TCCCATCACC AGGGTCATCG AGTTCATCAC CCTCGAAGAG 660
 AACAAGCCCA CAAGGCCTGT CATCGTCAGC CCCGCCAATG AAACCATGGA GGTGGACCTC 720
 GGCAGCCAGA TCCAGCTGAT CTGCAACGTG ACAGGCCAGC TCAGCGACAT TGCCTACTGG 780
 20 AAGTGGAACG GCTCCGTGAT CGACGAAGAT GATCCCGTGC TGGGCGAGGA CTACTATAGC 840
 GTGGAGAACC CCGCCAACAA AAGAAGGAGC ACCCTGATCA CCGTGCTGAA CATCAGCGAG 900
 ATCGAGTCCA GATTCTATAA GCATCCTTTC ACCTGCTTTG CCAAGAACAC CCACGGCATC 960
 GACGCCGCTT ACATCCAGCT GATCTATCCC GTGACCAACG GATCCGGTGG AGGTGACAAA 1020
 ACTCACACAT GCCCACCCTG CCCAGCTCCG GAACTCCTGG GCGGACCGTC AGTCTTCCTC 1080
 25 TTCCCCCCTAA AACCCAAGGA CACCCTCATG ATCTCCCGGA CCCCTGAGGT CACATGCGTG 1140
 GTGGTGGACG TGAGCCACGA AGACCCTGAG GTCAAGTTCA ACTGGTACGT GGACGGCGTG 1200
 GAGGTGCATA ATGCCAAGAC AAAGCCGCGG GAGGAGCAGT ACAACAGCAC GTACCGTG TG 1260
 GTCAGCGTCC TCACCGTCCT GCACCAGGAC TGGCTGAATG GCAAGGAGTA CAAGTGCAAG 1320
 GTCTCCAACA AAGCCCTCCC AGCCCCCATC GAGAAAACCA TCTCCAAAGC CAAAGGGCAG 1380
 30 CCCCAGAGAAC CACAGGTGTG TACCCTGCCC CCATCCCGGG ATGAGCTGAC CAAGAACCAG 1440
 GTCAGCCTGA GTTGCGCGGT CAAAGGCTTC TATCCCAGCG ACATCGCCGT GGAGTGGGAG 1500
 AGCAATGGGC AGCCGGAGAA CAACTACAAG ACCACGCCTC CCGTGTTGGA CTCCGACGGC 1560
 TCCTTCAAGC TCGTCAGCAA GCTCACCCTG GACAAGAGCA GGTGGCAGCA GGGGAACGTC 1620
 TTCTCATGCT CCGTGATGCA TGAGGCTCTG CACAACCACT ACACGCAGAA GAGCCTCTCC 1680

CTGTCTCCGG GTAAA

1695

hIL-1RAcP-hIgG1-Fc polypeptide (SEQ ID NO. 5)

5 MTLLWCVVSL YFYGILQSDA SERCDDWGLD TMRQIQVFED EPARIKCPLF EHFLKFNYST 60
 AHSAGLTLIW YWTRQDRDLE EPINFRLPEN RISKEKDVWL FRPTLLNDTG NYTCMLRNTT 120
 YCSKVAFFLE VVQKDSCFNS PMKLPVHKLY IEYGIQRITC PNVDGYFPSS VKPTITWYMG 180
 CYKIQNFNNV IPEGMNL SFL IALISNNGNY TCVVTYPENG RTFHLTRTLT VKVVGSPKNA 240
 VPPVIHSPND HVVYEKEPGE ELLIPCTVYF SFLMSRNEV WWTIDGKKPD DITIDVTINE 300
 10 SISHSRTEDE TRTQILSIKK VTSEDLKRSY VCHARSAKGE VAKAAKVQK VPAPRYTVGS 360
 GGGDKTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW 420
 YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS 480
 KAKGQPREPQ VYTLPPCRDE LTKNQVSLWC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV 540
 LDSDGSFFLY SALTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK 590
 15

hIL-1RAcP-hIgG1-Fc DNA (SEQ ID NO. 6)

