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(54) Title: TOLL LIKE RECEPTOR 3 MODULATORS, METHODS AND USES

(57) Abstract: Toll Like Receptor 3 (TLR3) modulators, such as antibodies, polynucleotides encoding TLR3 antibodies or fragments thereof, and methods of making and using the foregoing are disclosed.

Nucleotide sequence for C1130 heavy chain variable region

ATGGAATGTAACTGGATACTTCCTTTTATTCTGTCGGTAATTTCAGGGGTCTACTCAGAGGTTC
AGCTCCAGCAGTCTGGGACTGTGCTGGCAAGGCCTGGGGCTTCCGTGAAGATGTCCTGCAAGGC
TTCTGGCTACAGGTTTTCCAGCTACGGGATGCACTGGGTAAAACAGAGGCCTGGACAGGGTCTA
GAATGGATTGGTGCTATTTATCCTGGAAACAATGATATTACTTATACTCAGAAGTTCAAGGGCA
AGGCCAAACTGACTGCAGTCACATCCGCCAGCACTACCTACATGGAACTCAGCAGCCTGACAAA
TGAAGACTCTGCGGTCTATTACTGTTCAACTCTAATGTTTGCTTATTGGGGCCAAGGGACTCTG
GTCACTGTCACTGCA (SEQ ID NO: 5)

Amino Acid sequence for C1130 Heavy Chain variable region

MECNWILPFILSVISGVYSEVQLQQSGTVLARPGASVKMSCKASGYRFSSYGMHWVKQRPGQGL
EWIGAIYPGNNDITYTQKFKGKAKLTAVTSASTTYMELSSLTNEDSAVYYCSTLMFAYWGQGTL
VTVTA (SEQ ID NO: 6)

Signal Sequence
MECNWILPFILSVISGVYS (SEQ ID NO: 7)

FR1
EVQLQQSGTVLARPGASVKMSCKAS (SEQ ID NO: 8)

CDR1
GYRFSSYGMH (SEQ ID NO: 9)

FR2
WVKQRPGQGLEWIG (SEQ ID NO: 10)

CDR2
AIYPGNNDITYTQKFKG (SEQ ID NO: 11)

FR3
KAKLTAVTSASTTYMELSSLTNEDSAVYYCST (SEQ ID NO: 12)

CDR3
LMFAY (SEQ ID NO: 13)

Mouse J HC
WGQGTLVTVTA (SEQ ID NO: 14)

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TOLL LIKE RECEPTOR 3 MODULATORS, METHODS AND USES

Field of the Invention

The present invention relates to Toll Like Receptor 3 (TLR3) modulators such as antibodies.

Background of the Invention

Recognition of foreign antigens by mammalian cells can be mediated by a set of innate immune receptors called Toll-like receptors (TLRs). TLRs recognize conserved patterns derived from microbial pathogens identified as pathogen-associated molecular patterns (PAMPs) (Barton *et al.*, *Science* 300:1524-1525, 2003). Interaction of a TLR with a PAMP results in a signaling cascade involving NF-κB activation and transcription of cytokine gene expression. Ten human toll-like receptors and five TLR adaptor proteins have been identified.

TLRs are able to expand their repertoire of ligands by forming homo- or heterodimers as well as binding different adaptor proteins. For example, TLR3 binds dsRNA, an intermediate in viral replication. TLR3 also interacts with PolyI:C, a synthetic dsRNA analog, and mRNA from necrotic cells. Activation of TLR3 leads to the secretion of Type I interferons, which are important in the control of viral infection. A full-length human TLR3 amino acid sequence and encoding polynucleotide sequence are shown in SEQ ID NOs: 1 and 2, respectively. TLRs TLR7, TLR8, and TLR9 also have nucleic acid ligands; activation of these TLRs can also lead to interferon secretion.

Type I interferons trigger signaling cascades to activate a set of immediate early-response genes (IFN-stimulated genes or ISGs) and have proven useful in the clinic. The resulting antiviral activities include mRNA translation inhibition, RNA editing, and RNA degradation (Samuel *et al.*, *Clin Microbiol Rev*

14:778-809, 2001). Currently, a combination therapy of pegylated interferon and the broad-spectrum antiviral compound ribavirin is being used to treat hepatitis C infection (Manns *et al.*, *Lancet* 358:958-965, 2001).

The critical anti-viral role of Type I IFNs is further demonstrated by the evolution of viral resistance mechanisms to inhibit the production of Type I IFNs by infected host cells. For example, the NS1 protein of influenza antagonizes IRF-3 activation and IFNβ production (Donelan *et al.*, *J Virol* 78: 11574-11582, 2004) and the A52R poxvirus protein associates with IRAK2 and TRAF6 to block signaling downstream of TLR3 (Harte *et al.*, *J Exp Med*, 197:343-351, 2003). Thus, therapies based on triggering TLR activation or enhancing TLR-mediated signaling pathways increase endogenous IFNα/β production and assist the host in the control of acute viral infections.

The use of TLR agonists to modulate the outcome of an immune response is currently being investigated for therapeutic use (O'Neill, *Curr Opin Pharm* 3:396-403, 2003; Schetter *et al.*, *Curr Opin Drug Discov Devel* 7:204-210, 2004). For example, CpG oligodinucleotides (ODN), a TLR9 ligand, are capable of stimulating the production of type I IFN and a T_H1 response (Krieg, *Annu Rev Immunol* 20:709-60, 2002), a finding that suggests the possible use of CpG ODN not only as a vaccine adjuvant but also for the treatment and or prevention of diseases that necessitate a potent T_H1 response. Another example is the synthetic TLR7 agonist imiquimod, an approved agent for the treatment of genital warts; its protective effect is thought to be mediated through the stimulation of inflammatory cytokines such as IFNα, TNFα and IL-1β (Saunder, *J Amer Acad Derm* 43: S6-S11, 2000). Overall, these findings show that TLR agonists are a novel class of immunomodulatory agents with the potential of having a significant therapeutic benefit.

Thus, a need exists for the identification of novel immunomodulatory agents that potentiate the effect of TLR agonists. Such novel TLR-based therapies are expected to have an advantage of providing a sustained immune response with less frequent dosing regimens.

Brief Description of the Drawings

Fig. 1 shows C1130 anti-hTLR3 mAb heavy chain variable region sequences.

Fig. 2 shows C1130 anti-hTLR3 mAb light chain variable region sequences.

Fig. 3 shows C1130 induced IL-8, MCP-1, MIP-1α, RANTES, and TNFα secretion by human peripheral blood mononuclear cells (PBMCs) at 24h.

Fig. 4 shows C1130 enhanced CpG-induced IFNα production at 24h.

Fig. 5 shows C1130 decreased R848-induced IL-10 production at 24h.

Fig. 6 shows C1130 recognition of cell-surface TLR3 on stably transfected HEK293 cells.

Fig. 7 shows C1130 recognition of cell-surface TLR3 on stably transfected A549-TLR3.2 cells.

Fig. 8 shows C1130 recognition of Cynomolgus macaque PBMCs.

Summary of the Invention

One aspect of the invention is an isolated antibody reactive with human Toll Like Receptor 3 (hTLR3) or its homologs that induces cellular production of a cytokine selected from the group consisting of IL-8, MCP-1, MIP1-α, RANTES and TNF-α.

Another aspect of the invention is an isolated antibody reactive with hTLR3 or its homologs that modifies an immune response to other Toll Like Receptor ligands.

Another aspect of the invention is an isolated antibody reactive with hTLR3 having the antigen binding ability of a monoclonal antibody comprising the amino acid sequences of the heavy chain complementarity determining regions (CDRs) as shown in SEQ ID NOs: 9, 11 and 13 and the amino acid sequences of the light chain CDRs as shown in SEQ ID NOs: 19, 21 and 23.

Another aspect of the invention is an isolated antibody reactive with hTLR3 comprising the amino acid sequences of the heavy chain complementarity determining regions (CDRs) as shown in SEQ ID NOs: 9, 11 and 13 and the amino acid sequences of the light chain CDRs as shown in SEQ ID NOs: 19, 21 and 23.

Another aspect of the invention is an isolated antibody reactive with hTLR3 comprising a heavy chain comprising the amino acid sequence shown in SEQ ID NO: 6 and a light chain comprising the amino acid sequence shown in SEQ ID NO: 16.

Another aspect of the invention is an isolated polynucleotide encoding an antibody heavy chain comprising the CDR amino acid sequences shown in SEQ ID NOs: 9, 11 and 13.

Another aspect of the invention is an isolated polynucleotide encoding an antibody light chain comprising the CDR amino acid sequences shown in SEQ ID NOs: 19, 21 and 23.

Another aspect of the invention is an isolated polynucleotide encoding an antibody heavy chain comprising the amino acid sequence shown in SEQ ID NO: 6.

