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DESCRIPTION

Description

FIELD OF THE INVENTION

[0001] Generally, the invention relates to the field of biological pharmaceuticals as well as their use in conditions associated with inflammatory disorders (e.g. rheumatoid arthritis, Crohn's disease, etc.), diabetes, cardiovascular disease and gout. More specifically, the invention relates to a heterodimeric IL-1R1/IL-1RAcP -derived composition that is capable of inhibiting IL-1β cytokine.

BACKGROUND

[0002] The interleukin-1 (IL-1) family of cytokines comprises 11 proteins (IL-1F1 to IL-1F11) encoded by 11 distinct genes in humans and mice. IL-1-type cytokines are major mediators of innate immune reactions, and blockade of the founding members IL-1 or IL-1β by the interleukin-1 receptor antagonist (IL-1RA) has demonstrated a central role of IL-1 in a number of human autoinflammatory diseases. IL-1 or IL-1β rapidly increase messenger RNA expression of hundreds of genes in multiple different cell types. The potent proinflammatory activities of IL-1and IL-1β are restricted at three major levels: (i) synthesis and release, (ii) membrane receptors, and (iii) intracellular signal transduction. This pathway summarizes extracellular and intracellular signaling of IL-1 or IL-1β, including positive- and negative-feedback mechanisms that amplify or terminate the IL-1 response. In response to ligand binding of the receptor, a complex sequence of combinatorial phosphorylation and ubiquitination events results in activation of nuclear factor kappa-B signaling and the 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-1β, MKP-1) by transcriptional and posttranscriptional mechanisms. Of note, most intracellular components that participate in the cellular response to IL-1 also mediate responses to other cytokines (IL-18 and IL-33), Toll-like-receptors (TLRs), and many forms of cytotoxic stresses (see Weber A., et al., Sci Signal., 2010 Jan 19;3(105)).

[0003] IL-1 and IL-1β independently bind the type I IL-1 receptor (IL-1R1), which is ubiquitously expressed. A third specific ligand, the IL-1 receptor antagonist (IL-1RA), binds the IL-1RI with similar specificity and affinity but does not activate the receptor and trigger downstream signaling. The IL-1 receptor accessory protein (IL-1RAcP) serves as a co-receptor that is required for signal transduction of IL-1/IL-1RI complexes, and this co-receptor is also

necessary for activation of IL-1R1 by other IL-1 family members, in particular IL-18 and IL-33. The type II IL-1 receptor (IL-1R2) binds IL-1 and IL-1β but lacks a signaling-competent cytosolic part and thus serves as a decoy receptor. The II,-1RA, the plasma membrane-anchored IL-1R2, and the naturally occurring "shed" domains of each of the extracellular IL-1 receptor chains (termed sIL-1RI, sIL-1RII, and sIL-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.

[0004] The initial step in IL-1 signal transduction is a ligand-induced conformational change in the first extracellular domain of the IL-1RI that facilitates recruitment of IL-1RacP. Through conserved cytosolic regions called Toll- and IL-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 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 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).

[0005] Overproduction of IL-1 is the cause of many inflammatory disorders. For example, IL-1 has been linked to the pathology of diabetes, cardiovascular disease, gout and certain types of arthritis (e.g. rheumatoid arthritis (RA)).

[0006] 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 IL1-RAcP, 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 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.

[0007] 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.

[0008] The article ECONOMIDES A.N. et al., "Cytokine traps: multi-component, high-affinity blockers of cytokine action", NATURE MEDICINE, NATURE PUB. CO, NEW YORK, vol. 9, no. 1, (2003-01-01), pages 47-52, XP002256034, discloses the engineering of separate constructs

encoding the extracellular domain of IL6Ra or gp130 fused to the Fc portion of human immunoglobulin 1. The article also report the design of a second generation of cytokine traps, wherein the extracellular domains of two distinct receptors are fused in line and then fused to Fc portion of human LgG1.

SUMMARY OF THE INVENTION

[0009] In certain aspects, the present invention provides for a heterodimeric protein composition capable of binding human IL-1β (GenBank: AAH08678.1). The protein composition comprises a first polypeptide which includes a first amino acid sequence which contains amino acids 18 through 333 of human IL1-R1 (GenBank: AAM88423.1), and a second amino acid sequence which contains a first mutant of a Fc portion of human immunoglobulin gamma-1 Fc (GenBank: J00228.1). The protein composition also comprises a second polypeptide which includes another first amino acid sequence containing amino acids 21 through 358 of human Il,1-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. In the protein composition, the first polypeptide comprises the amino acid sequence of SEQ ID N°1 and the second polypeptide comprises the amino acid sequence of SEQ ID N°2. The protein composition may be capable of exhibiting human IL-1β binding activity in an ELISA assay with an EC50 of about 50 ng/ml.

[0010] In certain aspects, the present invention provides for a therapeutic composition. The therapeutic composition comprises a heterodimeric protein composition capable of binding human IL-1β. The protein composition comprises a first polypeptide which includes a first amino acid sequence which contains amino acids 18 through 333 of human IL1-R1, and a second amino acid sequence which contains a first mutant of the Fc portion of human immunoglobulin gamma-1 Fc. The protein composition also 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.

[0011] In the protein composition, the first polypeptide comprises the amino acid sequence of SEQ ID N°1 and the second polypeptide comprises the amino acid sequence of SEQ ID N°2.

[0012] 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.

[0013] In certain aspects, the present invention provides for an isolated nucleic acid encoding

a polypeptide comprising an amino acid sequence of any one of SEQ ID N°1, SEQ ID N°2, SEQ ID N°3 and SEQ ID N°5.

[0014] The codon usage of the nucleic acid may be optimized for high expression of the polypeptide in a mammalian cell.

[0015] In certain aspects, the present invention provides for an isolated nucleic acid encoding a polypeptide comprising amino acid sequence of SEQ ID N°3 and comprising the sequence of SEQ ID N°4, or an isolated nucleic acid sequence encoding a polypeptide comprising amino acid sequence of SEQ ID N°5 and comprising the sequence of SEQ ID N°6.

[0016] The nucleic acid may comprise an expression vector.

[0017] In certain aspects, the present invention provides for an isolated nucleic acid of SEQ ID NO. 7.

[0018] 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.

[0019] In certain aspects, the present invention provides a substance for use in the treatment or prevention of a disease associated with modulation of activity of human IL-1β. The substance comprises a heterodimeric protein comprised of a first polypeptide containing amino acid sequence of SEQ ID NO. 1 and a second polypeptide containing amino acid sequence of SEQ ID NO. 2. The disease associated with modulation of activity of human IL-1β may be an arthritis, a gout, a rheumatoid arthritis, a Cryopyrin-Associated Periodic Syndromes (CAPS), a scleroderma, a diabetes, a atherosclerosis, a dry eye disease, an ocular allergy, or an uveitis.

[0020] In certain aspects the present invention provides for a heterodimeric protein composition capable of binding human, wherein said heterodimeric composition comprises a first polypeptide comprising the SEQ ID N°1 and a second polypeptide comprising the amino acid sequence of SEQ ID N °2. The protein composition is capable of inhibiting human IL-6 production in human lung fibroblasts in response to treating the fibroblasts with human IL-1β. The inhibiting is characterized by an IC50 value of about 2.3 pM.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] The following drawings and descriptions are provided to aid in the understanding of the invention:

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

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

Figure 3 shows representative transfection growth curve obtained in the process of generating stable cell lines for expressing the polypeptides of the present invention;

Figure 4 shown a size-exclusion HPLC analytical chromatogram of the sample containing heterodimers comprising polypeptide of SEQ ID NO. 1 and polypeptide of SEQ ID NO. 2 after the anion exchange chromatography purification step;

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

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

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-1β-mediated IL-6 production and IL-6 recovery from the culture medium where MRC5 cells were incubated for 24 hours with IL-1β 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 20 pg/ml to estimate IL-6 recovery;

Figure 9 shows the results of the measurements of IL-1β-mediated IL-6 production and IL-6 recovery from the culture medium where MRC5 cells were incubated for 24 hours with IL-1β at a final concentration of 50 pg/ml (IL-1 alone) or left untreated (Cells Ctrl). 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 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-1β-mediated IL-6 production with IL1R-FcV-RAcP-FcII heterodimer and IL-6 recovery from the culture medium where MRC5 cells were incubated for 24 hours with IL-1β 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 Il,1R-FcV-RAcP-FcП 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