ATGACTCTGC TGTGGTGCCT CGTGTCCCTC TACTTCTACG GCATCCTCCA GTCCGACGCC 60
 AGCGAGAGGT GCGATGACTG GGGCCTGGAC ACCATGAGGC AGATCCAGGT GTTTGAGGAC 120
 20 GAGCCTGCCA GGATTAAGTG CCCCTCTTTC GAGCACTTTC TGAAGTTCAA CTACAGCACC 180
 GCTCACAGCG CTGGCCTGAC ACTGATCTGG TACTGGACAA GGCAGGACAG GGATCTCGAG 240
 GAGCCCATCA ACTTCAGGCT GCCCAGAAAAC AGAATCAGCA AGGAGAAGGA CGTGCTGTGG 300
 TTCAGACCCA CCCTCCTCAA CGACACAGGC AACTACACCT GCATGCTCAG GAACACCACC 360
 TACTGCAGCA AGGTGGCCTT CCCTCTCGAG GTGGTCCAGA AGGACAGCTG CTTCAACAGC 420
 25 CCCATGAAGC TGCCCGTCCA TAAACTGTAC ATCGAGTACG GCATCCAGAG GATCACATGC 480
 CCCAACGTGG ACGGCTACTT CCCCAGCTCC GTGAAGCCCA CCATCACATG GTACATGGGC 540
 TGTTACAAAA TCCAGAACTT TAACAACGTC ATCCCCGAGG GCATGAATCT GTCCTTCCTG 600
 ATCGCCCTGA TCAGCAACAA CGGCAATTAC ACCTGCGTCG TGACCTACCC CGAAAACGGC 660
 AGGACCTTCC ACCTGACCAG GACCCTGACC GTGAAAGTCG TGGGAAGCCC CAAGAATGCC 720
 30 GTGCCCCCGG TGATCCATTC CCCCAACGAC CACGTGGTGT ACGAGAAGGA GCCTGGAGAG 780
 GAGCTGCTGA TCCCCTGCAC AGTGTACTTC TCCTTCCTGA TGGACTCCAG GAATGAAGTG 840
 TGGTGGACCA TCGACGGCAA GAAGCCTGAC GACATCACCA TCGATGTGAC CATCAACGAG 900
 AGCATCAGCC ACAGCAGGAC CGAGGACGAG ACCAGGACCC AGATCCTGAG CATCAAGAAA 960
 GTCACCAGCG AGGACCTCAA GAGAAGCTAC GTCTGTCACG CCAGAAGCGC CAAAGGCGAG 1020

GTGGCCAAGG CTGCTAAGGT GAAACAGAAA GTGCCCCTC CTAGGTACAC AGTCGGATCC 1080
 GGTGGAGGTG AAAAACTCA CACATGCCCA CCGTGCCCAG CTCCGGAACCT CCTGGGCGGA 1140
 CCGTCAGTCT TCCTCTTCCC CCCAAAACCC AAGGACACCC TCATGATCTC CCGGACCCCT 1200
 GAGGTCACAT GCGTGGTGGT GGACGTGAGC CACGAAGACC CTGAGGTCAA GTTCAACTGG 1260
 5 TACGTGGACG GCGTGGAGGT GCATAATGCC AAGACAAAGC CGCGGGAGGA GCAGTACAAC 1320
 AGCACGTACC GTGTGGTCAG CGTCCTCACC GTCCTGCACC AGGACTGGCT GAATGGCAAG 1380
 GAGTACAAGT GCAAGGTCTC CAACAAAGCC CTCCCAGCCC CCATCGAGAA AACCATCTCC 1440
 AAAGCCAAAG GGCAGCCCCG AGAACCACAG GTGTACACCC TGCCCCCATG TCGGGATGAG 1500
 CTGACCAAGA ACCAGGTCAG CCTGTGGTGC CTGGTCAAAG GCTTCTATCC CAGCGACATC 1560
 10 GCCGTGGAGT GGGAGAGCAA TGGGCAGCCG GAGAACAACCT ACAAGACCAC GCCTCCCGTG 1620
 TTGGAATCCG ACGGCTCCTT CTTCTCTAC AGCGCGCTCA CCGTGGACAA GAGCAGGTGG 1680
 CAGCAGGGGA ACGTCTTCTC ATGCTCCGTG ATGCATGAGG CTCTGCACAA CCACTACACG 1740
 CAGAAGAGCC TCTCCCTGTC TCCGGGTAAA 1770

15 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
 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
 20 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
 25 synthetase, a selectable marker widely used for establishing stable CHOK1 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.

30 hIL1-R1-hIgG1-Fc-II/ IL-1RAcP- hIgG1-Fc-V expression plasmid (SEQ ID NO. 7)

AGCTTGCCAC CATGAAGGTC CTGCTCAGGC TGATCTGCTT CATTGCCCTG CTCATCAGCA 60
 GCCTGGAAGC CGACAAGTGC AAGGAGAGGG AGGAGAAGAT CATCCTCGTC AGCTCCGCCA 120