Another aspect of the invention is an isolated polynucleotide encoding an antibody light chain comprising the amino acid sequence shown in SEQ ID NO: 16.

Other aspects of the invention include methods of treating or preventing viral infection comprising administering to a patient a therapeutically effective amount of an antibody of the invention in combination with an immune stimulant.

Another aspect of the invention is a method of treating cancer comprising administering to a patient a therapeutically

effective amount of an antibody of the invention in combination with an immune stimulant.

Another aspect of the invention is a method of treating inflammatory bowel disease comprising administering to a patient a therapeutically effective amount of an antibody of the invention in combination with an immune stimulant.

Other aspects of the invention include methods of treating or preventing a viral infection-associated symptom comprising administering to a patient a therapeutically effective amount of an antibody of the invention in combination with a Toll-Like Receptor 7 (TLR7) agonist.

Other aspects of the invention include methods of treating or preventing a pulmonary disease and pathogen-mediated exacerbation comprising administering to a patient a therapeutically effective amount of an antibody of the invention in combination with a TLR9 or TLR7 agonist.

Other aspects of the invention include methods of treating or preventing graft-versus-host disease (GVHD) comprising administering to a patient a therapeutically effective amount of an antibody of the invention in combination with a TLR9 or TLR7 agonist.

Other aspects of the invention include methods of treating or preventing autoimmune disease comprising administering to a patient a therapeutically effective amount of an antibody of the invention in combination with an immune treatment.

Detailed Description of the Invention

All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as though fully set forth.

The term "antibodies" as used herein is meant in a broad sense and includes immunoglobulin or antibody molecules including polyclonal antibodies, monoclonal antibodies including

murine, human, humanized and chimeric monoclonal antibodies and antibody fragments.

In general, antibodies are proteins or polypeptides that exhibit binding specificity to a specific antigen. Intact antibodies are heterotetrameric glycoproteins, composed of two identical light chains and two identical heavy chains. Typically, each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies between the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain and the light chain variable domain is aligned with the variable domain of the heavy chain. Antibody light chains of any vertebrate species can be assigned to one of two clearly distinct types, namely kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains. Immunoglobulins can be assigned to five major classes, namely IgA, IgD, IgE, IgG and IgM, depending on the heavy chain constant domain amino acid sequence. IgA and IgG are further sub-classified as the isotypes IgA_1, IgA_2, IgG_1, IgG_2, IgG_3 and IgG_4.

The term "antibody fragments" means a portion of an intact antibody, generally the antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', $F(ab')_2$ and Fv fragments, diabodies, single chain antibody molecules and multispecific antibodies formed from at least two intact antibodies.

The term "antigen" as used herein means any molecule that has the ability to generate antibodies either directly or

indirectly. Included within the definition of "antigen" is a protein-encoding nucleic acid.

"CDRs" are defined as the complementarity determining region amino acid sequences of an antibody which are the hypervariable regions of immunoglobulin heavy and light chains. See, *e.g.*, Kabat *et al.*, Sequences of Proteins of Immunological Interest, 4th ed., U.S. Department of Health and Human Services, National Institutes of Health (1987). There are three heavy chain and three light chain CDRs or CDR regions in the variable portion of an immunoglobulin. Thus, "CDRs" as used herein refers to all three heavy chain CDRs, or all three light chain CDRs or both all heavy and all light chain CDRs, if appropriate.

CDRs provide the majority of contact residues for the binding of the antibody to the antigen or epitope. CDRs of interest in this invention are derived from donor antibody variable heavy and light chain sequences, and include analogs of the naturally occurring CDRs, which analogs also share or retain the same antigen binding specificity and/or neutralizing ability as the donor antibody from which they were derived.

The term "homolog" means protein sequences having between 40% and 100% sequence identity to a reference sequence. Homologs of hTLR3 include polypeptides from other species that have between 40% and 100% sequence identity to a known hTLR3 sequence. Percent identity between two peptide chains can be determined by pair wise alignment using the default settings of the AlignX module of Vector NTI v.9.0.0 (Invitrogen Corp., Carslbad, CA).

The term "in combination with" as used herein means that the described agents can be administered to an animal together in a mixture, concurrently as single agents or sequentially as single agents in any order.

The term "mimetibody" as used herein means a protein having the generic formula (I):

$(V1\text{-}Pep\text{-}Lk\text{-}V2\text{-}Hg\text{-}C_H2\text{-}C_H3)(t)$

(I)

where V1 is a portion of an N-terminus of an immunoglobulin variable region, Pep is a polypeptide that binds to cell surface TLR3, Lk is a polypeptide or chemical linkage, V2 is a portion of a C-terminus of an immunoglobulin variable region, Hg is a portion of an immunoglobulin hinge region, C_H2 is an immunoglobulin heavy chain C_H2 constant region and C_H3 is an immunoglobulin heavy chain C_H3 constant region and t is independently an integer of 1 to 10. A mimetibody can mimic properties and functions of different types of immunoglobulin molecules such as IgG1, IgG2, IgG3, IgG4, IgA, IgM, IgD and IgE dependent on the heavy chain constant domain amino acid sequence present in the construct. In some mimetibody embodiments, V1 may be absent. A mimetibody of the present invention modulates TLR biological activity through binding to TLR-expressing cells.

The term "monoclonal antibody" (mAb) as used herein means an antibody (or antibody fragment) obtained from a population of substantially homogeneous antibodies. Monoclonal antibodies are highly specific, typically being directed against a single antigenic determinant. The modifier "monoclonal" indicates the substantially homogeneous character of the antibody and does not require production of the antibody by any particular method. For example, murine mAbs can be made by the hybridoma method of Kohler *et al., Nature 256*:495-497 (1975). Chimeric mAbs containing a light chain and heavy chain variable region derived from a donor antibody (typically murine) in association with light and heavy chain constant regions derived from an acceptor antibody (typically another mammalian species such as human) can be prepared by the method disclosed in U.S. Pat. No. 4,816,567. Humanized mAbs having CDRs derived from a non-human donor immunoglobulin (typically murine) and the remaining immunoglobulin-derived parts of the molecule being derived from

one or more human immunoglobulins, optionally having altered framework support residues to preserve binding affinity, can be obtained by the techniques disclosed in Queen *et al.*, *Proc. Natl. Acad. Sci. (USA)*, *86*:10029-10032 (1989) and Hodgson *et al.*, *Bio/Technology*, *9*:421 (1991).

Exemplary human framework sequences useful for humanization are disclosed at, *e.g.*, www. ncbi.nlm.nih.gov/entrez/query.fcgi; www. ncbi.nih.gov/igblast; www. atcc.org/phage/hdb.html; www. mrc-cpe.cam.ac.uk/ALIGNMENTS.php; www. kabatdatabase.com/top.html; ftp.ncbi.nih.gov/repository/kabat; www. sciquest.com; www. abcam.com; www. antibodyresource.com/onlinecomp.html; www. public.iastate.edu/~pedro/research_tools.html; www. whfreeman.com/immunology/CH05/kuby05.htm; www. hhmi.org/grants/lectures/1996/vlab; www. path.cam.ac.uk/~mrc7/mikeimages.html; mcb.harvard.edu/BioLinks/Immunology.html; www. immunologylink.com; pathbox.wustl.edu/~hcenter/index.html; www. appliedbiosystems.com; www.nal.usda.gov/awic/pubs/antibody; www. m.ehime-u.ac.jp/~yasuhito/Elisa.html; www. biodesign.com; www. cancerresearchuk.org; www. biotech.ufl.edu; www. isac-net.org; baserv.uci.kun.nl/~jraats/links1.html; www. recab.uni-hd.de/immuno.bme.nwu.edu; www. mrc-cpe.cam.ac.uk; www. ibt.unam.mx/vir/V_mice.html; http://www. bioinf.org.uk/abs; antibody.bath.ac.uk; www. unizh.ch; www. cryst.bbk.ac.uk/~ubcg07s; www. nimr.mrc.ac.uk/CC/ccaewg/ccaewg.html; www. path.cam.ac.uk/~mrc7/humanisation/TAHHP.html; www. ibt.unam.mx/vir/structure/stat_aim.html; www. biosci.missouri.edu/smithgp/index.html; www. jerini.de; imgt.cines.fr; and Kabat *et al.*, Sequences of Proteins of

Immunological Interest, U.S. Dept. Health (1987), each entirely incorporated herein by reference.

Fully human mAbs lacking any non-human sequences can be prepared from human immunoglobulin transgenic mice by techniques referenced in, *e.g.*, Lonberg *et al.*, *Nature 368*:856-859 (1994); Fishwild *et al.*, *Nature Biotechnology 14*:845-851 (1996) and Mendez *et al.*, *Nature Genetics 15*:146-156 (1997). Human mAbs can also be prepared and optimized from phage display libraries by techniques referenced in, *e.g.*, Knappik *et al.*, *J. Mol. Biol. 296*:57-86 (2000) and Krebs *et al.*, *J. Immunol. Meth. 254*:67-84 (2001).

