

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 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

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 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 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 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 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. 1. 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 upregulation 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 sequence of SEQ ID NO. 2; wherein the substance inhibits the activity of human IL-1 β . 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 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 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 one aspect, the present disclosure provides a use of a pharmaceutical composition in the manufacture of a medicament for treating or preventing a disease or condition associated with modulation of activity of human IL-1 β , the 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; wherein the pharmaceutical composition inhibits the activity of human IL-1 β .

In certain aspects, the present invention provides for a heterodimeric protein composition 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.

In one aspect, the present disclosure provides 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, wherein the composition comprises 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.

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;

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 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, 10 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. 15 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 HPFTCEFAKNT 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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30 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 VVQKDCSFNS 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 VAKAAKVQK VPAPRYTVGS GGGDKTHTCP PCPAPELLGG 360
5 PSVFLFPPKP KDTLMIS RTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN 420
STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPCRDE 480
LTKNQVSLWC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SALTVDKSRW 540
QQGNVFSCSV MHEALHNHYT QKSLSLSPGK 570

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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 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)

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25 MKVLLRLICF IALLISSLEA DKCKEREKI ILVSSANEID VRPCPLNPNE HKGTITWYKD 60
DSKTPVSTEQ ASRIHQHKEK LWFVPAKVED SGHYCVVRN SSYCLRIKIS AKFVENEPNL 120
CYNQAIFKQ KLPVAGDGGL VCPYMEFFKN ENNELPKLQW YKDCCKPLLLD 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

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VSLSCAVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG 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
 AACAAAGCCCA 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 AACCCTAAGGA CACCCTCATG ATCTCCCGGA CCCCTGAGGT CACATGCGTG 1140
 GTGGTGGACG TGAGCCACGA AGACCCTGAG GTCAAGTTCA ACTGGTACGT GGACGGCGTG 1200
 GAGGTGCATA ATGCCAAGAC AAAGCCGCGG GAGGAGCAGT ACAACAGCAC GTACCGTGTG 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