ACGAGATTGA TGTCAGGCCC TGCCCCCTCA ACCCCAATGA GCACAAGGGC ACAATCACCT 180
 GGTACAAGGA CGACAGCAAG ACCCCTGTCT CCACCGAGCA GGCCAGCAGA ATCCACCAGC 240
 ACAAAGAGAA GCTGTGGTTC GTGCCTGCCA AGGTGGAAGA CAGCGGCCAC TACTACTGTG 300
 TGGTGAGGAA CAGCTCCTAC TGCCTCAGGA TCAAGATCTC CGCCAAGTTC GTGGAGAACG 360
 5 AGCCCAACCT CTGTTACAAC GCTCAGGCTA TTTTCAAGCA AAAGCTCCCC GTGGCTGGAG 420
 ACGGAGGCCT GGTCTGTCCC TACATGGAGT TCTTCAAGAA TGAGAATAAT GAGCTCCCCA 480
 AGCTCCAGTG GTACAAGGAC TGTAAGCCTC TGCTCCTGGA CAACATCCAC TTCTCCGGCG 540
 TGAAGGACAG ACTGATCGTC ATGAACGTGG CCGAGAAGCA CAGGGGAAAC TACACCTGTC 600
 ACGCCTCCTA CACCTACCTC GGCAAGCAAT ATCCCATCAC CAGGGTCATC GAGTTCATCA 660
 10 CCCTCGAAGA GAACAAGCCC ACAAGGCCTG TCATCGTCAG CCCCGCCAAT GAAACCATGG 720
 AGGTGGACCT CGGCAGCCAG ATCCAGCTGA TCTGCAACGT GACAGGCCAG CTCAGCGACA 780
 TTGCCTACTG GAAGTGGAAAC GGCTCCGTGA TCGACGAAGA TGATCCCGTG CTGGGCGAGG 840
 ACTACTATAG CGTGGAGAAC CCCGCCAACA AAAGAAGGAG CACCCTGATC ACCGTGCTGA 900
 ACATCAGCGA GATCGAGTCC AGATTCTATA AGCATCCTTT CACCTGCTTT GCCAAGAACA 960
 15 CCCACGGCAT CGACGCCGCT TACATCCAGC TGATCTATCC CGTGACCAAC GGATCCGGTG 1020
 GAGGTGACAA AACTCACACA TGCCCACCGT GCCCAGCTCC GGAACCTCTG GGCGGACCGT 1080
 CAGTCTTCCT CTTCCCCCA AAACCCAAGG ACACCCTCAT GATCTCCCGG ACCCCTGAGG 1140
 TCACATGCGT GGTGGTGGAC GTGAGCCACG AAGACCCTGA GGTCAAGTTC AACTGGTACG 1200
 TGGACGGCGT GGAGGTGCAT AATGCCAAGA CAAAGCCGCG GGAGGAGCAG TACAACAGCA 1260
 20 CGTACCGTGT GGTGAGCGTC CTCACCGTCC TGCACCAGGA CTGGCTGAAT GGCAAGGAGT 1320
 ACAAGTGCAA GGTCTCCAAC AAAGCCCTCC CAGCCCCCAT CGAGAAAACC ATCTCCAAAG 1380
 CCAAAGGGCA GCCCCGAGAA CCACAGGTGT GTACCCTGCC CCCATCCCGG GATGAGCTGA 1440
 CCAAGAACCA GGTGAGCCTG AGTTGCGCGG TCAAAGGCTT CTATCCCAGC GACATCGCCG 1500
 TGGAGTGGGA GAGCAATGGG CAGCCGGAGA ACAACTACAA GACCACGCCT CCCGTGTTGG 1560
 25 ACTCCGACGG CTCCTTCAAG CTCGTCAGCA AGCTCACCGT GGACAAGAGC AGGTGGCAGC 1620
 AGGGGAACGT CTTCTCATGC TCCGTGATGC ATGAGGCTCT GCACAACCAC TACACGCAGA 1680
 AGAGCCTCTC CCTGTCTCCG GGTAAATAAT AGAATTCATT GATCATAATC AGCCATACCA 1740
 CATTTGTAGA GGTTTTACTT GCTTTAAAAA ACCTCCACA CCTCCCCCTG AACCTGAAAC 1800
 ATAAAATGAA TGCAATTGTT GTTGTTAACT TGTTTATTGC AGCTTATAAT GGTTACAAAT 1860
 30 AAAGCAATAG CATCACAAAT TTCACAAATA AAGCATTTTT TCACTGCAT TCTAGTTGTG 1920
 GTTTGTCCAA ACTCATCAAT GTATCTTATC ATGTCTGGCG GCCGCCGATA TTTGAAAATA 1980
 TGGCATATTG AAAATGTCGC CGATGTGAGT TTCTGTGTAA CTGATATCGC CATTTTTCCA 2040
 AAAGTGATTT TTGGGCATAC GCGATATCTG GCGATAGCGC TTATATCGTT TACGGGGGAT 2100
 GGCGATAGAC GACTTTGGTG ACTTGGGCGA TTCTGTGTGT CGCAAATATC GCAGTTTCGA 2160
 35 TATAGGTGAC AGACGATATG AGGCTATATC GCCGATAGAG GCGACATCAA GCTGGCACAT 2220