The present invention relates to TLR3 receptor binding agents capable of modulating TLR3 receptor-mediated signaling. Such binding agents include anti-TLR3 antibodies having the properties of binding a TLR3 receptor and modulating TLR3 receptor-mediated signaling.

One aspect of the invention is an antibody reactive with human Toll Like Receptor 3 (hTLR3) or hTLR3 homologs that induces cellular production of a cytokine selected from the group consisting of IL-8, MCP-1, MIP1-α, RANTES and TNF-α. These antibodies are useful as research reagents, diagnostic reagents and therapeutic agents. In particular, the antibodies of the invention are useful as therapeutic agents that can stimulate an immune response against foreign antigens.

Another aspect of the invention is an antibody reactive with hTLR3 or hTLR3 homologs that modulates a cytokine response induced by other TLR ligands. Modulation of a cytokine response results in potentiation or modification of the immune response to other TLR ligands including Cpg ODN and R848. For example, antibodies of the invention can enhance the production of Type 1 interferons such as interferon-α (IFN-α) when used in combination with TLR9 ligands such as CpG oligodinucleotides (CpG ODN).

Another aspect of the invention is an antibody reactive with hTLR3 or hTLR3 homologs that decreases the production of IL-10 produced by TLR7 agonists. For example, the antibodies of the invention significantly decrease the production of the anti-inflammatory cytokine IL-10 produced by the TLR7 agonist R848, also known as resiquimod. While not wishing to be bound to any particular theory, it is believed that the antibodies of the invention potentiate the inflammatory response to TLR7 agonists.

In one embodiment, the antibody of the invention is an isolated antibody reactive with hTLR3 having the antigen binding ability of a monoclonal antibody having the amino acid sequences of the heavy chain complementarity determining regions (CDRs) as set forth in SEQ ID NOs: 9 (CDR H1), 11 (CDR H2) and 13 (CDR H3) and the amino acid sequences of the light chain CDRs as shown in SEQ ID NOs: 19 (CDR L1), 21 (CDR L2) and 23 (CDR L3). An exemplary antibody is a monoclonal antibody having heavy chain CDR amino acid sequences as shown in SEQ ID NOs: 9, 11 and 13 and light chain CDR amino acid sequences as shown in SEQ ID NOs: 19, 21 and 23.

Another embodiment of the invention is an isolated polynucleotide encoding an antibody heavy chain having the CDR amino acid sequences shown in SEQ ID NOs: 9, 11 and 13 or a complementary nucleic acid. Other polynucleotides which, given the degeneracy of the genetic code or codon preferences in a given expression system, encode the heavy chain variable region CDRs shown in SEQ ID NOS: 9, 11 and 13 are also within the scope of the invention.

Another embodiment of the invention is an isolated polynucleotide encoding an antibody light chain having the CDR amino acid sequences shown in SEQ ID NOs: 19, 21 and 23 or a complementary nucleic acid. Other polynucleotides which, given the degeneracy of the genetic code or codon preferences in a given expression system, encode the light chain variable region

CDRs shown in SEQ ID NOS: 19, 21 and 23 are also within the scope of the invention.

Another embodiment of the invention is an isolated antibody reactive with hTLR3 comprising a heavy chain having the amino acid sequence shown in SEQ ID NO: 6 and a light chain having the amino acid sequence shown in SEQ ID NO: 16.

Another embodiment of the invention is an isolated polynucleotide encoding the amino acid sequence shown in SEQ ID NO: 6 or its complement. An exemplary polynucleotide encoding the amino acid sequence shown in SEQ NO: 6 has the sequence shown in SEQ ID NO: 5.

Another embodiment of the invention is an isolated polynucleotide encoding the amino acid sequence shown in SEQ ID NO: 16 or its complement. An exemplary polynucleotide encoding the amino acid sequence shown in SEQ NO: 16 has the sequence shown in SEQ ID NO: 15.

Exemplary antibodies may be antibodies of the IgG, IgD, IgGA or IgM isotypes. Additionally, such antibodies can be post-translationally modified by processes such as glycosylation, isomerization, aglycosylation or non-naturally occurring covalent modification such as the addition of polyethylene glycol moieties (pegylation) and lipidation. Such modifications may occur *in vivo* or *in vitro*. Fully human, humanized and affinity-matured antibody molecules or antibody fragments are within the scope of the invention as are mimetibodies, fusion proteins and chimeric proteins.

The antibodie of the invention may bind hTLR3 with a K_d less than or equal to about 10^{-7}, 10^{-8}, 10^{-9}, 10^{-10}, 10^{-11} or 10^{-12} M. The affinity of a given molecule for a hTLR3 receptor can be determined experimentally using any suitable method. Such methods may utilize Biacore or KinExA instrumentation, ELISA or competitive binding assays known to those skilled in the art.

Antibody molecules binding a given TLR3 homolog with a desired affinity can be selected from libraries of variants or fragments by techniques including antibody affinity maturation and other art-recognized techniques suitable for non-antibody molecules.

Another embodiment of the invention is a vector comprising at least one polynucleotide of the invention. Such vectors may be plasmid vectors, viral vectors, transposon based vectors or any other vector suitable for introduction of the polynucleotides of the invention into a given organism or genetic background by any means.

Another embodiment of the invention is a host cell comprising any of the polynucleotides of the invention such as a polynucleotide encoding a polypeptide comprising SEQ ID NO: 9, SEQ ID NO: 11 and SEQ ID NO: 13 and a polynucleotide encoding a polypeptide comprising SEQ ID NO: 19, SEQ ID NO: 21 and SEQ ID NO: 23. Such host cells may be eukaryotic cells, bacterial cells, plant cells or archeal cells. Exemplary eukaryotic cells may be of mammalian, insect, avian or other animal origins. Mammalian eukaryotic cells include immortalized cell lines such as hybridomas or myeloma cell lines such as SP2/0 (American Type Culture Collection (ATCC), Manassas, VA, CRL-1581), NS0 (European Collection of Cell Cultures (ECACC), Salisbury, Wiltshire, UK, ECACC No. 85110503), FO (ATCC CRL-1646) and Ag653 (ATCC CRL-1580) murine cell lines. An exemplary human myeloma cell line is U266 (ATTC CRL-TIB-196). Other useful cell lines include those derived from Chinese Hamster Ovary (CHO) cells such as CHO-K1 (ATCC CRL-61) or DG44.

Another embodiment of the invention is a method of making an antibody of the invention comprising culturing a host cell of the invention and recovering the antibody produced by the host cell. Such an antibody may be the hTLR3 antibody exemplified

below as mAb C1130 having heavy and light amino acid sequences as shown in SEQ ID NOs: 6 and 16, respectively.

The ability of the antibodies of the invention to potentiate CpG-mediated IFN-α production provides for various combination-type therapies. For example, the use of an antibody of the invention in combination with foreign antigens such as TLR agonist molecules or vaccine antigens will modulate an immune response and be useful in treating infections. Thus, another aspect of the invention is the use of an antibody of the invention in combination with other immune stimulants such as interferon or TLR9 agonists including, but not limited to, CpG ODN to stimulate and sustain an immune response as measured by enhanced production of Type I IFN (*e.g.*, IFNα) to prevent or treat viral infections including hepatitis viruses, herpes simplex virus, human immunodeficiency virus and human papilloma virus and other cutaneous and mucosal-associated infections. Also, the invention provides for use of an antibody of the invention in combination with other immune stimulants such as interferon or TLR9 agonists including, but not limited to, CpG ODN to treat cancers including multiple myeloma, chronic myelogenous leukemia, hairy cell leukemia, malignant melanoma, and sarcomas (including Kaposi's sarcoma). Further, the invention also provides for the use of an antibody of the invention in combination with other immune stimulants such as interferon or TLR9 agonists including but not limited to CpG ODN to treat inflammatory bowel diseases (*e.g.*, Crohn's disease and ulcerative colitis).

Another aspect of the invention is the use of an antibody of the invention in combination with a TLR7 agonist such as R848 (resiquimod) or imiquimod to provide for a combination therapy to prevent or treat viral infection-associated symptoms such as genital warts. The synthetic TLR7 agonist imiquimod has been approved by regulatory authorities for the treatment of genital

warts. Another aspect of the invention is the use of an antibody of the invention in combination with TLR9 or TLR7 agonists to prevent or treat pulmonary diseases including bacterial, fungal and viral pneumonias, and pathogen-mediated exacerbation of pulmonary diseases such as asthma, bronchitis and chronic obstructive pulmonary diseases. Yet another aspect of the invention is is the use of an antibody of the invention in combination with TLR9 or TLR7 agonists to prevent or treat graft-versus-host disease (GVHD). Yet another aspect of the invention is the use of an antibody of the invention in combination with immune treatments, such as interferon, to prevent or treat autoimmune diseases, including multiple sclerosis and lupus.