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

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

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

ATGACTCTGC TGTGGTGC GT 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 GCGGAAAAC AGAATCAGCA AGGAGAAGGA CGTGCTGTGG 300
 TTCAGACCCA CCCTCCTCAA CGACACAGGC AACTACACCT GCATGCTCAG GAACACCACC 360
 TACTGCAGCA AGGTGGCCTT CCCTCTCGAG GTGGTCCAGA AGGACAGCTG CTCAACAGC 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 CCCAACGAC 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
 TTGGACTCCG 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 mamalian 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	GAAGTGGAAC	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	GGAACTCCTG	GGCGGACCGT	1080
	CAGTCTTCCT	CTTCCCCCCA	AAACCCAAGG	ACACCCTCAT	GATCTCCCGG	ACCCCTGAGG	1140
	TCACATGCGT	GGTGGTGGAC	GTGAGCCACG	AAGACCCTGA	GGTCAAGTTC	AACTGGTACG	1200
	TGGACGGCGT	GGAGGTGCAT	AATGCCAAGA	CAAAGCCGCG	GGAGGAGCAG	TACAACAGCA	1260
20	CGTACCGTGT	GGTCAGCGTC	CTCACCGTCC	TGCACCAGGA	CTGGCTGAAT	GGCAAGGAGT	1320
	ACAAGTGCAA	GGTCTCCAAC	AAAGCCCTCC	CAGCCCCCAT	CGAGAAAACC	ATCTCCAAAG	1380
	CCAAAGGGCA	GCCCCGAGAA	CCACAGGTGT	GTACCCTGCC	CCCATCCCGG	GATGAGCTGA	1440
	CCAAGAACCA	GGTCAGCCTG	AGTTGCGCGG	TCAAAGGCTT	CTATCCCAGC	GACATCGCCG	1500
	TGGAGTGGGA	GAGCAATGGG	CAGCCGGAGA	ACAACTACAA	GACCACGCCT	CCCGTGTGTTG	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	ACCTCCCACA	CCTCCCCCTG	AACCTGAAAC	1800
	ATAAAATGAA	TGCAATTGTT	GTTGTTAACT	TGTTTATTGC	AGCTTATAAT	GGTTACAAAT	1860
30	AAAGCAATAG	CATCACAAAT	TTCACAAATA	AAGCATTTTT	TTCACTGCAT	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
	GGTTATATAG	CATAAATCAA	TATTGGCTAT	TGGCCATTGC	ATACGTTGTA	TCCATATCAT	2340
	AATATGTACA	TTTATATTGG	CTCATGTCCA	ACATTACCGC	CATGTTGACA	TTGATTATTG	2400
	ACTAGTTATT	AATAGTAATC	AATTACGGGG	TCATTAGTTC	ATAGCCCATA	TATGGAGTTC	2460
5	CGCGTTACAT	AACTTACGGT	AAATGGCCCG	CCTGGCTGAC	CGCCCAACGA	CCCCGCCCCA	2520
	TTGACGTCAA	TAATGACGTA	TGTTCCCAT	GTAACGCCAA	TAGGGACTTT	CCATTGACGT	2580
	CAATGGGTGG	AGTATTTACG	GTAAACTGCC	CACTTGGCAG	TACATCAAGT	GTATCATATG	2640