GGCCAATGCA TATCGATCTA TACATTGAAT CAATATTGGC CATTAGCCAT ATTATTCATT 2280
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 AATATGTACA TTTATATTGG CTCATGTCCA ACATTACCGC CATGTTGACA TTGATTATTG 2400
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 5 CGCGTTACAT AACTTACGGT AAATGGCCCG CCTGGCTGAC CGCCCAACGA CCCCCGCCCA 2520
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 CTAACAGTGG AGGCCAGACT TAGGCACAGC ACGATGCCCA CCACCACCAG TGTGCCGCAC 3660
 25 AAGGCCGTGG CGGTAGGGTA TGTGTCTGAA AATGAGCTCG GGGAGCGGGC TTGCACCGCT 3720
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CTACTGCAGC AAGGTGGCCT TCCCTCTCGA GGTGGTCCAG AAGGACAGCT GCTTCAACAG 4380
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 CCCCACGTG GACGGCTACT TCCCCAGCTC CGTGAAGCCC ACCATCACAT GGTACATGGG 4500
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 20 ATGTCGTAAC AACTCCGCCC CATTGACGCA AATGGGCGGT AGGCGTGTAC GGTGGGAGGT 11820
 CTATATAAGC AGAGCTCGTT TAGTGAACCG TCAGATCGCC TGGAGACGCC ATCCACGCTG 11880
 TTTTGACCTC CATAGAAGAC ACCGGGACCG ATCCAGCCTC CGCGGCCGGG AACGGTGCAT 11940
 TGGAACGCGG ATTCCCCGTG CCAAGAGTGA CGTAAGTACC GCCTATAGAG TCTATAGGCC 12000
 CACCCCTTG GCTTCTTATG CATGCTATAC TGTTTTTGGC TTGGGGTCTA TACACCCCG 12060
 25 CTTCTCATG TTATAGGTGA TGGTATAGCT TAGCCTATAG GTGTGGGTTA TTGACCATTA 12120
 TTGACCACTC CCCTATTGGT GACGATACTT TCCATTACTA ATCCATAACA TGGCTCTTTG 12180
 CCACAACCTC CTTTATTGGC TATATGCCAA TAACTGTCC TTCAGAGACT GACACGGACT 12240
 CTGTATTTTT ACAGGATGGG GTCTCATTTA TTATTTACAA ATTCACATAT ACAACACCAC 12300
 CGTCCCCAGT GCCCGCAGTT TTTATTAAAC ATAACGTGGG ATCTCCACGC GAATCTCGGG 12360
 30 TACGTGTTCC GGACATGGGC TCTTCTCCGG TAGCGGCGGA GCTTCTACAT CCGAGCCCTG 12420
 CTCCCATGCC TCCAGCGACT CATGGTCGCT CGGCAGCTCC TTGCTCCTAA CAGTGGAGGC 12480
 CAGACTTAGG CACAGCACGA TGCCCACCAC CACCAGTGTG CCGCACAAGG CCGTGGCGGT 12540
 AGGGTATGTG TCTGAAAATG AGCTCGGGGA GCGGGCTTGC ACCGCTGACG CATTTGGAAG 12600
 ACTTAAGGCA GCGGCAGAAG AAGATGCAGG CAGCTGAGTT GTTGTGTTCT GATAAGAGTC 12660
 35 AGAGGTAACCT CCCGTTGCGG TGCTGTTAAC GGTGGAGGGC AGTGTAGTCT GAGCAGTACT 12720

CGTTGCTGCC GCGCGGCCA CCAGACATAA TAGCTGACAG ACTAACAGAC TGTTCCTTTC 12780
CATGGGTCTT TTCTGCAGTC ACCGTCCTTG ACACGA 12816

In certain aspects, the present teachings provide for a mammalian expression system for
5 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
10 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-1 β modulation: arthritis, a gout, a
rheumatoid arthritis, a Cryopyrin-Associated Periodic Syndromes (CAPS), a scleroderma, a
15 diabetes, a atherosclerosis, a dry eye disease, an ocular allergy, or an uveitis.

EXAMPLES

The following Examples illustrate the forgoing aspects and other aspects of the present
20 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
25 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
30 through 333 of IL-1R1 or residues 1 through 358 of IL-1RAcP 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-1R-FcV and PKN001'-RAcP-FcII, respectively.

The gene encoding IL-1R1-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-1R-FcV.

Finally, the expression cassette for RAcP-FcII from PKN001'-RAcP-FcII was integrated into PKN002-IL-1R-FcV plasmid via NotI and SalI restriction sites and yielded a new recombinant construction that contained expression elements for both RAcP-FcII and IL-1R-FcV. The resulting clones were screened by NotI and SalI 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-IL1R-FcV-RAcP-FcII.