The methods of the invention may be used to treat an animal belonging to any genus. Examples of such animals include humans, mice, birds, reptiles, and fish.

Amounts of a given TLR3 antibody sufficient to treat a given condition can be readily determined. In the method of the invention the TLR3 antibody may be administered singly or in combination with at least one other TLR agonist molecule or vaccine antigen.

The mode of administration for therapeutic use of the antibodies of the invention may be any suitable route that delivers the agent to the host. The proteins, antibodies, antibody fragments and mimetibodies and pharmaceutical compositions of these agents are particularly useful for parenteral administration, *i.e.*, subcutaneously, intramuscularly, intradermally, intravenously or intranasally.

Antibodies of the invention may be prepared as pharmaceutical compositions containing an effective amount of the antibody as an active ingredient in a pharmaceutically acceptable carrier. An aqueous suspension or solution containing the antibody, preferably buffered at physiological

pH, in a form ready for injection is preferred. The compositions for parenteral administration will commonly comprise a solution of the antibody of the invention or a cocktail thereof dissolved in an pharmaceutically acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be employed, *e.g.*, 0.4% saline, 0.3% glycine and the like. These solutions are sterile and generally free of particulate matter. These solutions may be sterilized by conventional, well-known sterilization techniques (*e.g.*, filtration). The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, etc. The concentration of the antibody of the invention in such pharmaceutical formulation can vary widely, *i.e.*, from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., according to the particular mode of administration selected.

Thus, a pharmaceutical composition of the invention for intramuscular injection could be prepared to contain 1 mL sterile buffered water, and between about 1 ng to about 100 mg, *e.g.* about 50 ng to about 30 mg or more preferably, about 5 mg to about 25 mg, of an antibody of the invention. Similarly, a pharmaceutical composition of the invention for intravenous infusion could be made up to contain about 250 ml of sterile Ringer's solution, and about 1 mg to about 30 mg and preferably 5 mg to about 25 mg of an antibody of the invention. Actual methods for preparing parenterally administrable compositions are well known and are described in more detail in, for example, "Remington's Pharmaceutical Science", 15th ed., Mack Publishing Company, Easton, PA.

The antibodies of the invention, when in a pharmaceutical preparation, can be present in unit dose forms. The appropriate therapeutically effective dose can be determined readily by

those of skill in the art. A determined dose may, if necessary, be repeated at appropriate time intervals selected as appropriate by a physician during the treatment period.

The antibodies of the invention can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional immunoglobulins and protein preparations and art-known lyophilization and reconstitution techniques can be employed.

The present invention will now be described with reference to the following specific, non-limiting examples.

Example 1

Generation of anti-TLR3 mAbs

The anti-TLR3 mAb was generated using standard hybridoma technology in normal Balb/c mice (Kohler *et al.*, *J Immunol* 6:511-519, 1976). All animal procedures were performed in accordance with the guidelines established by the Institutional Animal Care and Use Committee. The mice were injected intradermally twice with plasmid DNA encoding amino acids 1-703 of human TLR3 (SEQ ID NO: 3). Amino acids 1-703 correspond to the predicted extracellular domain of hTLR3 (SEQ ID NO: 4). The mice received the plasmid DNA injections of 10 µg/mouse two weeks apart. The mice were boosted by intradermal injection with the extracellular domain of the purified recombinant human TLR3 protein. The first and second protein immunizations, with 15 µg protein, occurred two and four weeks after the second plasmid DNA injection. The third boost (10 µg protein) occurred five months later. Three days prior to the harvest of the spleens, the mice were injected intravenously with the TLR3 protein (15 µg/mouse). B cell fusions were performed using standard methods (Kohler *et al.*, *supra)*. Hybridomas were selected using media containing hypoxanthine-aminopterin-thymidine. Wells were screened by ELISA to detect anti-TLR3 antibodies. Positive wells were expanded and cloned using

limiting dilution. A large batch of antibody was prepared and purified using a Protein G column. The endotoxin levels were confirmed to be <1 EU/mg. mAb C1130 was generated in this manner. The antibody sequence is shown in Figs. 1 and 2.

Example 2

Isolation of Human Periperal Blood Mononuclear Cells

PBMCs were isolated from human blood. Whole blood was collected from a human donor into heparin-coated syringes. Approximately 50 mL of sterile Hank's Balanced Salt Solution (HBSS) (Invitrogen) was added to every 100 mL of blood. Thirty-eight mL of blood:HBSS were added to a 50 mL conical tube and 11 mL Ficoll-Paque Plus solution (Amersham) was slowly layered underneath. The tubes were centrifuged at 400x *g* for 40 minutes at room temperature. The centrifuge brake was turned off to preserve the gradient. The PBMCs form a white layer just above the Ficoll. The PBMCs from one conical were aspirated with a pipette into a new 50 mL conical. The tube was filled with HBSS to wash away the remainder of the Ficoll. The cells were spun at 600x *g* for 10 minutes. The supernatant was poured off and the pellet was resuspended in 10 mL Red Blood Cell Lysis Solution (Sigma) in a single tube. The tube was incubated at room temperature for ten minutes. The tube was brought to 50 mL with HBSS, and the cells were pelleted by centrifugation at 600x *g*. The cells were washed twice more with HBSS. After the final wash the pellet was resuspended in complete media: RPMI 1640 media/10% FBS/1X Non-Essential Amino Acids/ 1X Sodium Pyruvate/ 10 ug/mL gentamycin. Gentamycin was purchased from Sigma; the other media components were purchased from Invitrogen. An aliquot of the cells was removed and mixed with 50 µL trypan blue to obtain a live cell count. The cells were plated in 48-well plates at a concentration of 3 x 10^6 cells/well (0.5 mL/well).

Example 3

Determination of Anti-hTLR3 Antibody Effects on Cytokine/Chemokine Production

Purified antibodies were added to PBMCs to a final concentration of 20 µg/mL. The cells and the antibodies were incubated at 37°C for 30 minutes to one hour before the addition of 1 µg/mL CpG2216 (synthesized by Invitrogen), or 1 µg/mL R848 (Invivogen). CpG2216 has the sequence 5′-ggG GGA CGA TCG TCg ggg gg-3′. The bases in capital letters are linked by phosphodiester bonds and those in lowercase are linked by phosphorothioate bonds. R848, also known as resiquimod, is an imidazoquinolinamine, and is in the same compound class as imiquimod. Supernatants were harvested after 24h and frozen at -20°C.

Cytokine and chemokine concentrations in the supernatants were measured using Luminex technology. A Luminex Kit from Biosource was used to measure the following cytokines/chemokines: IL-1β, IL-6, IL-8, IL-10, IL-12, TNFα, IFNα, IFNγ, RANTES, MCP-1, MIP-1α and IP-10. In some instances, IFNα levels were measured using an ELISA kit (PBL Biomedical Labs). Statistical analysis was performed using two-factor analysis of variance with follow-up pairwise comparisons.

The results are shown in Fig. 3 and indicate that incubation of PBMCs with C1130 resulted in the production of IL-8, MCP-1, MIP-1 α, RANTES, and TNF α by the human cells (n=3 experiments). These effects were not seen in PBMCs incubated with another anti-TLR3 murine IgG1 antibody, C1068 that was generated in a similar manner to C1130. Lots of purified antibody were tested for endotoxin and the levels were below 1 EU/mg.

Example 4

Determination of Anti-hTLR3 Antibody Effects on IFNα Production

Since some TLRs are known to dimerize and/or use different adaptor proteins to alter ligand-binding specificity, PBMCs that were pretreated with the anti-hTLR3 antibody C1130 were stimulated with ligands for other TLRs, in particular CpG2216 as described in Example 3 to examine the effect of TLR3 modulation on the response to other TLR ligands. Since the ligands for TLR3 and TLR9 are nucleic acids, and both activate interferon secretion, it was hypothesized that they could share a signaling pathway. The results from three experiments are shown in Fig. 4 and indicate that PBMCs incubated with C1130 and CpG2216 secreted more IFNα than cells stimulated with CpG alone. The average increase was 7-fold.

Example 5

Determination of Anti-hTLR3 Antibody Effects on IL10 Production

The ligands for TLR7 and TLR8 are also nucleic acids. R848 (resiquimod) is a synthetic ligand for TLR7 and TLR8 in humans, which have been shown to recognize guanosine- and uridine-rich single-stranded RNA (Heil *et al.*, *Science* 5663: 1526, 2004) and was used to stimulate human PBMCs. Activation of TLR7, like TLR3, triggers the secretion of Type-I interferons. The results in Fig. 5 indicate that C1130 did not affect levels of IFNα secreted by PBMCs in response to R848. Though PBMCs usually secrete very high levels of IFNα in response to R848, in two of three experiments stimulation with R848 induced the production of ~500 pg/mL IFNα (a level low enough to presumably see any effect by an agonist or antagonist mAb). C1130 did affect R848-induced IL-10 levels. In three experiments, C1130 decreased R848-induced IL-10 by an average of 5-fold.