	CCAAGTACGC	CCCCTATTGA	CGTCAATGAC	GGTAAATGGC	CCGCCTGGCA	TTATGCCCAG	2700
	TACATGACCT	TATGGGACTT	TCCTACTTGG	CAGTACATCT	ACGTATTAGT	CATCGCTATT	2760
10	ACCATGGTGA	TGCGGTTTTG	GCAGTACATC	AATGGGCGTG	GATAGCGGTT	TGACTCACGG	2820
	GGATTTCCAA	GTCTCCACCC	CATTGACGTC	AATGGGAGTT	TGTTTTGGCA	CCAAAATCAA	2880
	CGGGACTTTC	CAAAATGTCT	TAACAACCTC	GCCCCATTGA	CGCAAATGGG	CGGTAGGCGT	2940
	GTACGGTGGG	AGGTCTATAT	AAGCAGAGCT	CGTTTAGTGA	ACCGTCAGAT	CGCCTGGAGA	3000
	CGCCATCCAC	GCTGTTTTGA	CCTCCATAGA	AGACACCGGG	ACCGATCCAG	CCTCCGCGGC	3060
15	CGGGAACGGT	GCATTGGAAC	GCGGATTCCC	CGTGCCAAGA	GTGACGTAAG	TACCGCCTAT	3120
	AGAGTCTATA	GGCCCACCCC	CTTGGCTTCT	TATGCATGCT	ATACTGTTTT	TGGCTTGGGG	3180
	TCTATACACC	CCCCTTCCT	CATGTTATAG	GTGATGGTAT	AGCTTAGCCT	ATAGGTGTGG	3240
	GTTATTGACC	ATTATTGACC	ACTCCCCTAT	TGGTGACGAT	ACTTTCCATT	ACTAATCCAT	3300
	AACATGGCTC	TTTGCCACAA	CTCTCTTTAT	TGGCTATATG	CCAATACACT	GTCCTTCAGA	3360
20	GACTGACACG	GACTCTGTAT	TTTTACAGGA	TGGGGTCTCA	TTTATTATTT	ACAAATTCAC	3420
	ATATACAACA	CCACCGTCCC	CAGTGCCCGC	AGTTTTTATT	AAACATAACG	TGGGATCTCC	3480
	ACGCGAATCT	CGGGTACGTG	TTCCGGACAT	GGGCTCTTCT	CCGGTAGCGG	CGGAGCTTCT	3540
	ACATCCGAGC	CCTGCTCCCA	TGCCTCCAGC	GACTCATGGT	CGCTCGGCAG	CTCCTTGCTC	3600
	CTAACAGTGG	AGGCCAGACT	TAGGCACAGC	ACGATGCCCA	CCACCACCAG	TGTGCCGCAC	3660
25	AAGGCCGTGG	CGGTAGGGTA	TGTGTCTGAA	AATGAGCTCG	GGGAGCGGGC	TTGCACCGCT	3720
	GACGCATTTG	GAAGACTTAA	GGCAGCGGCA	GAAGAAGATG	CAGGCAGCTG	AGTTGTTGTG	3780
	TTCTGATAAG	AGTCAGAGGT	AACTCCCGTT	GCGGTGCTGT	TAACGGTGGA	GGGCAGTGTA	3840
	GTCTGAGCAG	TACTCGTTGC	TGCCGCGCGC	GCCACCAGAC	ATAATAGCTG	ACAGACTAAC	3900
	AGACTGTTCC	TTTCCATGGG	TCTTTTCTGC	AGTCACCGTC	CTTGACACGA	AGCTTGCCAC	3960
30	CATGACTCTG	CTGTGGTGCG	TCGTGTCCCT	CTACTTCTAC	GGCATCCTCC	AGTCCGACGC	4020
	CAGCGAGAGG	TGCGATGACT	GGGGCCTGGA	CACCATGAGG	CAGATCCAGG	TGTTTGAGGA	4080
	CGAGCCTGCC	AGGATTAAGT	GCCCCCTCTT	CGAGCACTTT	CTGAAGTTCA	ACTACAGCAC	4140
	CGCTCACAGC	GCTGGCCTGA	CACTGATCTG	GTACTGGACA	AGGCAGGACA	GGGATCTCGA	4200
	GGAGCCCATC	AACTTCAGGC	TGCCCCGAAA	CAGAATCAGC	AAGGAGAAGG	ACGTGCTGTG	4260
35	GTTCAGACCC	ACCCTCCTCA	ACGACACAGG	CAACTACACC	TGCATGCTCA	GGAACACCAC	4320