Recombinant plasmid PKN012-IL1R-FcV-RAcP-FcII combines expression cassettes for both RAcP-FcII and IL-1R-FcV. The plasmid can be used for co-expressing RAcP-FcII and IL-1R-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 IL1R-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 IL1R-FcV/RAcP-FcII heterodimer production. The plasmid map for PKN012-IL1R-FcV-RAcP-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-hIgG1-Fc polypeptide of SEQ ID NO. 1 and hIL-1RAcP-hIgG1-Fc polypeptide of SEQ ID NO. 2 has been generated through standard cell biology protocols. The expression plasmid PKN012-IL1R-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 4×10^6 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 ug of plasmid DNA in 100 ul 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 1.43×10^7 cells/ml;
- 0.7 ml cells (10^7 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 IL1R-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 IL1R-FcV-RAcP-FcII productivity. Based on these studies, it was determined that selected highest productivity cell line was expressing IL1R-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

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

Example 3: Purification of polypeptides of the present invention.

hIL1-R1-hIgG1-Fc polypeptide of SEQ ID NO. 1 and hIL-1RAcP-hIgG1-Fc polypeptide of
5 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 hIL1-R1-hIgG1-
Fc/ hIL-1RAcP-hIgG1-Fc polypeptides, was applied to a Protein A affinity column. The affinity
10 purification step was carried out according to the procedure outlined in Table 2. The Protein A
eluate containing hIL1-R1-hIgG1-Fc/ hIL-1RAcP-hIgG1-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.
15 The conductivity is adjusted to about 5.5 mS/cm with deionized water (dH₂O) if needed.

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
20 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-R1-hIgG1-Fc/ hIL-1RAcP-hIgG1-Fc
25 SDS-PAGE under non-reducing conditions, while lanes 4, 6 – under reducing conditions. Lanes 2,
5 show molecular weight markers. hIL1-R1-hIgG1-Fc/ hIL-1RAcP-hIgG1-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
30 mobile phase to reach protein concentration of about 5 mg/ml. The biologically relevant form of

hIL1-R1-hIgG1-Fc/ hIL-1RAcP-hIgG1-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

Step	Buffer	Vol CV	Flow cm/h
Rinse Before-use	dH ₂ O	3	150
Equilibration	10 mM NaPh, pH 6.0	5	150
Sample Load	Cell harvest	-	150
Wash 1	10 mM NaPh, pH 6.0	3	150
Wash 2	25 mM NaPh, 0.5 M NaCl, 5% Isopropanol pH 7.0	5	150
Re-Equilibration	10 mM NaPh, pH 6.0	3	150
Elution	20 mM Na-Citrate, pH 3.4	4	150
Re-Equilibration	10 mM PB, pH 6.0	3	150
CIP	0.1 M NaOH (contact time 15 min), reversed flow, CIP every 5 cycles	3	100
Rinse with NaCl	1M NaCl	3	150
Rinse After-use	dH ₂ O	3	150
Storage	20% (v/v) Ethanol, 20 mM NaPh, pH 7.0	3	150

Table 3: The operational procedure of AIEX

Step	Buffer	Vol CV	Flow cm/h
Rinse Before-use	dH ₂ O	3	150
Recharge	10 mM Tris-HCl, 1 M NaCl, pH 8	3	150
Equilibration	10 mM Tris-HCl, 50 mM NaCl, pH 8	3	150
Sample Load	Prepared Q Load	-	150
Wash	10 mM Tris-HCl, 50 mM NaCl, pH 8	3	150
Elution	10 mM Tris-HCl, 0.35 M NaCl, pH 8.0	3	150
CIP	1 M NaOH (contact time 1 hr), reversed-flow	3	40
Regeneration	10 mM Tris-HCl, 1 M NaCl, pH 8.0	3	150
Rinse After-use	dH ₂ O	3	150
Storage	20% (v/v) Ethanol	3	150

Table 4: The Operational Procedure of SEC-HPLC

Mobile Phase:	20 mM phosphate, 300 mM NaCl, pH 7.4
Flow Rate:	0.5 mL/min
Column:	G2000 SWxl, 7.8mm×300mm, TOSOH Bioscience
Guard column:	TSK Guard SWxl, 6.0mm×40mm, TOSOH Bioscience
Column Temperature:	25 °C
Sampler temperature:	Rome temperature
Injection Volume:	10 µl
Detector Wavelength:	280 nm
Run Time:	30 min

5

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-1 β (PrimGene, Shanghai, China; Cat#: 101-01B). 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: Characterization of inhibition of IL-1 β -induced IL-6 production with IL1R-FcV-RAcP-FcII heterodimer in human cells.