Example 6

Recognition of Epithelial Cell Surface TLR3 by Antibody C1130

Flow cytometry analyses were conducted on a Fluorescence-Activated Cell Sorter (FACS) instrument. C1130 antibody was

conjugated to APC using a Zenon mouse IgG1 labeling kit according to the manufacturer's protocol. Five microliters of labeling reagent per 1μg of mAb were incubated for 5 minutes at room temperature and protected from light. Blocking reagent was added at a ratio of 5μl to 1μg of mAb according to the manufacturer's protocol. Zenon-labeled antibodies were used within 30 minutes of conjugation. HEK293 (human embryonic kidney epithelial cells) that were stably transfected with human TLR3 were purchased from Invivogen.

293-TLR3 were fixed by 15 minute incubation in Cytofix either prior to or following staining. Approximately $1x10^6$ cells in 50 μl were incubated with APC-labeled antibody in 96-well round bottom plate for 30-60 minutes on ice. Cells were washed 3 times in PBS+1% FBS by centrifugation at 1600rpm for 2 minutes. Data acquisition was performed on a Becton-Dickinson FACSCalibur instrument and data analysis was performed using WinList (Verity Software House, Topsham, ME).

The results in Fig. 6 show that antibody C1130 binds to surface TLR3 on 293-hTLR3 cells fixed either before or after staining. A commercially available PE-labeled anti-hTLR3 (clone 3.7) was used as a positive control and Zenon APC-labeled mouse IgG1 as a negative control. These data demonstrate that the anti-hTLR3 antibody C1130 recognizes epithelial cells.

Example 7

Recognition of Lung Epithelial Cell Surface TLR3 by Antibody C1130

A549 cells, a human lung epithelial cell line, were obtained from the American Type Culture Collection (ATCC Accession No. CCL-185). Cells were transfected with a mammalian expression vector encoding a neomycin selectable marker along with a full-length copy of the human TLR3 gene under the control of the cytomegalovirus (CMV) promoter using Lipofectamine 2000 reagent (Invitrogen, Inc). A549 cells were also transfected in

parallel with the vector plasmid DNA-only (encoding neomycin resistance) as a control. Twenty-four hours post-transfection, cells were then trypsinized and seeded at dilution of 1:20 in media containing neomycin (G418) at 0.5 mg/ml. Cell clones appeared after 2 weeks growth in selection media containing G418. Cell colonies from each transfection were separately pooled. A549 cell lines derived from transfection and selection with the full-length human TLR3 expression vector (A549-hTLR3) or vector control (A549-neo) were maintained in growth media containing 0.5 mg/ml of G418.

C1130 antibody was conjugated to APC as described in Example 7. A549-TLR3.2 cells were fixed by 15 minute incubation in Cytofix either prior to or following staining. Approximately $1x10^6$ cells in 50 µl were incubated with APC-labeled antibody in 96-well round bottom plate for 30-60 minutes on ice. Cells were washed 3 times in PBS+1% FBS by centrifugation at 1600rpm for 2 minutes. Data acquisition and analysis was performed as described in Example 7. The results in Fig. 7 shows that C1130 binds to surface TLR3 on A549-TLR3.2 cells fixed either before or after staining. A commercially available PE-labeled anti-TLR3 (Clone 3.7) was used as a positive control and Zenon APC-labeled mouse IgG1 as a negative control. The ability of C1130 to recognize lung epithelial cells indicates that it has potential therapeutic use in pulmonary infections.

Example 8

Recognition of Cynomolgus White Blood Cells by Antibody C1130

Whole blood from Cynomolgus macaques was diluted 1:10 in FACSLyse buffer and incubated for 15 minutes at room temperature to lyse the red blood cells. Cells were then washed in PBS+1% FBS 4 times by centrifugation at 1400 rpm for 8 minutes. The resulting cell pellet was resuspended in PBS+1% FBS and counted manually using a hemacytometer. Cell viability was determined

by staining a sample cell population with 0.2% trypan blue. All samples tested were at least 95% viable.

The total cell pellet was resuspended in 1-2 ml of PBS supplemented with 10% FBS and kept at either 4°C or 37°C to evaluate for differences in receptor internalization. Fifty microliters (approximately $2x10^6$ cells) were distributed to each well in 96-well round bottom plates. FITC, PE and APC labeled mAbs were added at 1μg per well, incubated for at least 30 minutes at either 4°C or 37°C and protected from light. Cells were then washed 3 times in PBS + 1% FBS by centrifugation at 1600 rpm for 2 minutes. After the final wash the cells were resuspended in Cytofix buffer and incubated for 15 minutes at either 4°C or 37°C. Following paraformaldehyde fixation in the Cytofix buffer, the cells were washed once by centrifugation at 1600 rpm for 2 minutes and resuspended in 200μl of PBS+1% FBS. Samples were either read immediately or stored overnight at 4°C before acquisition. Data acquisition and analysis was performed as described in Example 7.

The results shown in Fig. 8 indicate that C1130 binds to cynomolgus macaque (cyno) CD11b positive cells, CD83 positive cells, CD86 positive cells, and CD3 positive cells. CD83 is found on B cells and dendritic cells and CD3 is found only on T-cells, indicating that C1130 recognizes different cell populations in PBMCs.

The present invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

CLAIMS

1. An isolated antibody reactive with human Toll Like Receptor 3 (hTLR3) or its homologs that induces cellular production of a cytokine selected from the group consisting of IL-8, MCP-1, MIP1-α, RANTES and TNF-α.

2. An isolated antibody reactive with hTLR3 or its homologs that modifies an immune response to other Toll Like Receptor ligands.

3. An isolated antibody reactive with hTLR3 having the antigen binding ability of a monoclonal antibody comprising the amino acid sequences of the heavy chain complementarity determining regions (CDRs) as shown in SEQ ID NOs: 9, 11 and 13 and the amino acid sequences of the light chain CDRs as shown in SEQ ID NOs: 19, 21 and 23.

4. An isolated antibody reactive with hTLR3 comprising the amino acid sequences of the heavy chain complementarity determining regions (CDRs) as shown in SEQ ID NOs: 9, 11 and 13 and the amino acid sequences of the light chain CDRs as shown in SEQ ID NOs: 19, 21 and 23.

5. An isolated antibody reactive with hTLR3 comprising a heavy chain comprising the amino acid sequence shown in SEQ ID NO: 6 and a light chain comprising the amino acid sequence shown in SEQ ID NO: 16.

6. An isolated polynucleotide encoding an antibody heavy chain comprising the CDR amino acid sequences shown in SEQ ID NOs: 9, 11 and 13.

7. An isolated polynucleotide encoding an antibody light chain comprising the CDR amino acid sequences shown in SEQ ID NOs: 19, 21 and 23.

8. An isolated polynucleotide encoding an antibody heavy chain comprising the amino acid sequence shown in SEQ ID NO: 6.

9. The polynucleotide of claim 7 comprising the sequence shown in SEQ ID NO: 5.

10. An isolated polynucleotide encoding an antibody light chain comprising the amino acid sequence shown in SEQ ID NO: 16.

11. The polynucleotide of claim 10 comprising the sequence shown in SEQ ID NO: 15.

12. A vector comprising at least one polynucleotide of claims 6, 7, 8, 9, 10 or 11.

13. A host cell comprising the vector of claim 12.

14. A method of making an antibody reactive with hTLR3 comprising culturing the host cell of claim 13 and recovering the antibody produced by the host cell.

15. A hybridoma cell line that produces the antibody of claim 5.

16. A method of treating viral infection comprising administering to a patient a therapeutically effective amount of the antibody of claim 1 in combination with an immune stimulant.

17. The method of claim 16 wherein the immune stimulant is interferon or a Toll-Like Receptor-9 (TLR9) agonist.

18. The method of claim 16 wherein the viral infection is hepatitis viruses, herpes simplex virus, human immunodeficiency virus, human papilloma virus or other cutaneous and mucosal-associated infections.

19. A method of preventing viral infection comprising administering to a patient a prophylactically effective amount of the antibody of claim 1 in combination with an immune stimulant.

20. The method of claim 19 wherein the immune stimulant is interferon or a Toll-Like Receptor-9 (TLR9) agonist.

21. The method of claim 19 wherein the viral infection is hepatitis viruses, herpes simplex virus, human immunodeficiency virus, human papilloma virus or other cutaneous or mucosal-associated infections.

22. A method of treating cancer comprising administering to a patient a therapeutically effective amount of the antibody of claim 1 in combination with an immune stimulant.