	CTACTGCAGC	AAGGTGGCCT	TCCCTCTCGA	GGTGGTCCAG	AAGGACAGCT	GCTTCAACAG	4380
	CCCCATGAAG	CTGCCCCTCC	ATAAACTGTA	CATCGAGTAC	GGCATCCAGA	GGATCACATG	4440
	CCCCAACGTG	GACGGCTACT	TCCCCAGCTC	CGTGAAGCCC	ACCATCACAT	GGTACATGGG	4500
	CTGTTACAAA	ATCCAGAACT	TTAACAACGT	CATCCCCGAG	GGCATGAATC	TGTCCTTCCT	4560
5	GATCGCCCTG	ATCAGCAACA	ACGGCAATTA	CACCTGCGTC	GTGACCTACC	CCGAAAACGG	4620
	CAGGACCTTC	CACCTGACCA	GGACCCTGAC	CGTGAAAGTC	GTGGGAAGCC	CCAAGAATGC	4680
	CGTGCCCCCC	GTGATCCATT	CCCCCAACGA	CCACGTGGTG	TACGAGAAGG	AGCCTGGAGA	4740
	GGAGCTGCTG	ATCCCCTGCA	CAGTGTACTT	CTCCTTCCTG	ATGGACTCCA	GGAATGAAGT	4800
	GTGGTGGACC	ATCGACGGCA	AGAAGCCTGA	CGACATCACC	ATCGATGTGA	CCATCAACGA	4860
10	GAGCATCAGC	CACAGCAGGA	CCGAGGACGA	GACCAGGACC	CAGATCCTGA	GCATCAAGAA	4920
	AGTCACCAGC	GAGGACCTCA	AGAGAAGCTA	CGTCTGTCAC	GCCAGAAGCG	CCAAAGGCGA	4980
	GGTGGCCAAG	GCTGCTAAGG	TGAAACAGAA	AGTGCCCGCT	CCTAGGTACA	CAGTCGGATC	5040
	CGGTGGAGGT	GACAAAACCTC	ACACATGCCC	ACCGTGCCCA	GCTCCGGAAC	TCCTGGGCGG	5100
	ACCGTCAGTC	TTCTCTTTCC	CCCCAAAACC	CAAGGACACC	CTCATGATCT	CCCGGACCCC	5160
15	TGAGGTCACA	TGCGTGGTGG	TGGACGTGAG	CCACGAAGAC	CCTGAGGTCA	AGTTCAACTG	5220
	GTACGTGGAC	GGCGTGGAGG	TGCATAATGC	CAAGACAAAG	CCGCGGGAGG	AGCAGTACAA	5280
	CAGCACGTAC	CGTGTGGTCA	GCGTCCTCAC	CGTCCTGCAC	CAGGACTGGC	TGAATGGCAA	5340
	GGAGTACAAG	TGCAAGGTCT	CCAACAAAGC	CCTCCCAGCC	CCCATCGAGA	AAACCATCTC	5400
	CAAAGCCAAA	GGGCAGCCCC	GAGAACCACA	GGTGTACACC	CTGCCCCCAT	GTCGGGATGA	5460
20	GCTGACCAAG	AACCAGGTCA	GCCTGTGGTG	CCTGGTCAAA	GGCTTCTATC	CCAGCGACAT	5520
	CGCCGTGGAG	TGGGAGAGCA	ATGGGCAGCC	GGAGAACAAC	TACAAGACCA	CGCCTCCCGT	5580
	GTTGGACTCC	GACGGCTCCT	TCTTCCTCTA	CAGCGCGCTC	ACCGTGGACA	AGAGCAGGTG	5640
	GCAGCAGGGG	AACGTCTTCT	CATGCTCCGT	GATGCATGAG	GCTCTGCACA	ACCACTACAC	5700
	GCAGAAGAGC	CTCTCCCTGT	CTCCGGGTAA	ATAATAGAAT	TCATTGATCA	TAATCAGCCA	5760
25	TACCACATTT	GTAGAGGTTT	TACTTGCTTT	AAAAAACCTC	CCACACCTCC	CCCTGAACCT	5820
	GAAACATAAA	ATGAATGCAA	TTGTTGTTGT	TAAGTTGTTT	ATTGCAGCTT	ATAATGGTTA	5880
	CAAATAAAGC	AATAGCATCA	CAAATTTTAC	AAATAAAGCA	TTTTTTTTCAC	TGCATTCTAG	5940
	TTGTGGTTTTG	TCCAAACTCA	TCAATGTATC	TTATCATGTC	TGGATCCTCT	ACGCCGGACG	6000
	CATCGTGGCC	GGCATCACCG	GCGCCACAGG	TGCGGTTGCT	GGCGCCTATA	TCGCCGACAT	6060
30	CACCGATGGG	GAAGATCGGG	CTCGCCACTT	CGGGCTCATG	AGCGCTTGTT	TCGGCGTGGG	6120
	TATGGTGGCA	GGCCCCGTGG	CCGGGGGACT	GTTGGGCGCC	ATCTCCTTGC	ATGCACCATT	6180
	CCTTGCGGCG	GCGGTGCTCA	ACGGCCTCAA	CCTACTACTG	GGCTGCTTCC	TAATGCAGGA	6240
	GTCGCATAAG	GGAGAGCGTC	GACCTCGGGC	CGCGTTGCTG	GCGTTTTTCC	ATAGGCTCCG	6300
	CCCCCCTGAC	GAGCATCACA	AAAATCGACG	CTCAAGTCAG	AGGTGGCGAA	ACCCGACAGG	6360
35	ACTATAAAGA	TACCAGGCGT	TTCCCCCTGG	AAGCTCCCTC	GTGCGCTCTC	CTGTTCCGAC	6420