The present Example demonstrates potent functional (inhibitory) properties of IL1R-FcV-RAcP-FcII heterodimer in targeting human IL-1 β . As a functional comparator, previously characterized mouse monoclonal antibodies against IL-1 β were used. Human lung fibroblasts MRC5 produce IL-6 in response to treatment with IL-1 β . Quantification of IL-1 β -induced IL-6 production in MRC5 cells was used as the assay functional output. Inhibition of IL-6 production by IL1R-FcV-RAcP-FcII heterodimer and by control anti IL-1 β antibody provides a quantitative measure of inhibitory properties of the IL1R-FcV-RAcP-FcII heterodimer preparation.

Polypeptides of IL1R-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. The protein concentration of substantially pure IL1R-FcV-RAcP-FcII heterodimer in the sample used was 0.9 mg/ml in 100 mM NaCl, 25 mM NaH₂PO₄/Na₂HPO₄, 25 mM Arginine hydrochloride, 1% Sucrose; pH=6.3.

The following materials were used in the assay:

Cells: MRC5 cells, Human Lung Fibroblasts, ATCC Cat # CCL-171, Lot # 59474707.

Medium: DMEM, Dulbecco's Modification of Eagle's Medium, high glucose (4.5 g/L), Mediatech, Cat # 10-017-CM, Lot # 10017204, supplemented with L-glutamine and 1x penn/strep and 10% Fetal Bovine Serum, Omega Scientific, Cat # FB-05, Lot # 105104

Reagents: IL-1 β , Human recombinant, E. coli-derived, Ala117-Ser269, Accession # NP_000567, R&D systems, Cat # 201-LB, Lot # ADM1411062; Monoclonal antibodies against human IL-1 β , clone #8516, R&D systems, Cat # MAB201, Lot # AWE1011081; Human IL-6 Quantakine Immunoassay, R&D systems, Cat # D6050, Lot # 300070.

The following procedures were utilized in the assay:

Cell Maintenance:

1. Propagate MRC5 cells in the DMEM containing 10% FBS on T75 flasks according to the manufacturer's recommendations. Record the passage number;
2. Trypsinize cells, re-suspend in DMEM containing 10% FBS;
3. Test cell viability using Guava ViaCount Reagent. Standard viability should be >85%, if less, do not use this batch of cells;
4. Prepare dilutions of the cells for plating at desired density of 2 x 10³ cells/well per well of 48-well plate or 5 x 10³ per ml;
5. Allow cells to attach for 16-24 hours at 37°C, 5% CO₂, then remove medium;
6. Replace with fresh DMEM medium supplemented with 10% FBS, 0.4 ml/well for 48-well plate, incubate 5-10 min and replace the medium again with the same volume;

7. Add to the wells IL-1 β , Test Substances pre-mixed with IL-1 β and appropriate controls;
8. Incubate the cells after treatment for 24 hours then harvest the supernatants;
9. Centrifuge the supernatants at 300 x g for 10 min, collect cleared supernatants and use them for ELISA either directly or with 1/3 dilution if appropriate;

ELISA Protocol:

Principle of the assay: the assay employs the quantitative sandwich enzyme immunoassay technique. A microplate is pre-coated with a monoclonal antibody specific for IL-6.

Standards and samples are pipetted into the wells of the microplate and any IL-6 present binds to the immobilized antibodies. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for IL-6 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of bound IL-6. The color development is stopped and the color intensity is measured.

Assay procedure:

1. Prepare all reagents and working standards as described in manufacturer's manual (http://www.rndsystems.com/product_results.aspx?k=D6050)
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 μ L of Assay Diluent RD1W to each well.
4. Add 100 μ L of Standard, sample, or control per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 μ L) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 200 μ L of IL-6 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.

7. Repeat the aspiration/wash as in step 5.
8. Add 200 μ L of Substrate Solution to each well. Incubate for 20 minutes at room temperature. Protect from light.
9. Add 50 μ L of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Following are the experimental data obtained:

Inhibition of IL-6 production by control anti-IL-1 β antibodies:

1. MRC5 cells (Passage 5) were plated into a 48-well plate (Costar, Cat # 3548) at 2×10^3 cells per well, 0.4 ml per well, and incubated for 24 hours;
2. On the day of the assay, medium from the wells was aspirated and replaced with 0.4 ml of DMEM supplemented with 10% FBS, incubated 10-15 min and replaced with the same amount of the same fresh medium again;
3. IL-1 β was used at a final concentration of 50 pg/ml throughout the experiment;
4. Anti-human IL-1 β antibodies were used at final concentrations of 0.192, 0.96, 4.8, 24, 120 and 600 ng/ml;
5. Both IL-1 β and anti-human IL-1 β antibodies were prepared as 10x concentrated solutions;
6. Prior to adding the test substances to the cells, 55 μ L of 10x IL-1 β was mixed with 55 μ L of 10x corresponding anti-human IL-1 β antibody solution (or appropriate control) and incubated at room temperature for 30 min;
7. After the incubation, 100 μ L of each mixture was added to the wells containing 0.4 ml of growth medium;