23. The method of claim 22 wherein the immune stimulant is interferon or a Toll-Like Receptor 9 (TLR9) agonist.

24. The method of claim 22 wherein the cancer is multiple myeloma, chronic myelogenous leukemia, hairy cell leukemia, malignant melanoma or sarcomas.

25. A method treating inflammatory bowel disease comprising administering to a patient a therapeutically effective amount of the antibody of claim 1 in combination with an immune stimulant.

26. The method of claim 25 wherein the immune stimulant is interferon or a TLR9 agonist.

27. The method of claim 25 wherein the inflammatory bowel disease is Crohn's disease or ulcerative colitis.

28. A method of treating a viral infection-associated symptom comprising administering to a patient a therapeutically effective amount of the antibody of claim 1 in combination with a Toll-Like Receptor 7 (TLR7) agonist.

29. The method of claim 28 wherein the TLR7 agonist is imiquimod or resiquimod.

30. The method of claim 28 wherein the viral infection-associated symptom is genital warts.

31. A method preventing a viral infection-associated symptom comprising administering to a patient a prophylactically effective amount of the antibody of claim 1 in combination with a Toll-Like Receptor 7 (TLR7) agonist.

32. The method of claim 31 wherein the TLR7 agonist is imiquimod or resiquimod.

33. The method of claim 31 wherein the viral infection-associated symptom is genital warts.

34. A method of treating a pulmonary disease and pathogen-mediated exacerbation comprising administering to a patient a therapeutically effective amount of the antibody of claim 1 in combination with a TLR9 or TLR7 agonist.

35. The method of claim 34 wherein the TLR7 agonist is imiquimod or resiquimod.

36. The method of claim 34 wherein the pulmonary disease is bacterial, fungal or viral pneumonias, and pathogen-mediated exacerbation is asthma, bronchitis or chronic obstructive pulmonary diseases.

37. A method preventing a pulmonary disease and pathogen-mediated exacerbation comprising administering to a patient a prophylactically effective amount of the antibody of claim 1 in combination with a TLR9 or TLR7 agonist.

38. The method of claim 37 wherein the TLR7 agonist is imiquimod or resiquimod.

39. The method of claim 37 wherein the pulmonary disease is bacterial, fungal or viral pneumonias, and pathogen-mediated exacerbation is asthma, bronchitis or chronic obstructive pulmonary diseases.

40. A method of treating graft-versus-host disease (GVHD) comprising administering to a patient a therapeutically effective amount of the antibody of claim 1 in combination with a TLR9 or TLR7 agonist.

41. The method of claim 40 wherein the TLR7 agonist is imiquimod or resiquimod.

42. A method preventing GVHD comprising administering to a patient a prophylactically effective amount of the antibody of claim 1 in combination with a TLR9 or TLR7 agonist.

43. The method of claim 42 wherein the TLR7 agonist is imiquimod or resiquimod.

44. A method of treating autoimmune disease comprising administering to a patient a therapeutically effective amount of the antibody of claim 1 in combination with an immune treatment.

45. The method of claim 44 wherein the immune treatment is inteferon.

46. The method of claim 44 wherein the autoimmune disease is multiple sclerosis or lupus.

47. A method preventing an autoimmune disease comprising administering to a patient a prophylactically effective amount of the antibody of claim 1 in combination with an immune treatment.

48. The method of claim 47 wherein the immune treatment is interferon.

49. The method of claim 47 wherein the autoimmune disease is multiple sclerosis or lupus.

Figure 1

Nucleotide sequence for C1130 heavy chain variable region

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AGGCCAAACTGACTGCAGTCACATCCGCCAGCACTACCTACATGGAACTCAGCAGCCTGACAAA
TGAAGACTCTGCGGTCTATTACTGTTCAACTCTAATGTTTGCTTATTGGGGCCAAGGGACTCTG
GTCACTGTCACTGCA (SEQ ID NO: 5)

Amino Acid sequence for C1130 Heavy Chain variable region

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EWIGAIYPGNNDITYTQKFKGKAKLTAVTSASTTYMELSSLTNEDSAVYYCSTLMFAYWGQGTL
VTVTA (SEQ ID NO: 6)

Signal Sequence

MECNWILPFILSVISGVYS (SEQ ID NO: 7)

FR1

EVQLQQSGTVLARPGASVKMSCKAS (SEQ ID NO: 8)

CDR1

GYRFSSYGMH (SEQ ID NO: 9)

FR2

WVKQRPGQGLEWIG (SEQ ID NO: 10)

CDR2

AIYPGNNDITYTQKFKG (SEQ ID NO: 11)

FR3

KAKLTAVTSASTTYMELSSLTNEDSAVYYCST (SEQ ID NO: 12)

CDR3

LMFAY (SEQ ID NO: 13)

Mouse J HC

WGQGTLVTVTA (SEQ ID NO: 14)

Figure 2

Nucleotide sequence for C1130 Light Chain variable region

ATGGACATGAGGGTTCCTGCTCACGTTTTTGGCTTCTTGTTGCTCTGGTTTCCAGGTACCAGAT
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CACTTGTCGGGCAAGTCAGGAAATTAGTGATCACTTAAGTTGGCTTCAGCAGAAATCGGGTGGA
ACTATTAAACGCCTGGTCTATGCCGCATCCACTTTAGATTCTGGTGTCCCAAAAAGGTTCAGTG
GCAGTAGGTCTGGGTCAGACTTTTCTCTCACCATCAGCAGCCTTGAGTCTGAAGATTTTGCAGA
CTATTACTGTCTACGATATGATAATTATCCGTGGACGTTCGGTGCAGGCACCAGGCTGGAAATC
AGA (SEQ ID NO: 15)

Amino Acid sequence for C1130 Light Chain variable region

MDMRVPAHVFGFLLLWFPGTRCDIQMTQSPSSLSASLGERVSLTCRASQEISDHLSWLQQKSGG
TIKRLVYAASTLDSGVPKRFSGSRSGSDFSLTISSLESEDFADYYCLRYDNYPWTFGAGTRLEI
R (SEQ ID NO: 16)

Signal sequence

MDMRVPAHVFGFLLLWFPGTRC (SEQ ID NO: 17)

FR1

DIQMTQSPSSLSASLGERVSLTC (SEQ ID NO: 18)

CDR1

RASQEISDHLS (SEQ ID NO: 19)

FR2

WLQQKSGGTIKRLVY (SEQ ID NO: 20)

CDR2

AASTLDS (SEQ ID NO: 21)

FR3

GVPKRFSGSRSGSDFSLTISSLESEDFADYYC (SEQ ID NO: 22)

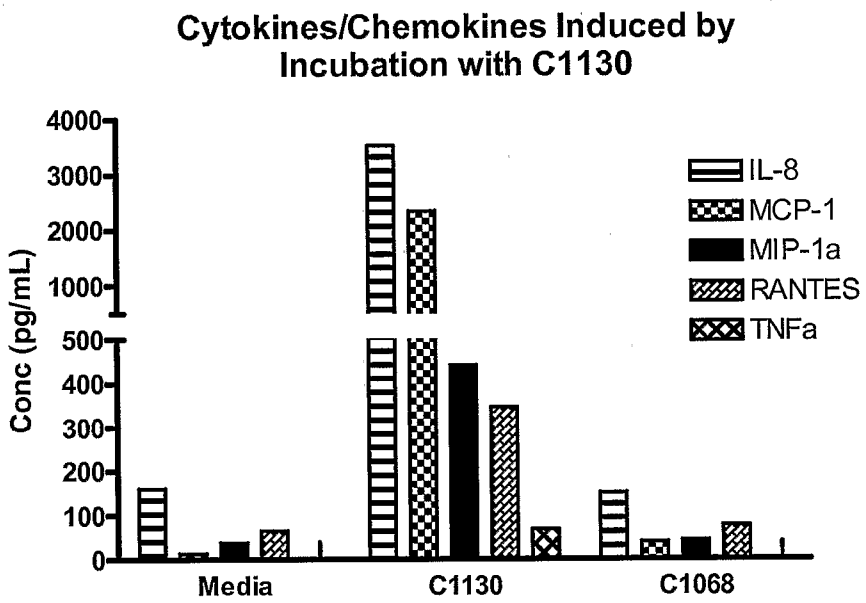
CDR3

LRYDNYPWT (SEQ ID NO: 23)

Mouse J KAPPA

FGAGTRLEIR (SEQ ID NO: 24)