	CCTGCCGCTT	ACCGGATACC	TGTCCGCTT	TCTCCCTTCG	GGAAGCGTGG	CGCTTTCTCA	6480
	TAGCTCACGC	TGTAGGTATC	TCAGTTCGGT	GTAGGTCGTT	CGCTCCAAGC	TGGGCTGTGT	6540
	GCACGAACCC	CCCGTTTCAGC	CCGACCGCTG	CGCCTTATCC	GGTAACTATC	GTCTTGAGTC	6600
	CAACCCGGTA	AGACACGACT	TATCGCCACT	GGCAGCAGCC	ACTGGTAACA	GGATTAGCAG	6660
5	AGCGAGGTAT	GTAGGCGGTG	CTACAGAGTT	CTTGAAGTGG	TGGCCTAACT	ACGGCTACAC	6720
	TAGAAGAACA	GTATTTGGTA	TCTGCGCTCT	GCTGAAGCCA	GTTACCTTCG	GAAAAAGAGT	6780
	TGGTAGCTCT	TGATCCGGCA	AACAAACCAC	CGCTGGTAGC	GGTGGTTTTT	TTGTTTGCAA	6840
	GCAGCAGATT	ACGCGCAGAA	AAAAAGGATC	TCAAGAAGAT	CCTTTGATCT	TTTCTACGGG	6900
	GTCTGACGCT	CAGTGGAACG	AAAACTCACG	TTAAGGGATT	TTGGTCATGA	GATTATCAAA	6960
10	AAGGATCTTC	ACCTAGATCC	TTTTAAATTA	AAAATGAAGT	TTTAAATCAA	TCTAAAGTAT	7020
	ATATGAGTAA	ACTTGGTCTG	ACAGTTACCA	ATGCTTAATC	AGTGAGGCAC	CTATCTCAGC	7080
	GATCTGTCTA	TTTCGTTTCAT	CCATAGTTGC	CTGACTCCCC	GTCGTGTAGA	TAACCTACGAT	7140
	ACGGGAGGGC	TTACCATCTG	GCCCCAGTGC	TGCAATGATA	CCGCGAGACC	CACGCTCACC	7200
	GGCTCCAGAT	TTATCAGCAA	TAAACCAGCC	AGCCGGAAGG	GCCGAGCGCA	GAAGTGGTCC	7260
15	TGCAACTTTA	TCCGCCTCCA	TCCAGTCTAT	TAATTGTTGC	CGGGAAGCTA	GAGTAAGTAG	7320
	TTCGCCAGTT	AATAGTTTGC	GCAACGTTGT	TGCCATTGCT	ACAGGCATCG	TGGTGTACAG	7380
	CTCGTCGTTT	GGTATGGCTT	CATTGAGCTC	CGGTTCCCAA	CGATCAAGGC	GAGTTACATG	7440
	ATCCCCCATG	TTGTGCAAAA	AAGCGGTTAG	CTCCTTCGGT	CCTCCGATCG	TTGTCAGAAG	7500
	TAAGTTGGCC	GCAGTGTTAT	CACTCATGGT	TATGGCAGCA	CTGCATAATT	CTCTTACTGT	7560
20	CATGCCATCC	GTAAGATGCT	TTTCTGTGAC	TGGTGAGTAC	TCAACCAAGT	CATTCTGAGA	7620
	ATAGTGTATG	CGGCGACCGA	GTTGCTCTTG	CCCGGCGTCA	ATACGGGATA	ATACCGCGCC	7680
	ACATAGCAGA	ACTTTAAAAG	TGCTCATCAT	TGGAAAACGT	TCTTCGGGGC	GAAAACCTCT	7740
	AAGGATCTTA	CCGCTGTTGA	GATCCAGTTC	GATGTAACCC	ACTCGTGCAC	CCAACCTGATC	7800
	TTCAGCATCT	TTTACTTTCA	CCAGCGTTTC	TGGGTGAGCA	AAAACAGGAA	GGCAAAATGC	7860
25	CGCAAAAAAG	GGAATAAGGG	CGACACGGAA	ATGTTGAATA	CTCATACTCT	TCCTTTTTTCA	7920
	ATATTATTGA	AGCATTTATC	AGGGTTATTG	TCTCATGAGC	GGATACATAT	TTGAATGTAT	7980
	TTAGAAAAAT	AAACAAATAG	GGGTTCGCG	CACATTTCCC	CGAAAAGTGC	CACCTGACGT	8040
	CTAAGAAACC	ATTATTATCA	TGACATTAAC	CTATAAAAAT	AGGCGTATCA	CGAGGCCCTG	8100
	ATGGCTCTTT	GCGGCACCCA	TCGTTCGTAA	TGTTCCGTGG	CACCGAGGAC	AACCCTCAAG	8160
30	AGAAAATGTA	ATCACACTGG	CTCACCTTCG	GGTGGGCCTT	TCTGCGTTTA	TAAGGAGACA	8220
	CTTTATGTTT	AAGAAGGTTG	GTAAATTCCT	TGCGGCTTTG	GCAGCCAAGC	TAGATCCGGC	8280
	TGTGGAATGT	GTGTCAGTTA	GGGTGTGGAA	AGTCCCCAGG	CTCCCCAGCA	GGCAGAAGTA	8340
	TGCAAAGCAT	GCATCTCAAT	TAGTCAGCAA	CCAGGTGTGG	AAAGTCCCCA	GGCTCCCCAG	8400
	CAGGCAGAAG	TATGCAAAGC	ATGCATCTCA	ATTAGTCAGC	AACCATAGTC	CCGCCCCCTAA	8460
35	CTCCGCCCAT	CCCGCCCCTA	ACTCCGCCCA	GTTCCGCCCA	TTCTCCGCCC	CATGGCTGAC	8520