8. The cells were incubated in the presence of the test substances for 24 hours and supernatants were collected, centrifuged at 300 x g for 10 min and used for IL-6 ELISA assay;
9. To test for recovery of IL-6 in the presence of anti-IL-1 β antibody, the latter was used at a final concentration of 600 ng/ml and were spiked with IL-6 standard to a final concentration of 20 pg/ml.

The calibration curve obtained in the IL-1 antibody mediated inhibition of IL-6 production assay is shown in **Figure 7**. The results of the measurements of IL-1 β -mediated IL-6 production and of IL-6 recovery from the culture medium in the experiment are shown in **Figure 8** and **Figure 9**. The obtained numerical values for IL-6 production along with their standard deviations are presented in **Table 5**.

Table 5: Summary of IL-6 production values.

Sample ID	Average, pg/ml	StDev pg/ml
Cells Control	1.8	0.001
IL-1 alone, 50 pm/ml	149.2	1.2
Anti-IL-1 Ab + 30 pg/ml IL-6	22.8	0.2
IL-1 + 0.192 ng/ml anti IL-1 Abs	152.5	2.5
IL-1 + 0.96 ng/ml anti IL-1 Abs	122.1	2.3
IL-1 + 4.8 ng/ml anti IL-1 Abs	33.4	0.4
IL-1 + 24 ng/ml anti IL-1 Abs	3.37	0.09
IL-1 + 120 ng/ml anti IL-1 Abs	2.44	0.02
IL-1 + 600 ng/ml anti IL-1 Abs	2.16	0.03

The foregoing experiment revealed that: (1) the control anti-IL-1 β antibody is a very potent inhibitor of IL-1 β signaling pathway with an IC₅₀ of about 2.1 ng/ml or about 14 pM; (2) plating cell density of 2×10^3 cells per well 24 hours prior to the assay setup is adequate; (3) recovery of IL-6 spiked into the culture medium at a final concentration of 20 pg/ml is about 114%.

Inhibition of IL-6 production by IL1R-FcV-RAcP-FcII heterodimer preparation:

1. MRC5 cells (Passage 6) were plated into 48-well plate (Costar, Cat # 3548) at 2 x 10³ cells per well, 0.4 ml per well and incubated for 24 hours;
2. Plating for this experiment was carried out in duplicates. Each plating duplicate was measured in duplicates on ELISA plate resulting in 4 experimental points per treatment
3. On the day of the assay, medium from the wells was aspirated and replaced with 0.4 ml of DMEM supplemented with 10% FBS, incubated 10-15 min and replaced with the same amount of the same fresh medium again;
4. IL-1 β was used at a final concentration of 50 pg/ml throughout the experiment;
5. IL1R-FcV-RAcP-FcII heterodimer was used at final concentrations of 0.0536, 0.134, 0.335, 0.839, 2.097, 5.24, 13.1, 32.8, 81.9, 204.8, 512, 1280, 3200 and 20000 ng/ml;
6. Both IL-1 β and IL1R-FcV-RAcP-FcII heterodimer was prepared as 10x concentrated solutions;
7. Prior to adding the test substances to the cells, 120 μ l of 10x IL-1 β /IL-1F2 was mixed with 120 μ l of 10x corresponding IL1R-FcV-RAcP-FcII heterodimer solution (or appropriate control) and incubated at room temperature for 30 min;
8. After the incubation, 100 μ l of each mixture was added to the wells containing 0.4 ml of growth medium;
9. The cells were incubated in the presence of the test substances for 24 hours and supernatants were collected, centrifuged at 300 x g for 10 min and used for IL-6 ELISA assay.

The calibration curve obtained in the IL1R-FcV-RAcP-FcII heterodimer mediated inhibition of IL-6 production assay is shown in **Figure 10**. The results of the measurements of IL1R-FcV-RAcP-FcII heterodimer -mediated IL-6 production and of IL-6 recovery from the culture medium in the experiment are shown in **Figure 11** and **Figure 12**. The obtained numerical values for IL-6 production along with their standard deviations are presented in **Table 6**.

Table 6: Summary of IL-6 production values.