[0022] The teachings disclosed herein are based, in part, upon engineering of a heterodimeric protein assembly that is capable of binding to human IL-1 β and attenuating its function. The heterodimeric protein assembly of the present teachings comprises extracellular portions of IL1-R1 (GenBank: AAM88423.1) and of IL-1RAcP (GenBank: BAA25421.1), or functional fragments thereof. Each, the IL1-R1 portion and the IL-1RAcP portion, is fused to a distinct mutant of Fc portion of the human Ig Gamma-1 (GenBank: J00228.1). The two distinct Fc mutants in the heterodimeric protein assembly are engineered as to favor the heteromeric dimer formation between the two Fc mutants over any homomeric assembly. To enable recombinant production of the heterodimeric protein assembly of the present teachings, a DNA expression vector has been constructed for overproducing the heterodimeric protein assembly in a heterologous protein expression system, and mammalian cells have been prepared stably expressing the heterodimeric protein assembly to a high expression level. A protein purification procedure has been devised allowing obtaining a physiologically relevant substantially pure preparation of the heterodimeric protein assembly of the present teachings. Thus purified protein molecule demonstrates a high degree of specific activity in an *in vitro* Enzyme-Linked Immunosorbent Assay (ELISA) using human IL-1β (GenBank: AAH08678.1). Unexpectedly, the protein molecule exhibits an acceptable pharmacokinetics profile upon subcutaneous animal administration, while not resulting in any body weight loss or adverse clinical events.

[0023] The terms used in this specification generally have their ordinary meanings in the art, within the context of this invention and in the specific context where each term is used. Certain terms are discussed below or elsewhere in the specification, to provide additional guidance to the practitioner in describing the compositions and methods of the invention and how to make and use them. The scope or meaning of any use of a term will be apparent from the specific

context in which the term is used. "About" and "approximately" shall generally mean an acceptable degree of error for the quantity measured given the nature or precision of the measurements. Typically, exemplary degrees of error are within 20 percent (%), preferably within 10%, and more preferably within 5% of a given value or range of values. Alternatively, and particularly in biological systems, the terms "about" and "approximately" may mean values that are within an order of magnitude, preferably within 5- fold and more preferably within 2-fold of a given value. Numerical quantities given herein are approximate unless stated otherwise, meaning that the term "about" or "approximately" can be inferred when not expressly stated.

[0024] The methods disclosed herein may include steps of comparing sequences to each other, including wild-type sequence to one or more mutants (sequence variants). Such comparisons typically comprise alignments of polymer sequences, e.g., using sequence alignment programs and/or algorithms that are well known in the art (for example, BLAST, FASTA and MEGALIGN, to name a few). The skilled artisan can readily appreciate that, in such alignments, where a mutation contains a residue insertion or deletion, the sequence alignment will introduce a "gap" (typically represented by a dash, or "A") in the polymer sequence not containing the inserted or deleted residue.

[0025] The methods disclosed herein 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.

[0026] "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.

[0027] 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.

[0028] 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 described herein 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

described herein may be post-translationally modified without substantially effecting its biological function.

[0029] In certain aspects, functional variants of the heterodimeric protein assemblies described herein 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.*

[0030] In certain aspects, the heterodimeric protein assemblies described herein comprise an extracellular portion of IL1-R1, or a functional fragment thereof, fused with a IgG-Fc domain, and an extracellular portion IL-1RAcP, or a functional fragment thereof, fused with another IgG-Fc domain. The IgG-Fc domain and the 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 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.

[0031] In an example embodiment, illustratively shown in **Figure 1,** the extracellular portion of IL1-R1 fused with IgG-Fc domain (Fc-II) via a flexible linker may comprise the amino acid sequence of SEQ. ID NO. 1, while IL-1RAcP fused with another IgG-Fc domain (Fc-V) via a flexible linker may comprise the amino acid sequence of SEQ. ID NO. 2.

HIL1-R1-hIgG1-Fc polypeptide (SEQ ID NO. 1)

```
LEADKCKERE EKIILVSSAN EIDVRPCPLN PNEHKGTITW YKDDSKTPVS TEQASRIHQH 60
KEKLWFVPAK VEDSGHYYCV VRNSSYCLRI KISAKFVENE PNLCYNAQAI FKQKLPVAGD 120
GGLVCPYMEF FKNENNELPK LQWYKDCKPL 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
TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY 420
KCKVSNKALP APIEKTISKA KGQPREPQVC TLPPSRDELT KNQVSLSCAV KGFYPSDIAV 480
EWESNGQPEN NYKTTPPVLD SDGSFKLVSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK 540
SLSLSPGK                                                        548
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hIL-1RAcP-hIgG1-Fc polypeptide (SEQ ID NO. 2)

```
SERCDDWGLD TMRQIQVFED EPARIKCPLF EHFLKFNYST AHSAGLTLIW YWTRQDRDLE 60
EPINFRLPEN RISKEKDVLW FRPTLLNDTG NYTCMLRNTT YCSKVAFPLE VVQKDSCFNS 120

PMKLPVHKLY IEYGIQRITC PNVDGYFPSS VKPTITWYMG CYKIQNFNNV IPEGMNLSFL 180
IALISNNGNY TCVVTYPENG RTFHLTRTLT VKVVGSPKNA VPPVIHSPND HVVYEKEPGE 240
ELLIPCTVYF SFLMDSRNEV WWTIDGKKPD DITIDVTINE SISHSRTEDE TRTQILSIKK 300
VTSEDLKRSY VCHARSAKGE VAKAAKVKQK VPAPRYTVGS GGGDKTHTCP PCPAPELLGG 360
PSVFLFPPKP KDTLMISRTP 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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[0032] In certain aspects, the present teachings provides for a recombinant DNA molecule having an open reading frame coding for a polypeptide comprising the leading 333 amino acids of the human IL1-R1 fused with IgG-Fc domain (Fc-II) via a flexible linker, and for another recombinant DNA molecule having an open reading frame coding for another polypeptide comprising the leading 358 amino acids of the human IL-1RAcP fused with another IgG-Fc domain (Fc-V) via a flexible linker.

[0033] 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)

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

VSLSCAVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFKLVSKLTV DKSRWQQGNV 540
FSCSVMHEAL HNHYTQKSLS LSPGK                                       565
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HIL1-R1-hIgG1-Fc DNA (SEQ ID NO. 4)

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ATGAAGGTCC TGCTCAGGCT GATCTGCTTC ATTGCCCTGC TCATCAGCAG CCTGGAAGCC 60
GACAAGTGCA AGGAGAGGGA GGAGAAGATC ATCCTCGTCA GCTCCGCCAA CGAGATTGAT 120
GTCAGGCCCT GCCCCCTCAA CCCCAATGAG CACAAGGGCA CAATCACCTG GTACAAGGAC 180
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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
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
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 GCCCACCGTG CCCAGCTCCG GAACTCCTGG GCGGACCGTC AGTCTTCCTC 1080
TTCCCCCCAA AACCCAAGGA 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
CCCCGAGAAC CACAGGTGTG TACCCTGCCC CCATCCCGGG ATGAGCTGAC CAAGAACCAG 1440
GTCAGCCTGA GTTGCGCGGT CAAAGGCTTC TATCCCAGCG ACATCGCCGT GGAGTGGGAG 1500
AGCAATGGGC AGCCGGAGAA CAACTACAAG ACCACGCCTC CCGTGTTGGA CTCCGACGGC 1560
TCCTTCAAGC TCGTCAGCAA GCTCACCGTG GACAAGAGCA GGTGGCAGCA GGGGAACGTC 1620
TTCTCATGCT CCGTGATGCA TGAGGCTCTG CACAACCACT ACACGCAGAA GAGCCTCTCC 1680

CTGTCTCCGG GTAAA                                                  1695
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hIL-1RAcP-hIgG1-Fc polypeptide (SEQ ID NO. 5)