	TAATTTTTTT	TATTTATGCA	GAGGCCGAGG	CCGCCTCGGC	CTCTGAGCTA	TTCCAGAAGT	8580
	AGTGAGGAGG	CTTTTTTGGG	GGCCTAGGCT	TTTGCAAAAA	GCTAGCTTGG	GGCCACCGCT	8640
	CAGAGCACCT	TCCACCATGG	CCACCTCAGC	AAGTTCCCAC	TTGAACAAAA	ACATCAAGCA	8700
	AATGTACTTG	TGCCTGCCCC	AGGGTGAGAA	AGTCCAAGCC	ATGTATATCT	GGGTTGATGG	8760
5	TACTGGAGAA	GGACTGCGCT	GCAAAACCCG	CACCCTGGAC	TGTGAGCCCA	AGTGTGTAGA	8820
	AGAGTTACCT	GAGTGGAATT	TTGATGGCTC	TAGTACCTTT	CAGTCTGAGG	GCTCCAACAG	8880
	TGACATGTAT	CTCAGCCCTG	TTGCCATGTT	TCGGGACCCC	TTCCGCAGAG	ATCCCAACAA	8940
	GCTGGTGTTT	TGTGAAGTTT	TCAAGTACAA	CCGGAAGCCT	GCAGAGACCA	ATTTAAGGCA	9000
	CTCGTGTAAT	CGGATAATGG	ACATGGTGAG	CAACCAGCAC	CCCTGGTTTG	GAATGGAACA	9060
10	GGAGTATACT	CTGATGGGAA	CAGATGGGCA	CCCTTTTGGT	TGGCCTTCCA	ATGGCTTTCC	9120
	TGGGCCCCAA	GGTCCGTATT	ACTGTGGTGT	GGGCGCAGAC	AAAGCCTATG	GCAGGGATAT	9180
	CGTGGAGGCT	CACTACCGCG	CCTGCTTGTA	TGCTGGGGTC	AAGATTACAG	GAACAAATGC	9240
	TGAGGTCATG	CCTGCCCAGT	GGGAAGTCCA	AATAGGACCC	TGTGAAGGAA	TCCGCATGGG	9300
	AGATCATCTC	TGGGTGGCCC	GTTTCATCTT	GCATCGAGTA	TGTGAAGACT	TTGGGGTAAT	9360
15	AGCAACCTTT	GACCCCAAGC	CCATTCCTGG	GAAGTGAAT	GGTGCAGGCT	GCCATACCAA	9420
	CTTTAGCACC	AAGGCCATGC	GGGAGGAGAA	TGGTCTGAAG	CACATCGAGG	AGGCCATCGA	9480
	GAAACTAAGC	AAGCGGCACC	GGTACCACAT	TCGAGCCTAC	GATCCCAAGG	GGGGCCTGGA	9540
	CAATGCCCCG	GGTCTGACTG	GGTCCACGA	AACGTCCAAC	ATCAACGACT	TTTCTGCTGG	9600
	TGTCGCCAAT	CGCAGTGCCA	GCATCCGCAT	TCCCCGGAAT	GTCGGCCAGG	AGAAGAAAGG	9660
20	TTACTTTGAA	GACCGCGGCC	CCTCTGCCAA	TTGTGACCCC	TTTGCAGTGA	CAGAAGCCAT	9720
	CGTCCGCACA	TGCCTTCTCA	ATGAGACTGG	CGACGAGCCC	TTCCAATACA	AAAACCTAAT	9780
	AGACTTTGAG	TGATCTTGAG	CCTTTCCTAG	TTCATCCCAC	CCCGCCCCAG	AGAGATCTTT	9840
	GTGAAGGAAC	CTTACTTCTG	TGGTGTGACA	TAATTGGACA	AACTACCTAC	AGAGATTTAA	9900
	AGCTCTAAGG	TAAATATAAA	ATTTTAAAGT	GTATAATGTG	TTAAACTACT	GATTCTAATT	9960
25	GTTTGTGTAT	TTTAGATTCC	AACCTATGGA	ACTGATGAAT	GGGAGCAGTG	GTGGAATGCC	10020
	TTTAATGAGG	AAAACCTGTT	TTGCTCAGAA	GAAATGCCAT	CTAGTGATGA	TGAGGCTACT	10080
	GCTGACTCTC	AACATTCTAC	TCCTCCAAAA	AAGAAGAGAA	AGGTAGAAGA	CCCCAAGGAC	10140
	TTTCCTTCAG	AATTGCTAAG	TTTTTTGAGT	CATGCTGTGT	TTAGTAATAG	AACTCTTGCT	10200
	TGCTTTGCTA	TTTACACCAC	AAAGGAAAAA	GCTGCACTGC	TATACAAGAA	AATTATGGAA	10260
30	AAATATTCTG	TAACCTTTAT	AAGTAGGCAT	AACAGTTATA	ATCATAACAT	ACTGTTTTTT	10320
	CTTACTCCAC	ACAGGCATAG	AGTGTCTGCT	ATTAATAACT	ATGCTCAAAA	ATTGTGTACC	10380
	TTTAGCTTTT	TAATTTGTAA	AGGGGTTAAT	AAGGAATATT	TGATGTATAG	TGCCTTGACT	10440
	AGAGATCATA	ATCAGCCATA	CCACATTTGT	AGAGGTTTTA	CTTGCTTTAA	AAAACCTCCC	10500
	ACACCTCCCC	CTGAACCTGA	AACATAAAAT	GAATGCAATT	GTTGTTGTTA	ACTTGTTTAT	10560
35	TGCAGCTTAT	AATGGTTACA	AATAAAGCAA	TAGCATCACA	AATTTACAAA	ATAAAGCATT	10620