Figure 3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2000
1000
500
400
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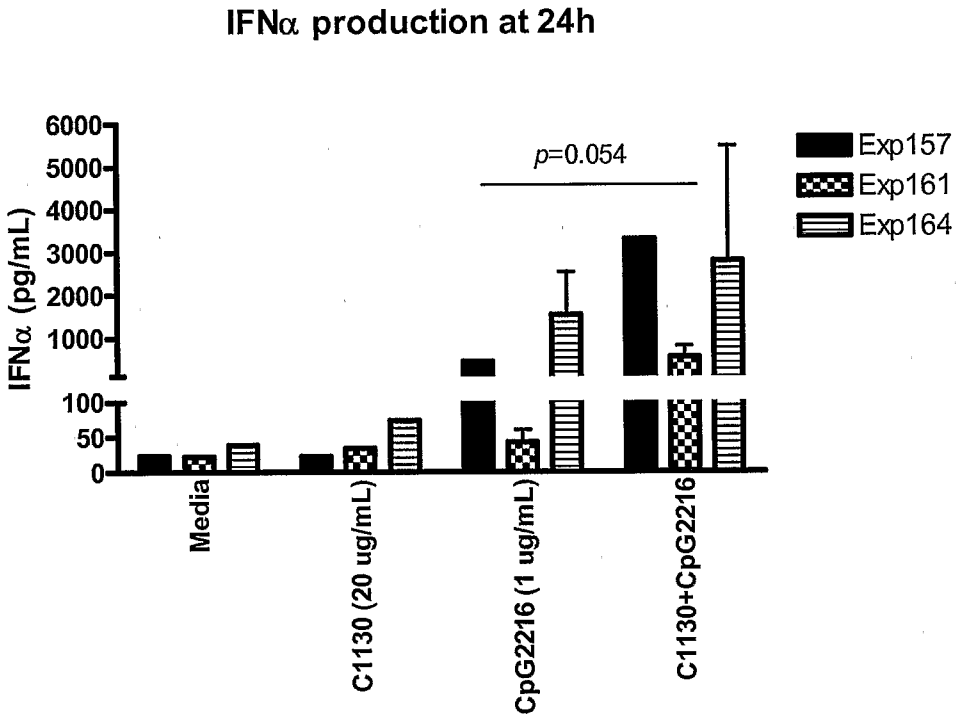
C1068
IL-8
MCP-1
MIP-1a
RANTES
TNFa

Figure 4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IFNα (pg/mL)
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4000
3000
2000
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100
50
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Media
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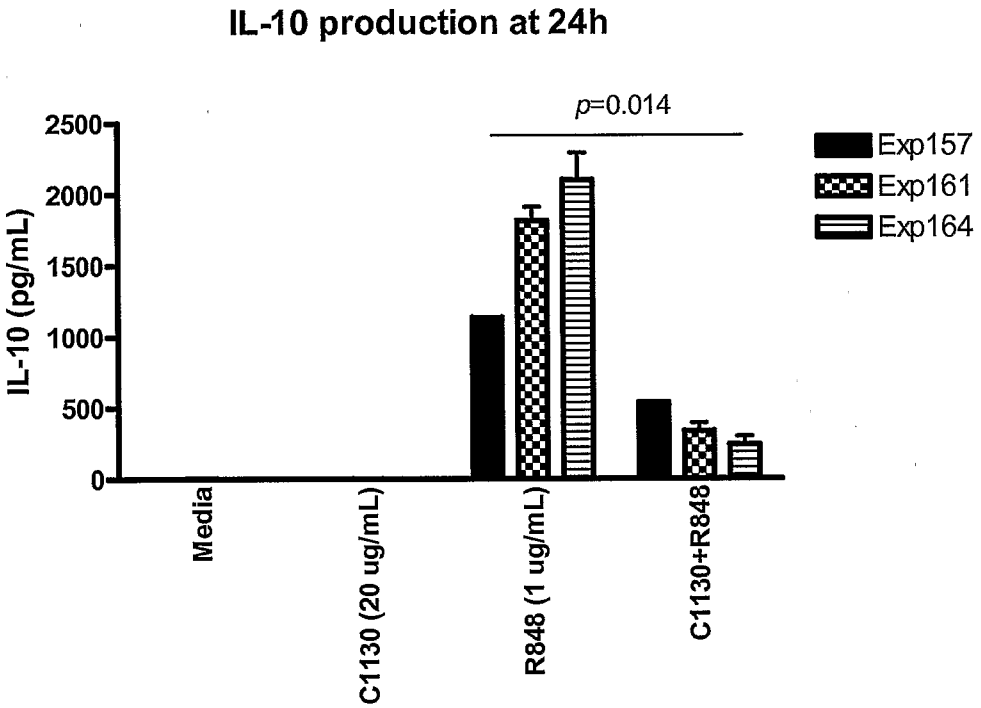
CpG2216 (1 ug/mL)
C1130+CpG2216

Figure 5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IL-10 (pg/mL)
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2000
1500
1000
500
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Exp157
Exp161
Exp164

Figure 6

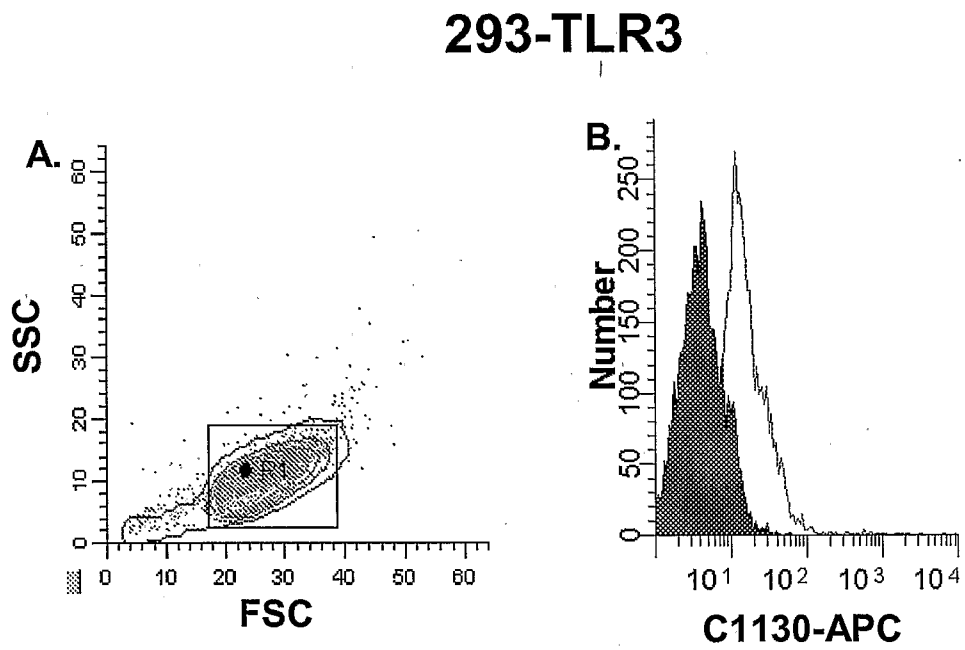
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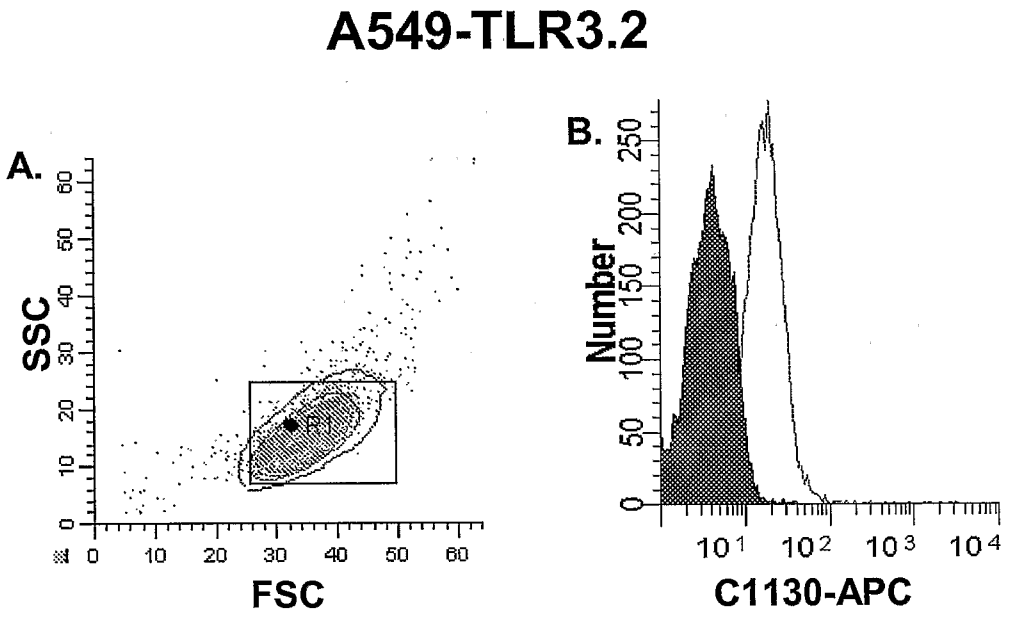
Figure 7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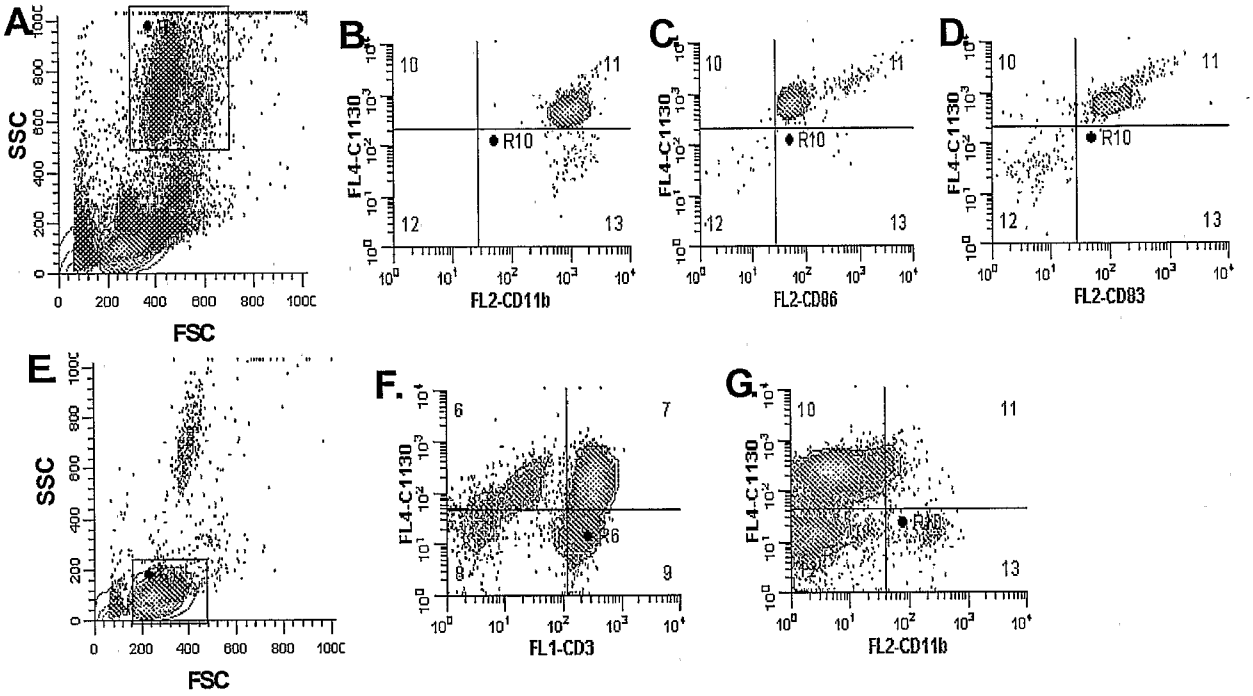
C1130-APC