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MTLLWCVVSL YFYGILQSDA SERCDDWGLD TMRQIQVFED EPARIKCPLF EHFLKFNYST 60
AHSAGLTLIW YWTRQDRDLE EPINFRLPEN RISKEKDVLW FRPTLLNDTG NYTCMLRNTT 120
YCSKVAFPLE VVQKDSCFNS PMKLPVHKLY IEYGIQRITC PNVDGYFPSS VKPTITWYMG 180
CYKIQNFNNV IPEGMNLSFL IALISNNGNY TCVVTYPENG RTFHLTRTLT VKVVGSPKNA 240
VPPVIHSPND HVVYEKEPGE ELLIPCTVYF SFLMDSRNEV WWTIDGKKPD DITIDVTINE 300
SISHSRTEDE TRTQILSIKK VTSEDLKRSY VCHARSAKGE VAKAAKVKQK 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
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hIL-1RAcP-hIgG1-Fc DNA (SEQ ID NO. 6)

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ATGACTCTGC TGTGGTGCGT CGTGTCCCTC TACTTCTACG GCATCCTCCA GTCCGACGCC 60
AGCGAGAGGT GCGATGACTG GGGCCTGGAC ACCATGAGGC AGATCCAGGT GTTTGAGGAC 120
GAGCCTGCCA GGATTAAGTG CCCCCTCTTC GAGCACTTTC TGAAGTTCAA CTACAGCACC 180
GCTCACAGCG CTGGCCTGAC ACTGATCTGG TACTGGACAA GGCAGGACAG GGATCTCGAG 240
GAGCCCATCA ACTTCAGGCT GCCCGAAAAC AGAATCAGCA AGGAGAAGGA CGTGCTGTGG 300
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TTCAGACCCA CCCTCCTCAA CGACACAGGC AACTACACCT GCATGCTCAG GAACACCACC 360
TACTGCAGCA AGGTGGCCTT CCCTCTCGAG GTGGTCCAGA AGGACAGCTG CTTCAACAGC 420
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
GTGCCCCCCG 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 GTGCCCGCTC CTAGGTACAC AGTCGGATCC 1080
GGTGGAGGTG ACAAAACTCA CACATGCCCA CCGTGCCCAG CTCCGGAACT CCTGGGCGGA 1140
CCGTCAGTCT TCCTCTTCCC CCCAAAACCC AAGGACACCC TCATGATCTC CCGGACCCCT 1200
GAGGTCACAT GCGTGGTGGT GGACGTGAGC CACGAAGACC CTGAGGTCAA GTTCAACTGG 1260
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
GCCGTGGAGT GGGAGAGCAA TGGGCAGCCG GAGAACAACT ACAAGACCAC GCCTCCCGTG 1620
TTGGACTCCG ACGGCTCCTT CTTCCTCTAC AGCGCGCTCA CCGTGGACAA GAGCAGGTGG 1680
CAGCAGGGGA ACGTCTTCTC ATGCTCCGTG ATGCATGAGG CTCTGCACAA CCACTACACG 1740
CAGAAGAGCC TCTCCCTGTC TCCGGGTAAA                                  1770
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[0034] In certain aspects, the present disclosure 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 linker. This plasmid comprises two cytomegalovirus (CMV) promoters to drive transcription of the two genes coding for said polypeptide and said another polypeptide, each followed by a transcription termination sequence and a polyadenylation sequence. The plasmid also contains an origin of replication and a gene conferring ampicillin resistance, for supporting plasmid propagation and selection in bacteria. The plasmid further contains a gene for Glutamine synthetase, a selectable marker widely used for establishing stable CHOK1 and NSO cell lines. The plasmid of the present disclosure is illustratively shown in **Figure 2.**

[0035] In an example embodiment, the mammalian expression plasmid of the present teachings comprises the nucleotide sequence of SEQ ID NO. 7.

HIL1-R1-hIgG1-Fc-II/ IL-1RAcP- hIgG1-Fc-V expression plasmid (SEQ ID NO. 7)

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AGCTTGCCAC CATGAAGGTC CTGCTCAGGC TGATCTGCTT CATTGCCCTG CTCATCAGCA 60
GCCTGGAAGC CGACAAGTGC AAGGAGAGGG AGGAGAAGAT CATCCTCGTC AGCTCCGCCA 120
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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
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
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
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
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 CCCGTGTTGG 1560
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
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
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AATATGTACA TTTATATTGG CTCATGTCCA ACATTACCGC CATGTTGACA TTGATTATTG 2400
ACTAGTTATT AATAGTAATC AATTACGGGG TCATTAGTTC ATAGCCCATA TATGGAGTTC 2460
CGCGTTACAT AACTTACGGT AAATGGCCCG CCTGGCTGAC CGCCCAACGA CCCCCGCCCA 2520
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CGGGAACGGT GCATTGGAAC GCGGATTCCC CGTGCCAAGA GTGACGTAAG TACCGCCTAT 3120
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TCTATACACC CCCGCTTCCT CATGTTATAG GTGATGGTAT AGCTTAGCCT ATAGGTGTGG 3240
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ACGCGAATCT CGGGTACGTG TTCCGGACAT GGGCTCTTCT CCGGTAGCGG CGGAGCTTCT 3540
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GTTTGTGTAT TTTAGATTCC AACCTATGGA ACTGATGAAT GGGAGCAGTG GTGGAATGCC 10020
TTTAATGAGG AAAACCTGTT TTGCTCAGAA GAAATGCCAT CTAGTGATGA TGAGGCTACT 10080
GCTGACTCTC AACATTCTAC TCCTCCAAAA AAGAAGAGAA AGGTAGAAGA CCCCAAGGAC 10140
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TGCTTTGCTA TTTACACCAC AAAGGAAAAA GCTGCACTGC TATACAAGAA AATTATGGAA 10260
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
TGCAGCTTAT AATGGTTACA AATAAAGCAA TAGCATCACA AATTTCACAA ATAAAGCATT 10620

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TTTTTCACTG CATTCTAGTT GTGGTTTGTC CAAACTCATC AATGTATCTT ATCATGTCTG 10680
GATCTAGCTT CGTGTCAAGG ACGGTGACTG CAGTGAATAA TAAAATGTGT GTTTGTCCGA 10740
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TCGCCGATAG AGATGGCGAT ATTGGAAAAA TCGATATTTG AAAATATGGC ATATTGAAAA 10860
TGTCGCCGAT GTGAGTTTCT GTGTAACTGA TATCGCCATT TTTCCAAAAG TGATTTTTGG 10920
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GTAATCAATT ACGGGGTCAT TAGTTCATAG CCCATATATG GAGTTCCGCG TTACATAACT 11340
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GACGTATGTT CCCATAGTAA CGCCAATAGG GACTTTCCAT TGACGTCAAT GGGTGGAGTA 11460
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TTTTGACCTC CATAGAAGAC ACCGGGACCG ATCCAGCCTC CGCGGCCGGG AACGGTGCAT 11940
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TTGACCACTC CCCTATTGGT GACGATACTT TCCATTACTA ATCCATAACA TGGCTCTTTG 12180
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CTGTATTTTT ACAGGATGGG GTCTCATTTA TTATTTACAA ATTCACATAT ACAACACCAC 12300
CGTCCCCAGT GCCCGCAGTT TTTATTAAAC ATAACGTGGG ATCTCCACGC GAATCTCGGG 12360
TACGTGTTCC GGACATGGGC TCTTCTCCGG TAGCGGCGGA GCTTCTACAT CCGAGCCCTG 12420
CTCCCATGCC TCCAGCGACT CATGGTCGCT CGGCAGCTCC TTGCTCCTAA CAGTGGAGGC 12480
CAGACTTAGG CACAGCACGA TGCCCACCAC CACCAGTGTG CCGCACAAGG CCGTGGCGGT 12540
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ACTTAAGGCA GCGGCAGAAG AAGATGCAGG CAGCTGAGTT GTTGTGTTCT GATAAGAGTC 12660
AGAGGTAACT CCCGTTGCGG TGCTGTTAAC GGTGGAGGGC AGTGTAGTCT GAGCAGTACT 12720

CGTTGCTGCC GCGCGCGCCA CCAGACATAA TAGCTGACAG ACTAACAGAC TGTTCCTTTC 12780
CATGGGTCTT TTCTGCAGTC ACCGTCCTTG ACACGA                          12816
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[0036] In certain aspects, the present teachings provide for a mammalian expression system for production of a heterodimeric protein assembly comprising a polypeptide comprising amino acid residues 18 through 333 of the human IL1-R1 fused with IgG-Fc domain (Fc-II) via a flexible linker, and another polypeptide comprising amino acid residues 21 through 358 of the

human IL-1RAcP fused with another IgG-Fc domain (Fc-V) via a flexible linker.