	TTTTTCACTG	CATTCTAGTT	GTGGTTTGTG	CAAACATCATC	AATGTATCTT	ATCATGTCTG	10680
	GATCTAGCTT	CGTGTCAAGG	ACGGTGAAGT	CAGTGAATAA	TAAAATGTGT	GTTTGTCCGA	10740
	AATACGCGTT	TTGAGATTTT	TGTCGCCGAC	TAAATTCATG	TCGCGCGATA	GTGGTGTTTA	10800
	TCGCCGATAG	AGATGGCGAT	ATTGGAAAAA	TCGATATTTG	AAAATATGGC	ATATTGAAAA	10860
5	TGTCGCCGAT	GTGAGTTTCT	GTGTAAGTGA	TATCGCCATT	TTTCCAAAAG	TGATTTTTTG	10920
	GCATACGCGA	TATCTGGCGA	TAGCGCTTAT	ATCGTTTACG	GGGGATGGCG	ATAGACGACT	10980
	TTGGTGACTT	GGGCGATTCT	GTGTGTCGCA	AATATCGCAG	TTTCGATATA	GGTGACAGAC	11040
	GATATGAGGC	TATATCGCCG	ATAGAGGCGA	CATCAAGCTG	GCACATGGCC	AATGCATATC	11100
	GATCTATACA	TTGAATCAAT	ATTGGCCATT	AGCCATATTA	TTCATTGGTT	ATATAGCATA	11160
10	AATCAATATT	GGCTATTGGC	CATTGCATAC	GTTGTATCCA	TATCATAATA	TGTACATTTA	11220
	TATTGGCTCA	TGTCCAACAT	TACCGCCATG	TTGACATTGA	TTATTGACTA	GTTATTAATA	11280
	GTAATCAATT	ACGGGGTTCAT	TAGTTCATAG	CCCATATATG	GAGTTCCGCG	TTACATAACT	11340
	TACGGTAAAT	GGCCCGCCTG	GCTGACCGCC	CAACGACCCC	CGCCCATTTA	CGTCAATAAT	11400
	GACGTATGTT	CCCATAGTAA	CGCCAATAGG	GACTTTCCAT	TGACGTCAAT	GGGTGGAGTA	11460
15	TTTACGGTAA	ACTGCCCCTG	TGGCAGTACA	TCAAGTGTAT	CATATGCCAA	GTACGCCCCC	11520
	TATTGACGTC	AATGACGGTA	AATGGCCCGC	CTGGCATTAT	GCCCAGTACA	TGACCTTATG	11580
	GGACTTTTCT	ACTTGGCAGT	ACATCTACGT	ATTAGTCATC	GCTATTACCA	TGGTGATGCG	11640
	GTTTTTGGCAG	TACATCAATG	GGCGTGGATA	GCGGTTTGAC	TCACGGGGAT	TTCCAAGTCT	11700
	CCACCCCATT	GACGTCAATG	GGAGTTTGTG	TTGGCACCAA	AATCAACGGG	ACTTTCCAAA	11760
20	ATGTCGTAAC	AACTCCGCCC	CATTGACGCA	AATGGGCGGT	AGGCGTGTAC	GGTGGGAGGT	11820
	CTATATAAGC	AGAGCTCGTT	TAGTGAACCG	TCAGATCGCC	TGGAGACGCC	ATCCACGCTG	11880
	TTTTTGACCTC	CATAGAAGAC	ACCGGGACCG	ATCCAGCCTC	CGCGGCCGGG	AACGGTGCAT	11940
	TGGAACGCGG	ATTCCCCGTG	CCAAGAGTGA	CGTAAGTACC	GCCTATAGAG	TCTATAGGCC	12000
	CACCCCTTGT	GCTTCTTATG	CATGCTATAC	TGTTTTTTGG	TTGGGGTCTA	TACACCCCGG	12060
25	CTTCCTCATG	TTATAGGTGA	TGGTATAGCT	TAGCCTATAG	GTGTGGGTGA	TTGACCATTA	12120
	TTGACCACTC	CCCTATTGGT	GACGATACTT	TCCATTACTA	ATCCATAACA	TGGCTCTTTG	12180
	CCACAACCTCT	CTTTATTGGC	TATATGCCAA	TACACTGTCC	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	AGAGGTAAC	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
purification step was carried out according to the procedure outlined in Table 2. The Protein A
10 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	dH2O	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	dH2O	3	150
Storage	20% (v/v) Ethanol, 20 mM NaPh, pH 7.0	3	150