Sample ID	Average, pg/ml	StDev pg/ml	Calibration Range
Cells Control	13.97	0.23	
IL-1 alone, 50 pm/ml Plate 1	548.9	7.29	R
IL-1 alone, 50 pm/ml Plate 2	563.1	1.87	R
IL-1 + 0.054 ng/ml heterodimer	322.9	15.69	R
IL-1 + 0.134 ng/ml heterodimer	256.2	12.51	
IL-1 + 0.335 ng/ml heterodimer	180.2	8.69	
IL-1 + 0.834 ng/ml heterodimer	108.6	3.86	
IL-1 + 2.09 ng/ml heterodimer	46.7	1.39	
IL-1 + 5.24 ng/ml heterodimer	21.9	1.26	
IL-1 + 13.11 ng/ml heterodimer	12.9	0.24	
IL-1 + 32.8 ng/ml heterodimer	13.9	0.29	
IL-1 + 81.92 ng/ml heterodimer	13.4	1.04	
IL-1 + 204.8 ng/ml heterodimer Pl. 1	13.4	0.64	
IL-1 + 512 ng/ml heterodimer	12.9	0.65	
IL-1 + 1280 ng/ml heterodimer	13.9	0.16	
IL-1 + 3200 ng/ml heterodimer	16.1	3.03	
IL-1 + 8000 ng/ml heterodimer	16.9	1.09	
IL-1 + 20000 ng/ml heterodimer	22.5	0.55	
heterodimer alone 20000 ng/ml	20.9	1.04	
IL-1 + 204.8 ng/ml heterodimer Pl. 2	13.9	0.68	
heterodimer + 30 pg/ml IL-6 Spike	27.4	1.2	

R – indicates that the value fell outside the assay calibration range

Each experimental point was plated in duplicate and IL-6 production was further measured in duplicate resulting in 4 experimental reads per concentration point. The obtained data demonstrated high reproducibility of the experimental procedure and the assay. The foregoing experiment revealed that the IC50 value for IL1R-FcV-RAcP-FcII heterodimer in this experiment
5 was about 0.3 mg/ml or about 2.4 pM.

Example 5: Pharmacokinetics (PK) of IL1R-FcV-RAcP-FcII heterodimer after subcutaneous administration in mice.

10 Polypeptides of IL1R-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
15 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
20 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
25 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
30 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 (C_{max}) 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 7.

Table 7: Mean Human-Fc Protein Concentration ± SEM (µg/mL) at each Time Post-Administration

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

*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

5 [^] Bleed via orbital sinus

[#] Bleed via terminal cardiac puncture

Example 6: Evaluation of the toxicity of IL1R-FcV-RAcP-FcII heterodimer following 28-days repeated dosing in C57BL6 mice.

10 Following is a repeat dose murine toxicity evaluation study results summary. Treatment of C57BL6 mice with polypeptides of IL1R-FcV-RAcP-FcII heterodimer (SEQ ID NO.1 and SEQ ID NO. 2) test article by twice weekly subcutaneous injection at 10, 30 and 100 mg/kg dose levels was well tolerated in the study. There were no unscheduled deaths prior to completion of the study. Test article treatment was not associated with morbidity or clinical signs of toxicity. There was no
15 consistent gender or treatment related effect on body weight. There was a finding in increased food consumption which was considered to be a possible effect of treatment but this was not considered to be adverse.

 Although there was evidence of functional changes in parameters related to clinical biochemistry, these were not considered to be of toxicological significance as there was evidence
20 of reversibility in most of these parameters, although not complete in some. In the absence of toxicokinetic evaluation it was not possible to assess the significance of apparent non-reversibility of some of these differences. However, none of the differences was associated with histopathological change or was considered to be adverse. The differences observed were of a type that is typically reversible and it is possible that the reversibility period was no sufficient to
25 demonstrate this.

 It was concluded that the No-Observed Adverse Effect Level (NOAEL) in the mouse 28-day IL1R-FcV-RAcP-FcII heterodimer dosing study was therefore considered to be 100 mg/kg.

 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
30 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
5 equivalents, and the specification, along with such variations.

What is claimed is:

1. A heterodimeric protein composition capable of binding human IL-1 β , said protein composition comprising:

5 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;
10 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
15 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-1 β 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-1 β , said heterodimeric protein composition comprising:

a first polypeptide comprising
30 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.

13. An isolated nucleic acid of SEQ ID NO. 7.

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-1 β , 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.

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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.

10 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.

15 29. A method of treating or preventing a disease or condition associated with modulation of activity of human IL-1 β , the method comprising administering to a patient in need for treating or preventing a disease associated with modulation of activity of human IL-1 β 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.

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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.

25 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).

30 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.

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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.

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40. A heterodimeric protein composition capable of binding human IL-1 β , said protein composition being capable of inhibiting human IL-6 production in human lung fibroblasts in response to treating the fibroblasts with human IL-1 β , said inhibiting having an IC₅₀ value of about 2.3 pM.

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