[0037] In an example embodiment, the mammalian expression system of the present invention comprises Chinese hamster ovary cells (CHO-K1) harboring a plasmid comprising nucleotide sequence of SEQ ID NO. 7.

EXAMPLES

[0038] The following Examples illustrate the forgoing aspects and other aspects of the present teachings. These non-limiting Examples are put forth so as to provide those of ordinary skill in the art with illustrative embodiments as to how the compounds, compositions, articles, devices, and/or methods claimed herein are made and evaluated.

[0039] Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperature, etc.) but some errors and deviations should be accounted for.

[0040] **Example 1:** Construction of plasmids for expression of polypeptides of the present invention.

[0041] Optimized gene sequences comprising sequences encoding residues amino acid residues 1 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.

[0042] 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.

[0043] Finally, the expression cassette for RAcP-FcII from PKN001 '-RAcP-FcII was integrated into PKN002-II,-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.

[0044] 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-II,1R-FcV-RAcP-FcII is illustratively shown in Figure 2.

[0045] **Example 2:** Generation of stable cell lines for expressing polypeptides of the present invention.

[0046] A stable clone of CHO-K1 cells co-expressing hIL1-R1-hIgG1-Fc polypeptide of SEQ ID NO. 1 and hIL-IRAcP-hIgGI-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

[0047] 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

[0048] 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).

[0049] 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.

[0050] 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.

[0051] 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.

[0052] HIL1-R1-hIgG1-Fc polypeptide of SEQ ID NO. 1 and hIL-1RAcP-hIgG1-Fc polypeptide of SEQ ID NO. 2 were co-expressed in CHO-K1 essentially as described in foregoing Example 2. Cells were harvested and lysed utilizing well established protocols. After cell lysate clarification, the supernatant at protein concentration of about 0.4 mg/ml, containing expressed HIL1-R1-hIgG1-Fc/ hIL-IRAcP-hIgGI-Fc polypeptides, was applied to a Protein A affinity column. The affinity purification step was carried out according to the procedure outlined in Table 2. The Protein A eluate containing 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.

[0053] After material incubation for about 45 minutes at pH 3.6 at room temperature its pH is adjusted to about 7.9-8.1 with 2 M Tris-HCl pH 9.5 (~ 3.5% v/v of the diluted Pro A eluate). The low pH treated Protein A eluate is pH-adjusted to pH of about 8.0 using 1 M Tris-HCl, pH 9.0. The conductivity is adjusted to about 5.5 mS/cm with deionized water (dH2O) if needed.

[0054] In order to reduce the contents of DNA, HCP, endotoxin and potential viral contaminants, the pH adjusted Protein A column eluate was further purified by anion-exchange chromatography (AIEX) utilizing Q Sepharose resin. The AIEX step is operated according to a step-elution procedure outlined in Table 3. Pooled elution peak fractions are concentrated by microfiltration to a concentration of about 20 mg/ml, followed by addition of a 20% Sucrose stock solution to a final Sucrose concentration of about 1% (w/v) and freezing at -80°C.

[0055] 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 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.

[0056] The SEC-HPLC operational procedure is outlined in Table 4. Loading sample is diluted with mobile phase to reach protein concentration of about 5 mg/ml. The biologically relevant form of hIL1-R1-hIgG1-Fc/ hIL-IRAcP-hIgGI-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

Step	Buffer	Vol CV	Flow cm/h
Wash 1	10 mM NaPh, pH 6.0	3	150
Wash 2	25 mM NaPh, 0.5 MNaCl, 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 AIEX

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 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

[0057] The activity of thus purified sample, which was expressed and purified essentially as described in this example, was tested in a standard ELISA assay using commercially available human IL-1β (PrimGene, Shanghai, China; Cat#: 101-01B). 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.

[0058] **Example 4:** Characterization of inhibition of IL-1β-induced IL-6 production with IL1R-FcV-RAcP-FcII heterodimer in human cells.

[0059] The present Example demonstrates potent functional (inhibitory) properties of IL1R-FcV-RAcP-FcII heterodimer in targeting human IL-1β. As a functional comparator, previously characterized mouse monoclonal antibodies against IL-1β were used. Human lung fibroblasts MRC5 produce IL-6 in response to treatment with IL-1β. Quantification of IL-1β-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-1β antibody provides a quantitative measure of inhibitory properties of the IL1R-FcV-RAcP-FcII heterodimer preparation.

[0060] Polypeptides of II,1R-FcV-RAcP-FcП 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 NaH_2PO_4/Na_2HPO_4, 25 mM Arginine hydrochloride, 1% Sucrose; pH=6.3.

[0061] 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-1β, Human recombinant, E. coli-derived, Ala1 17-Ser269, Accession # NP_000567, R&D systems, Cat # 201-LB, Lot # ADM1411062; Monoclonal antibodies against human IL-1β, clone #8516, R&D systems, Cat # MAB201, Lot # AWE1011081;

Human IL-6 Quantakine Immunoassay, R&D systems, Cat # D6050, Lot # 300070.

[0062] The following procedures were utilized in the assay:

Cell Maintenance:

[0063]

1. 1. Propagate MRC5 cells in the DMEM containing 10% FBS on T75 flasks according to the manufacturer's recommendations. Record the passage number;
2. 2. Trypsinize cells, re-suspend in DMEM containing 10% FBS;
3. 3. Test cell viability using Guava ViaCount Reagent. Standard viability should be >85%, if less, do not use this batch of cells;
4. 4. Prepare dilutions of the cells for plating at desired density of 2 × 103 cells/well per well of 48-well plate or 5 × 103 per ml;
5. 5. Allow cells to attach for 16-24 hours at 37°C, 5% CO_2, then remove medium;
6. 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. 7. Add to the wells IL-1β, Test Substances pre-mixed with IL-1β and appropriate controls;
8. 8. Incubate the cells after treatment for 24 hours then harvest the supernatants;
9. 9. Centrifuge the supernatants at 300 × g for 10 min, collect cleared supernatants and use them for ELISA either directly or with 1/3 dilution if appropriate;

ELISA Protocol:

[0064] 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.

[0065] 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:

[0066]

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 µL of Assay Diluent RD1W to each well.

4. Add 100 µL 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 µL) 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 µL 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 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature. Protect from light.

9. Add 50 µL 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.

[0067] Following are the experimental data obtained:

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

[0068]

1. 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. 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. 3. IL-1β was used at a final concentration of 50 pg/ml throughout the experiment;
4. 4. Anti-human IL-1β antibodies were used at final concentrations of 0.192, 0.96, 4.8, 24, 120 and 600 ng/ml;
5. 5. Both IL-1β and anti-human IL-1β antibodies were prepared as 10x concentrated solutions;
6. 6. Prior to adding the test substances to the cells, 55 µl of 10x IL-1β was mixed with 55 µl of 10x corresponding anti-human IL-1β antibody solution (or appropriate control) and incubated at room temperature for 30 min;
7. 7. After the incubation, 100 µl of each mixture was added to the wells containing 0.4 ml of growth medium;
8. 8. The cells were incubated in the presence of the test substances for 24 hours and supernatants were collected, centrifuged at 300 × g for 10 min and used for IL-6 ELISA assay;
9. 9. To test for recovery of IL-6 in the presence of anti-IL-1β 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.

[0069] 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

[0070] The foregoing experiment revealed that: (1) the control anti-IL-1β antibody is a very potent inhibitor of IL-1β 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-FeII heterodimer preparation:

[0071]

1. 1. MRC5 cells (Passage 6) were plated into 48-well plate (Costar, Cat # 3548) at 2 × 103 cells per well, 0.4 ml per well and incubated for 24 hours;
2. 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. 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. 4. IL-1β was used at a final concentration of 50 pg/ml throughout the experiment;
5. 5. II,1R-FcV-RAcP-FcΠ 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. 6. Both IL-1β and IL1R-FcV-RAcP-FcII heterodimer was prepared as 10x concentrated solutions;
7. 7. Prior to adding the test substances to the cells, 120 µl of 10x IL-1β/IL-1F2 was mixed with 120 µl of 10x corresponding II,1R-FcV-RAcP-FcΠ heterodimer solution (or appropriate control) and incubated at room temperature for 30 min;
8. 8. After the incubation, 100 µl of each mixture was added to the wells containing 0.4 ml of growth medium;
9. 9. The cells were incubated in the presence of the test substances for 24 hours and supernatants were collected, centrifuged at 300 × g for 10 min and used for IL-6 ELISA assay.