Table 3: The operational procedure of AIEEX

Step	Buffer	Vol CV	Flow cm/h
Rinse Before-use	dH2O	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	dH2O	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 SWx1, 7.8mm×300mm, TOSOH Bioscience
Guard column:	TSK Guard SWx1,6.0mm×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.

10

Example 4: Characterization of inhibition of IL-1 β -induced IL-6 production with IL1R-FcV-RAcP-FcII heterodimer in human cells.

15

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.

20

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 x 10³ 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 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.

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 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 (Tmax) was 36 hours post treatment. The half-life (T1/2) 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:

a first polypeptide comprising

a first amino acid sequence comprising amino acids 18 through 333 of human IL1-R1 (GenBank: AAM88423.1), 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 (GenBank: BAA2542 1.1), 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; and

wherein 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.

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. 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

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; and

wherein 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. The therapeutic composition of claim 3, 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.

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

6. An isolated nucleic acid encoding a polypeptide comprising amino acid sequence of SEQ ID NO. 5.

7. The nucleic acid of claim 5 or claim 6, wherein the codon usage is optimized for high expression of said polypeptide in a mammalian cell.

8. The nucleic acid of claim 5, wherein the nucleic acid sequence comprises the sequence of SEQ ID NO. 4.

9. The nucleic acid of claim 6, wherein the nucleic acid sequence comprises the sequence of SEQ ID NO. 6.

10. The nucleic acid of claim 8 or claim 9, wherein said nucleic acid comprises an expression vector.
11. An isolated nucleic acid of SEQ ID NO. 7.
12. An in vitro 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. 5.
13. The expression system of claim 12, wherein said expression vector is harbored in a mammalian cell.
14. The expression system of claim 13, wherein said mammalian cell is a CHO cell.
15. The expression system of claim 13, 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.
16. Use of a substance for manufacture of a medicament for the treatment or prevention of a disease associated with upregulation 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; wherein the substance inhibits the activity of human IL-1 β .
17. Use of a pharmaceutical composition in the manufacture of a medicament for treating or preventing a disease or condition associated with upregulation of activity of human IL-1 β , the 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; wherein the pharmaceutical composition inhibits the activity of human IL-1 β .
18. The use according to claim 16 or claim 17, wherein said disease is an arthritis.
19. The use according to claim 16 or claim 17, wherein said disease is a gout.

20. The use according to claim 16 or claim 17, wherein said disease is a rheumatoid arthritis.
21. The use according to claim 16 or claim 17, wherein said disease is a Cryopyrin-Associated Periodic Syndromes (CAPS).
22. The use according to claim 16 or claim 17, wherein said disease is a scleroderma.
23. The use according to claim 16 or claim 17, wherein said disease is a diabetes.
24. The use according to claim 16 or claim 17, wherein said disease is atherosclerosis.
25. The use according to claim 16 or claim 17, wherein said disease is a dry eye disease.
26. The use according to claim 16 or claim 17, wherein said disease is an ocular allergy.
27. The use according to claim 16 or claim 17, wherein said disease is an uveitis.
28. 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, wherein the composition comprises 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.

IL-1 β INHIBITOR COMPOSITION AND USE THEREOF

ABSTRACT

A therapeutic composition is described that can be used for treating or prevention of diseases association with modulation of activity of human IL-1 β . In certain aspects, the disclosed invention is based, on engineering of a heterodimeric protein assembly that is capable of binding to human IL-1 β and attenuating its function. The heterodimeric protein assembly comprises an extracellular portions of human IL1-R1 and of human IL-1RAcP, or their functional fragments. 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. 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. DNA expression vectors and expression systems for overproducing the polypeptides in mammalian cells are also provided for.

Figure 1