Figure 8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<212> PRT
<213> Homo sapiens

<400> 2

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Arg Leu Pro Ala Ala Asn Phe Thr Arg Tyr Ser Gln Leu Thr Ser Leu
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Asp Val Gly Phe Asn Thr Ile Ser Lys Leu Glu Pro Glu Leu Cys Gln
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Gln Leu Ser Asp Lys Thr Phe Ala Phe Cys Thr Asn Leu Thr Glu Leu
        115                 120                 125
His Leu Met Ser Asn Ser Ile Gln Lys Ile Lys Asn Asn Pro Phe Val
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Lys Gln Lys Asn Leu Ile Thr Leu Asp Leu Ser His Asn Gly Leu Ser
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Ser Thr Lys Leu Gly Thr Gln Val Gln Leu Glu Asn Leu Gln Glu Leu
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Ile Phe Ala Asn Ser Ser Leu Lys Lys Leu Glu Leu Ser Ser Asn Gln
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Ile Lys Glu Phe Ser Pro Gly Cys Phe His Ala Ile Gly Arg Leu Phe
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Leu Glu Tyr Asn Asn Ile Gln His Leu Phe Ser His Ser Leu His Gly
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565 570 575
Ile Pro Val Glu Val Phe Lys Asp Leu Phe Glu Leu Lys Ile Ile Asp
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Gln Val Ser Leu Lys Ser Leu Asn Leu Gln Lys Asn Leu Ile Thr Ser
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Ser His Tyr Leu Cys Asn Thr Pro Pro His Tyr His Gly Phe Pro Val
675 680 685
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690 695 700
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740 745 750
Gln Phe Glu Tyr Ala Ala Tyr Ile Ile His Ala Tyr Lys Asp Lys Asp
755 760 765
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770 775 780
Lys Phe Cys Leu Glu Glu Arg Asp Phe Glu Ala Gly Val Phe Glu Leu
785 790 795 800
Glu Ala Ile Val Asn Ser Ile Lys Arg Ser Arg Lys Ile Ile Phe Val
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Ile Thr His His Leu Leu Lys Asp Pro Leu Cys Lys Arg Phe Lys Val
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His His Ala Val Gln Gln Ala Ile Glu Gln Asn Leu Asp Ser Ile Ile
835 840 845
Leu Val Phe Leu Glu Glu Ile Pro Asp Tyr Lys Leu Asn His Ala Leu
850 855 860
Cys Leu Arg Arg Gly Met Phe Lys Ser His Cys Ile Leu Asn Trp Pro
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<211> 2109
<212> DNA
<213> Homo sapiens

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<212> PRT
<213> Homo sapiens

<400> 4

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		35					40					45			
Leu	Pro	Thr	Asn	Ile	Thr	Val	Leu	Asn	Leu	Thr	His	Asn	Gln	Leu	Arg
	50					55					60				
Arg	Leu	Pro	Ala	Ala	Asn	Phe	Thr	Arg	Tyr	Ser	Gln	Leu	Thr	Ser	Leu
65					70					75					80
Asp	Val	Gly	Phe	Asn	Thr	Ile	Ser	Lys	Leu	Glu	Pro	Glu	Leu	Cys	Gln
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Lys	Leu	Pro	Met	Leu	Lys	Val	Leu	Asn	Leu	Gln	His	Asn	Glu	Leu	Ser
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		115					120					125			
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	130					135					140				
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145					150					155					160
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260 265 270
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290 295 300
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305 310 315 320
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325 330 335
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340 345 350
Trp Leu Lys Cys Leu Glu His Leu Asn Met Glu Asp Asn Asp Ile Pro
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370 375 380
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385 390 395 400
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485 490 495
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500 505 510
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515 520 525
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545 550 555 560
Leu Ser His Leu His Ile Leu Asn Leu Glu Ser Asn Gly Phe Asp Glu
565 570 575
Ile Pro Val Glu Val Phe Lys Asp Leu Phe Glu Leu Lys Ile Ile Asp
580 585 590
Leu Gly Leu Asn Asn Leu Asn Thr Leu Pro Ala Ser Val Phe Asn Asn
595 600 605
Gln Val Ser Leu Lys Ser Leu Asn Leu Gln Lys Asn Leu Ile Thr Ser
610 615 620

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Val Glu Lys Lys Val Phe Gly Pro Ala Phe Arg Asn Leu Thr Glu Leu
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Asp Met Arg Phe Asn Pro Phe Asp Cys Thr Cys Glu Ser Ile Ala Trp
                645                 650                 655
Phe Val Asn Trp Ile Asn Glu Thr His Thr Asn Ile Pro Glu Leu Ser
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Arg Leu Phe Asp Thr Ser Ser Cys Lys Asp Ser Ala Pro Phe Glu
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<213> Mus musculus

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Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Arg Phe
        35                  40                  45
Ser Ser Tyr Gly Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu
    50                  55                  60
Glu Trp Ile Gly Ala Ile Tyr Pro Gly Asn Asn Asp Ile Thr Tyr Thr
65                  70                  75                  80
Gln Lys Phe Lys Gly Lys Ala Lys Leu Thr Ala Val Thr Ser Ala Ser
                85                  90                  95
Thr Thr Tyr Met Glu Leu Ser Ser Leu Thr Asn Glu Asp Ser Ala Val
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<213> Mus musculus

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35 40 45
Gln Glu Ile Ser Asp His Leu Ser Trp Leu Gln Gln Lys Ser Gly Gly
50 55 60
Thr Ile Lys Arg Leu Val Tyr Ala Ala Ser Thr Leu Asp Ser Gly Val
65 70 75 80
Pro Lys Arg Phe Ser Gly Ser Arg Ser Gly Ser Asp Phe Ser Leu Thr
85 90 95
Ile Ser Ser Leu Glu Ser Glu Asp Phe Ala Asp Tyr Tyr Cys Leu Arg
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<211> 22
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<213> Mus musculus

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<210> 18
<211> 23
<212> PRT
<213> Mus musculus

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<212> PRT
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