[0072] The calibration curve obtained in the II,1R-FcV-RAcP-FcΠ 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	

Sample ID	Average, pg/ml	StDev pg/ml	Calibration Range
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			

[0073] 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.

[0074] **Example 5:** Pharmacokinetics (PK) of IL1R-FcV-RAcP-FcII heterodimer after subcutaneous administration in mice.

[0075] Polypeptides of II,1R-FcV-RAcP-FcП 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.

[0076] 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.

[0077] 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.

[0078] 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.

[0079] 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.

[0080] 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

Group	Treatment	Bleeding Schedule (time post-administration)	Mean Human-Fc Protein Concentration [µg/mL]	SEM
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 Bleed via orbital sinus # Bleed via terminal cardiac puncture				

[0081] **Example 6:** Evaluation of the toxicity of Il,1R-FcV-RAcP-FcΠ heterodimer following 28-days repeated dosing in C57BL6 mice.

[0082] Following is a repeat dose murine toxicity evaluation study results summary. Treatment of C57BL6 mice with polypeptides of Il,1R-FcV-RAcP-FcΠ 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 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.

[0083] 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 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 demonstrate this.

[0084] 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.

[0085] The full scope of the invention should be determined by reference to the claims and the specification.

REFERENCES CITED IN THE DESCRIPTION

Cited references

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- US6472179B [0006]
- US20030143697A [0006]
- US7361350B [0006]
- US20050197293A [0006]

Non-patent literature cited in the description

- **WEBER A. et al.**Sci Signal., 2010, vol. 3, 105 [0002]
- Cytokine traps: multi-component, high-affinity blockers of cytokine action**ECONOMIDES A.N. et al.**NATURE MEDICINENATURE PUB. CO20030101vol. 9, 47-52 [0008]

PATENTKRAV

1. Heterodimerisk proteinsammensætning, der er i stand til at binde sig til human IL-1β, hvilken proteinsammensætning omfatter:

et første polypeptid, der omfatter

en første aminosyresekvens, der omfatter aminosyrerne 18 til og med 333 af human IL1 -R 1, og

en anden aminosyresekvens, der omfatter en første mutant af en Fc-del af human immunoglobulin gamma-1 Fc;

et andet polypeptid, der omfatter

endnu en første aminosyresekvens, der omfatter aminosyrerne 21 til og med 358 af human ILl-RAcP, og

endnu en anden aminosyresekvens, der omfatter en anden mutant af Fc-delen af human immunoglobulin gamma-1 Fc; og

hvor de første og andre mutanter er udvalgt med henblik på at favorisere heterodimerisk sammenkobling mellem de første og andre mutanter frem for en hvilken som helst homodimerisk sammenkobling,

hvor det første polypeptid omfatter aminosyresekvensen af SEQ ID NO. 1, og det andet polypeptid omfatter aminosyresekvensen af SEQ ID NO. 2.

2. Proteinsammensætning ifølge krav 1, hvor proteinsammensætningen udviser human IL- 1β-bindingsaktivitet i en ELISA-analyse med en EC50 på 50 ng/ml.

3. Terapeutisk sammensætning, hvilken sammensætning omfatter en heterodimerisk proteinsammensætning, der er i stand til at binde sig til human IL-1β, hvilken heterodimerisk proteinsammensætning omfatter:

et første polypeptid, der omfatter

en første aminosyresekvens, der omfatter aminosyrerne 18 til og med 333 af human IL1 -R 1, og

en anden aminosyresekvens, der omfatter en første mutant af en Fc-del af human immunoglobulin gamma-1 Fc;

et andet polypeptid, der omfatter

endnu en første aminosyresekvens, der omfatter aminosyrerne 21 til og med 358 af human ILl-RAcP, og

endnu en anden aminosyresekvens, der omfatter en anden mutant af Fc-delen af human immunoglobulin gamma-1 Fc; og

hvor de første og andre mutanter er udvalgt med henblik på at favorisere heterodimerisk sammenkobling mellem de første og andre mutanter frem for en hvilken som helst homodimerisk sammenkobling,

hvor det første polypeptid omfatter aminosyresekvensen af SEQ ID NO. 1, og det andet polypeptid omfatter aminosyresekvensen af SEQ ID NO. 2.

4. Terapeutisk sammensætning ifølge krav 3, hvor halveringstiden af den heterodimeriske proteinsammensætning i systemisk cirkulation hos mus efter en subkutan administration i en dosis på 5 mg/kg er mindst 88 timer, analyseret ved human Fc-ELISA.

5. Isoleret nukleinsyre, der koder for et polypeptid, der omfatter en aminosyresekvens ifølge en hvilken som helst af SEQ ID. 1, SEQ ID NO. 2, SEQ ID NO. 3 og SEQ ID NO. 5.

6. Nukleinsyre ifølge krav 5, hvor codon-anvendelsen er optimeret til høj ekspression af polypeptidet i en mammal celle.

7. Nukleinsyre ifølge krav 5, hvor nukleinsyresekvensen koder for et polypeptid, der omfatter aminosyresekvensen af SEQ ID NO. 3 og omfatter sekvensen af SEQ ID NO. 4, eller hvor nukleinsyresekvensen koder for et polypeptid, der omfatter aminosyresekvensen af SEQ ID NO. 5 og omfatter sekvensen af SEQ ID NO. 6.

8. Nukleinsyre ifølge krav 7, hvor nukleinsyren omfatter en ekspressionsvektor.

9. Isoleret nukleinsyre af SEQ ID NO. 7.

10. Heterologt ekspressionssystem, hvilket ekspressionssystem indeholder en ekspressionsvektor, der omfatter en nukleinsyresekvens, der koder for et første polypeptid, der omfatter aminosyresekvensen af SEQ ID NO. 3 og endnu en nukleinsyresekvens, der koder for et andet polypeptid, der omfatter aminosyresekvensen af SEQ ID NO. 5.

11. Ekspressionssystem ifølge krav 10, hvor ekspressionsvektoren er indeholdt i en mammal celle, fortrinsvis hvor den mammale celle er en CHO-celle.

12. Ekspressionssystem ifølge krav 11, hvor ekspressionssystemet er i stand til at udtrykke et heterodimerisk protein, der omfatter et første polypeptid, der omfatter aminosyresekvensen af SEQ ID NO. 1, og et andet polypeptid, der omfatter aminosyresekvensen af SEQ ID NO. 2, og hvor ekspressionsniveauet af det heterodimeriske protein er mindst 300 mg pr. liter cellekultur.

13. Heterodimerisk protein, der omfatter et første polypeptid, der omfatter aminosyresekvensen af SEQ ID NO. 1, og et andet polypeptid, der omfatter aminosyresekvensen af SEQ ID NO. 2, til anvendelse i behandlingen eller forebyggelsen af en sygdom, der er forbundet med modulering af aktivitet af human IL-1β, hvor sygdommen er en artritis, eller hvor sygdommen er en urinsyregigt, eller hvor sygdommen er en rheumatoid artritis, eller hvor sygdommen er et cryopyrin-associeret periodisk syndrom (CAPS), eller hvor sygdommen er en sklerodermi, eller hvor sygdommen er en diabetes, eller hvor sygdommen er aterosklerose, eller hvor sygdommen er sygdommen tørre øjne, eller hvor sygdommen er en okulær allergi, eller hvor sygdommen er en uveitis.

14. Heterodimerisk proteinsammensætning, der er i stand til at binde sig til human IL-1β, hvilken proteinsammensætning er i stand til at hæmme produktion af human IL-6 i humane lungefibroblaster som respons på behandling af fibroblasterne med human IL-1β, hvilken hæmning har en IC50-værdi på 2,3 pM, hvor den heterodimeriske proteinsammensætning omfatter et første polypeptid,

der omfatter aminosyresekvensen af SEQ ID NO. 1, og et andet polypeptid, der omfatter aminosyresekvensen af SEQ ID NO. 2.

DRAWINGS

Drawing

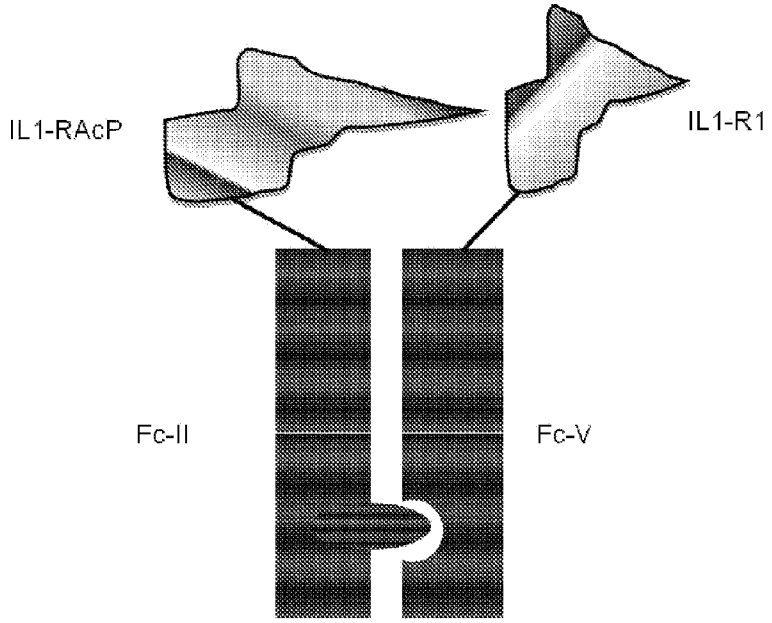


FIGURE 1

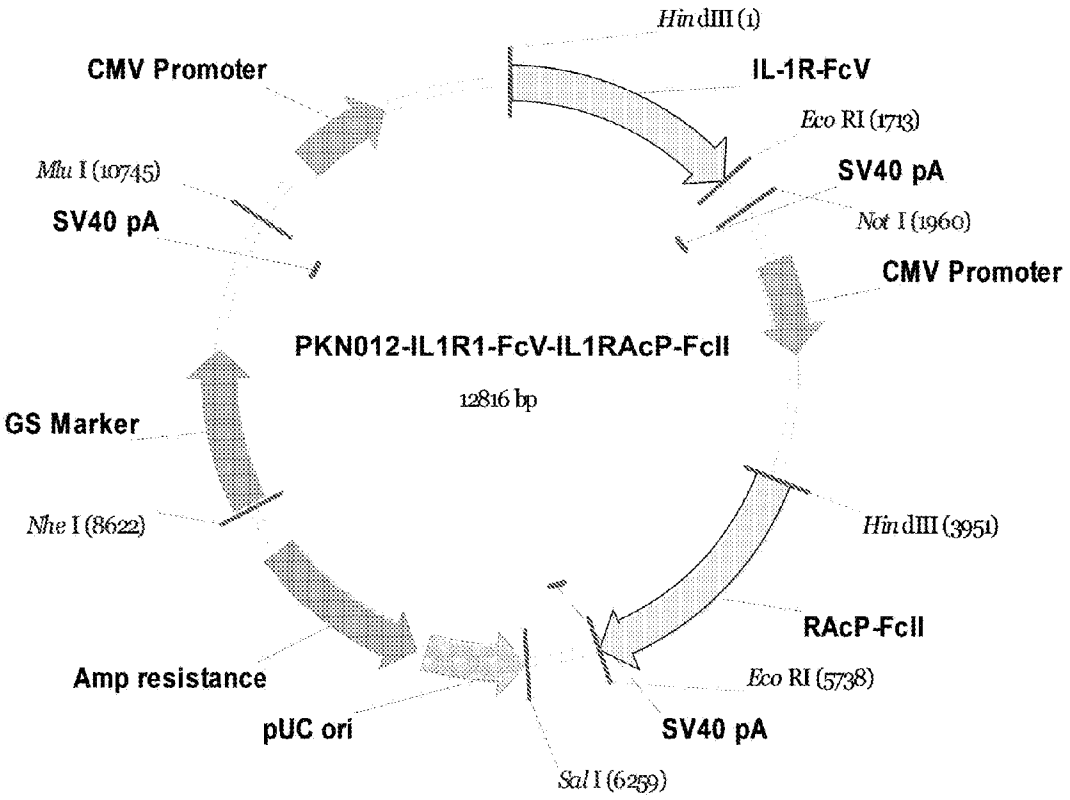

Hin dIII (1)
CMV Promoter
IL-1R-FcV
Eco RI (1713)
Mlu I (10745)
SV40 pA
SV40 pA
Not I (1960)
CMV Promoter
PKN012-IL1R1-FcV-IL1RAcP-FcII
12816 bp
GS Marker
Nhe I (8622)
Hin dIII (3951)
RAcP-FcII
Amp resistance
Eco RI (5738)
pUC ori
SV40 pA
Sal I (6259)

FIGURE 2

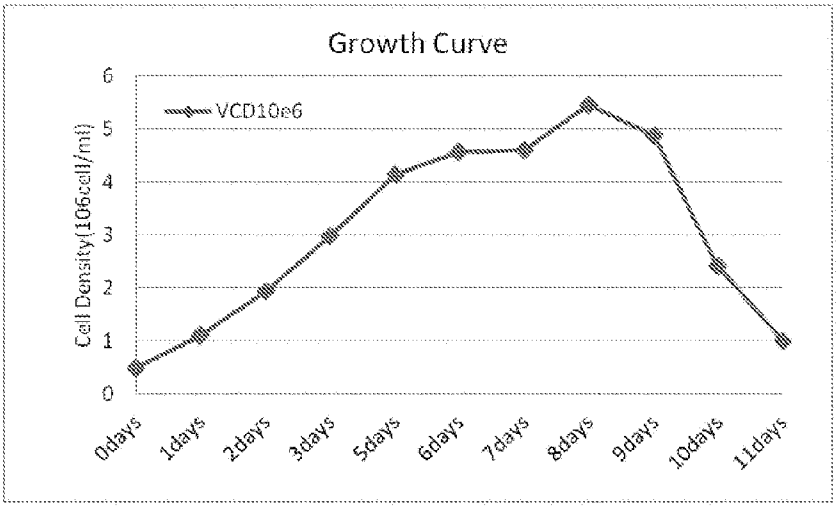
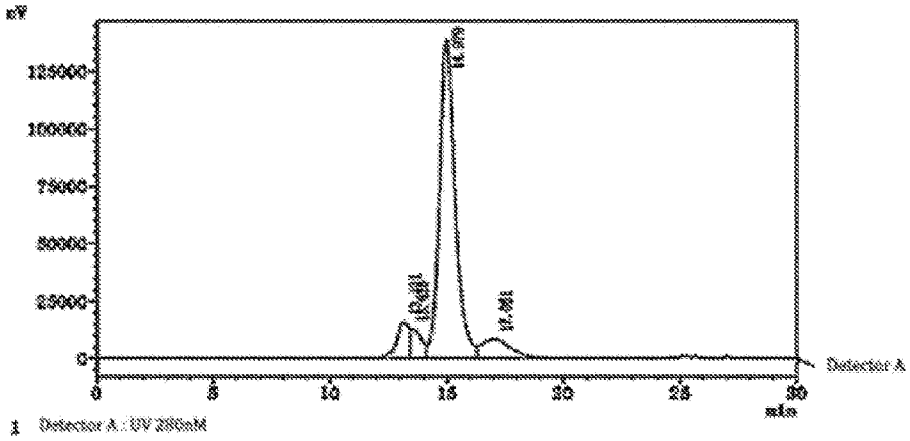

Growth Curve
VCD10e6
Cell Density(106cell/ml)
0
1
2
3
4
5
6
0days
1days
2days
3days
5days
6days
7days
8days
9days
10days
11days

FIGURE 3



Peak #	Retention Time	Peak Area	Peak Height	Peak Area %
1	13.131	582459	16051	6.992
2	13.425	428184	12833	5.140
3	14.979	6579146	138922	78.975
4	17.051	740921	8427	8.894
Total		8330710		100.000

FIGURE 4

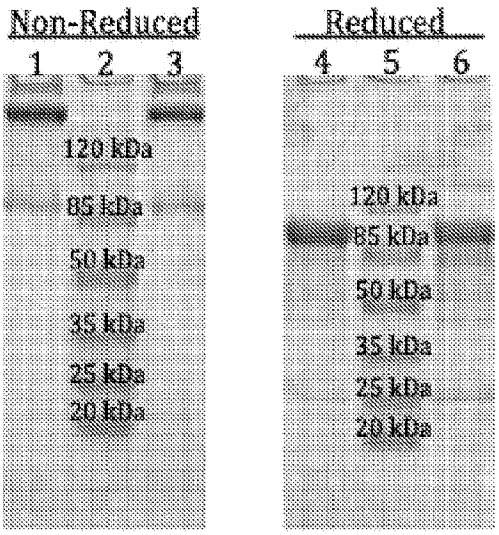
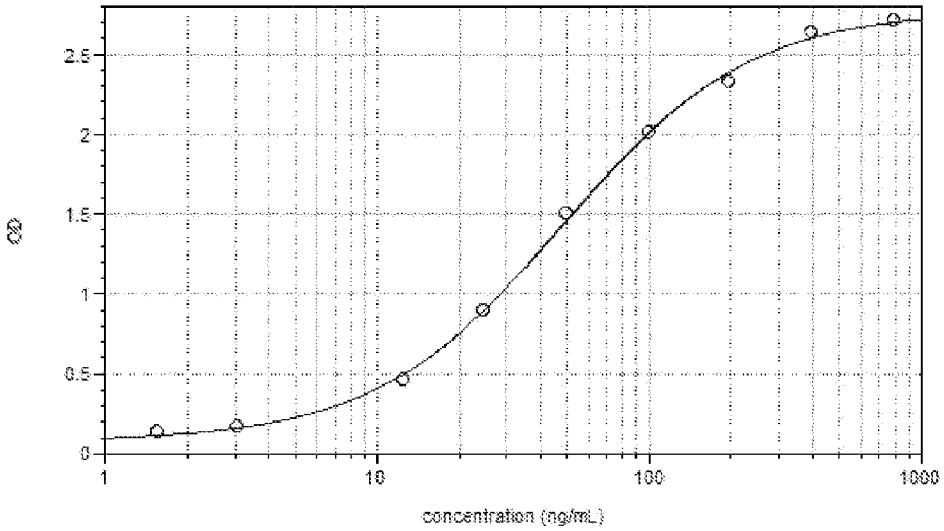

Non-Reduced
Reduced
1
2
3
4
5
6
120 kDa
85 kDa
50 kDa
35 kDa
25 kDa
20 kDa
120 kDa
85 kDa
50 kDa
35 kDa
25 kDa
20 kDa

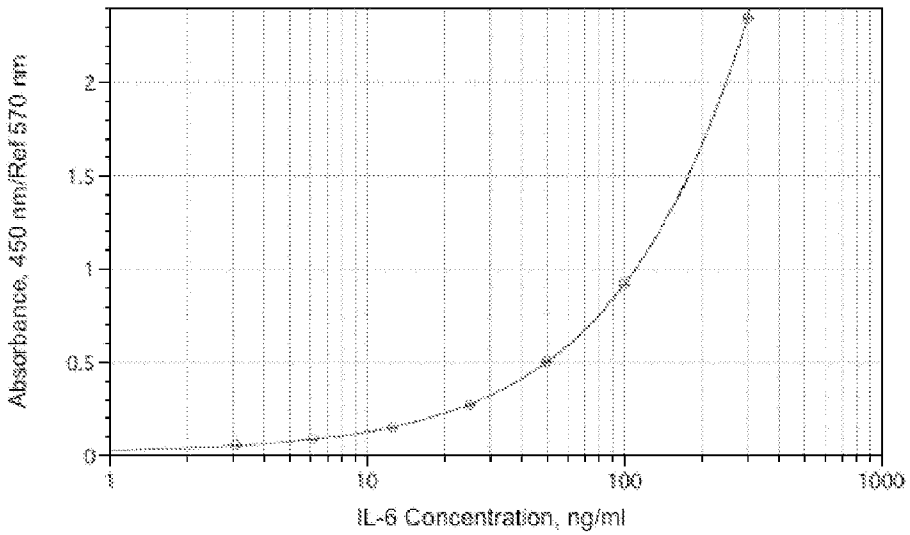
FIGURE 5



4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:	A	B	C	D	R^2
Plot#1 (STD3: conc. vs Values)	0.0751	1.26	48.3	2.78	0.999

Curve Fit Option - Fixed Weight Value

FIGURE 6



4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:	A	B	C	D	R^2
○ STD#1 (Standards: Conc vs AvgOD)	0.0128	0.921	3.33e+03	23.8	1

Curve Fit Option - Fixed Weight Value

FIGURE 7

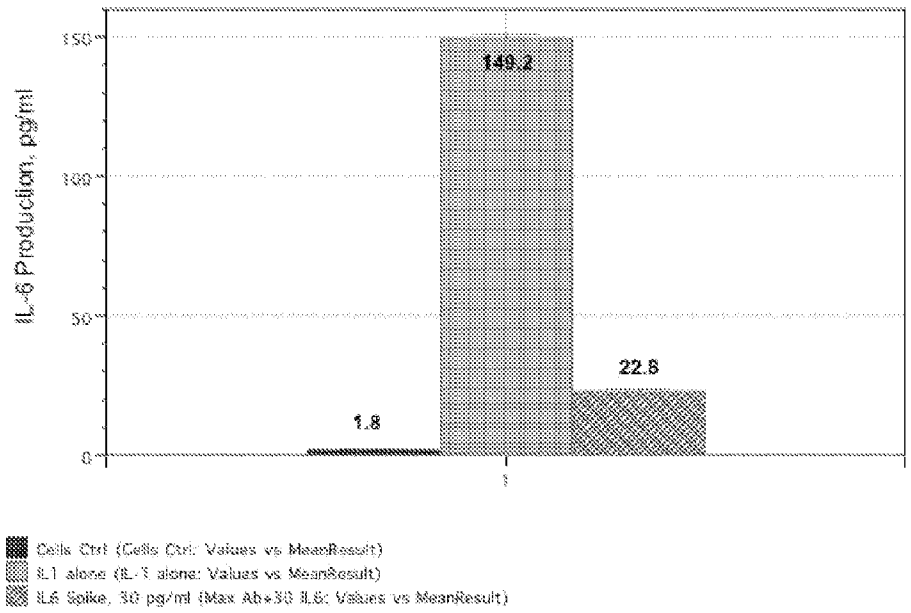
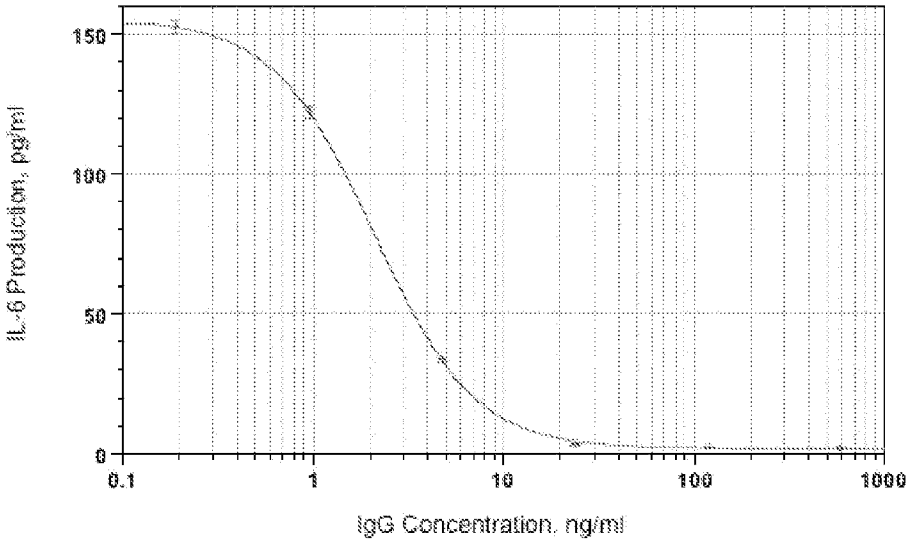

IL-6 Production, pg/ml
150
100
50
0
149.2
22.8
1.8
Cells Ctrl (Cells Ctrl: Values vs MeanResult)
IL1 alone (IL-1 alone: Values vs MeanResult)
IL6 Spike, 30 pg/ml (Max Ab+30 IL6: Values vs MeanResult)

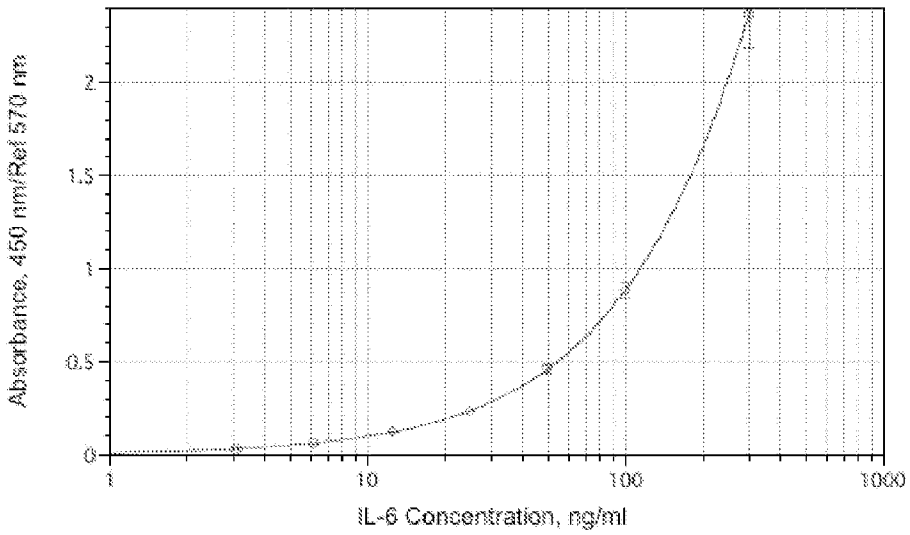
FIGURE 8



4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:	A	B	C	D	R^2
Inhibition Curve (IgG1 2*103: Dilution vs MeanResult)	155	1.65	2.1	1.73	1

Curve Fit Option - Fixed Weight Value

FIGURE 9



4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:	A	B	C	D	R^2
STD#1 (Standards: Conc vs AvgOD)	-0.000817	0.385	1.87e+03	16.7	1

Curve Fit Option - Fixed Weight Value

FIGURE 10

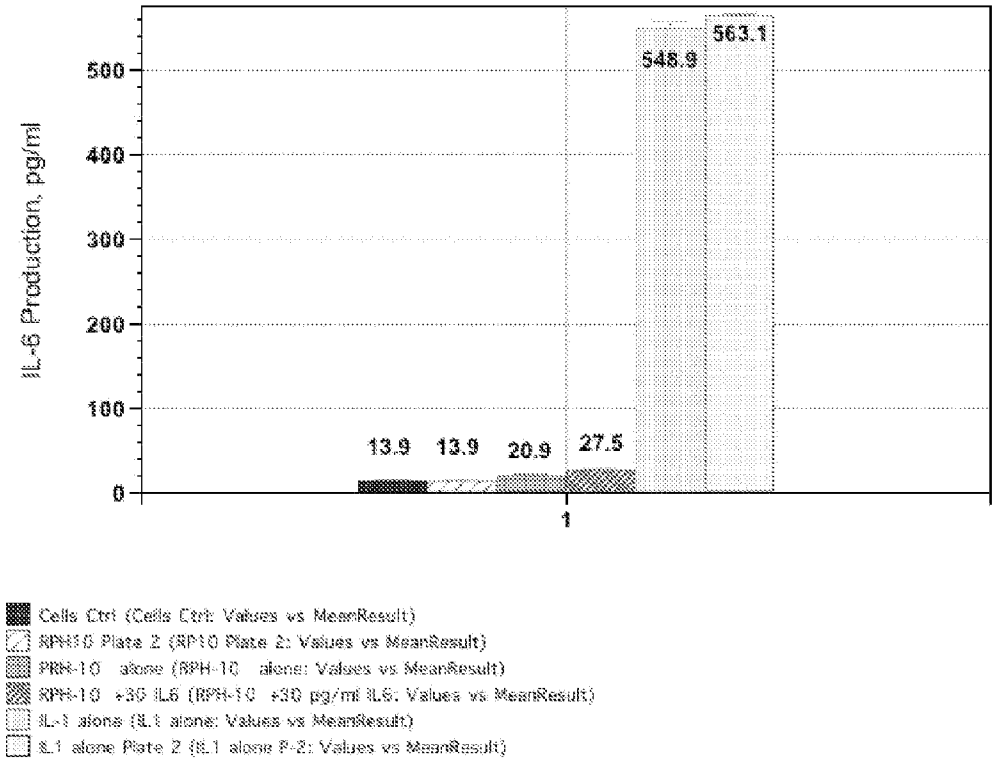

IL-6 Production, pg/ml
500
400
300
200
100
0
13.9
13.9
20.9
27.5
548.9
563.1
1
Cells Ctrl (Cells Ctrl: Values vs MeanResult)
RPH10 Plate 2 (RP10 Plate 2: Values vs MeanResult)
PRH-10 alone (RPH-10 alone: Values vs MeanResult)
RPH-10 +30 IL6 (RPH-10 +30 pg/ml IL6: Values vs MeanResult)
IL-1 alone (IL1 alone: Values vs MeanResult)
IL1 alone Plate 2 (IL1 alone P-2: Values vs MeanResult)

FIGURE 11

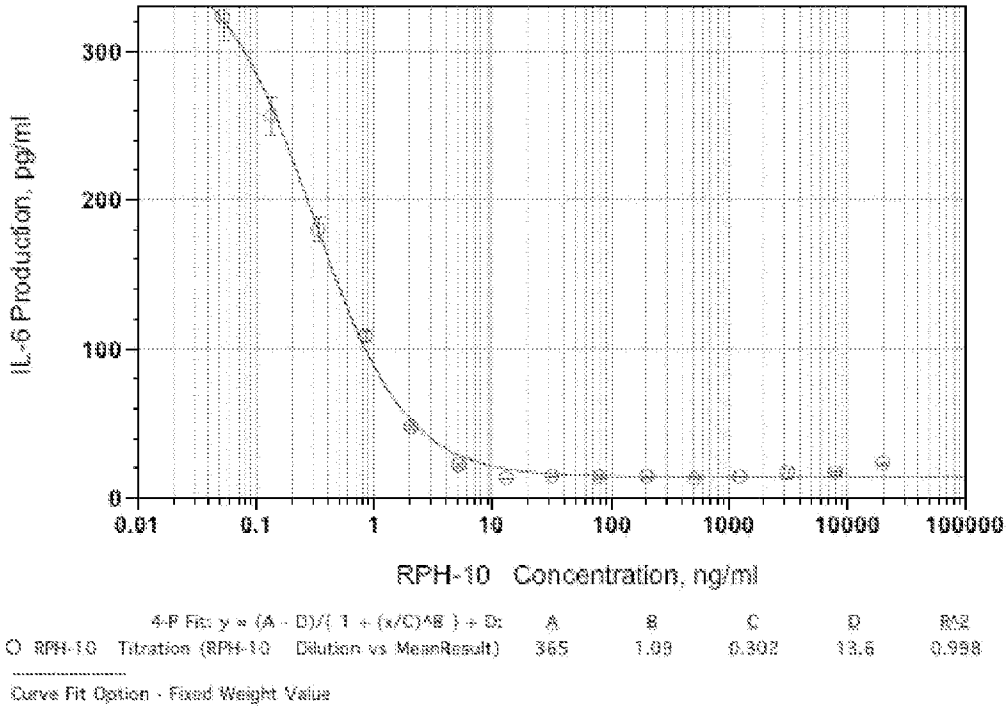

IL-6 Production, pg/ml
300
200
100
0
0.01
0.1
1
10
100
1000
10000
100000
RPH-10 Concentration, ng/ml
4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:
A
B
C
D
R^2
O RPH-10 Titration (RPH-10 Dilution vs MeanResult)
365
1.09
0.302
13.6
0.998
Curve Fit Option - Fixed Weight Value

FIGURE 12