Title: INTERLEUKIN 10 RECEPTOR (IL-10R) ANTIBODIES AND METHODS OF USE

Figures 9A

Abstract: The invention relates to IL-IO Receptor alpha (IL-10R α) antibodies and subsequences thereof, human and humanized IL-IO Receptor alpha (IL-10R α) antibodies and subsequences thereof, isolated and purified IL-IO Receptor alpha (IL-10R α) antibodies and subsequences thereof, compositions including IL-IO Receptor alpha (IL-10R α) antibodies and subsequences thereof, and methods that employ IL-IO Receptor alpha (IL-10R α) antibodies and subsequences thereof. The invention includes among other things, methods of treating a pathogen infection, pathogen reactivation, and methods of vaccinating or immunizing against a pathogen infection, which include, for example, administering an IL-IO Receptor alpha (IL-10R α) antibody or subsequence, to treat a pathogen infection, pathogen reactivation or for vaccination or immunization.
Interleukin 10 Receptor (IL-IOR) Antibodies and Methods of Use

Related Applications

[0001] This application claims priority to application serial no. 61/056,299, filed May 27, 2008, and is expressly incorporated by reference in its entirety.

Introduction

[0002] Interleukin 10 (IL-10) or cytokine synthesis inhibitory factor (CSIF) is secreted by dendritic cells (DC), macrophages, T cells, B cells, mast cells and keratinocytes at the late stage of an immune response to a pathogen, and has potent anti-inflammatory and immunosuppressive effects on hematopoietic cells (Redpath, et al., *Annu Rev Microbiol* 55:531 (2001)). IL-10 can inhibit the production of many cytokines, including EL-2, IFN-γ, TNF-α, IL-1, IL-4 and GM-CSF (Donnelly, et al., *J Interferon Cytokine Res* 19:563 (1999); Redpath, et al., *Annu Rev Microbiol* 55:531 (2001)). It can also downregulate the expression of MHC class II, ICAM-I, CD80 and CD86 on monocytes, thus reducing the T cell activating capacity of monocyte APC (OFarrell, et al., *Embo J* 17:1006 (1998); Donnelly, et al., *J Interferon Cytokine Res* 19:563 (1999); Moore, et al., *Annu Rev Immunol* 19:683 (2001)), while also increasing CD14 expression and responses to LPS (Rahimi, et al., *J Immunol* 174:7823 (2005)). IL-10 inhibits DC maturation and IL-12 production (Brossart, et al., *Cancer Res* 60:4485 (2000)), thus suppressing their capacity to induce a Th1 response. Furthermore, IL-10 promotes the generation of regulatory T cells (Tregs) (Moore, et al., *Annu Rev Immunol* 19:683 (2001)).


[0004] In humans, elevated IL-10 levels correlate with a number of chronic or progressive infectious diseases caused by intracellular pathogens, such as visceral leishmaniasis and mycobacteria. In addition, it has been reported that anti-IL-10 monoclonal antibodies can restore responses of pathogen-specific T
cells from infected patients in vitro (Moore, et al., Annu Rev Immunol 19:683 (2001)), suggesting that BL-10 mediates the T cell unresponsiveness or anergy in these chronic diseases. Elevated IL-10 serum levels have also been associated with several chronic viral infectious diseases, including Hepatitis B virus (HBV) (Geng, L., et al., J Viral Hepat 13:725 (2006)), Hepatitis C virus (HCV) (Vicari, et al., Immunol Rev 202:223 (2004)), HIV (Redpath, et al., Trends Microbiol 9:86 (2001)), and Cytomegalovirus (CMV) (Redpath, et al., Trends Microbiol 9:86 (2001)). IL-10 is one strategy viruses exploit to evade the immune response. Some viruses infect macrophages and induce cellular IL-10 production, whereas other viruses encode an IL-10 homolog (vEL-10), such as CMV and Epstein-Barr virus (EBV) (Liu, et al., / Immunol 158:604 (1997); Moore, et al., Annu Rev Immunol 19:683 (2001)). The exact molecular mechanisms by which persistent viral infection causes increased IL-10 secretion have not been determined. However, elevated IL-10 levels result in local and/or systemic suppression of inflammatory responses. In HBV and HCV chronically infected patients, increased IL-10 levels lead to diminished T cell activity, evidenced by the loss of proliferation and cytokine production in the presence of viral antigens in vitro. Moreover, this phenotype in HCV patients can be reversed with anti-DL-10R antibody in vitro (Rigopoulou, et al., AASLD Abstracts: 304A (2000); Rigopoulou, et al., Hepatology 42:1028 (2005)). Similar responses to IL-10 neutralization have been observed in vitro by PBMC from HIV infected patients (Clerici, et al., J Clin Invest 93:768 (1994); Brockman, et al., Blood (2009)).

IL-10 mediates its immunosuppressive activities through binding to the cellular IL-10 receptor (IL-10R). IL-10R is composed of two subunits, IL-10Rα (IL-10Ralpha or IL-10RI, CD210) and IL-10Rβ (EL-10Rbeta, IL-10R2), which are members of the class II cytokine receptor family (Donnelly, et al., J Interferon Cytokine Res 19:563 (1999); Moore, et al., Annu Rev Immunol 19:683 (2001)). Although not wishing to be bound by theory, binding of IL-10 to the heterodimeric IL-10R results in the activation of receptor-associated Jak1 and Tyk2 protein tyrosine kinases, and subsequent tyrosine phosphorylation and activation of DNA binding of signal transducer and activator of transcription 3 (STAT3) and STAT1. This signaling pathway ultimately results in suppression of proinflammatory cytokine production, the negative regulation of T cell, dendritic cell and macrophage activation and other immune suppressive effects.

IL-10Ra is the ligand binding subunit, and it binds IL-10 with high affinity (Kd ~ 35-200 pM). Human IL-10Rα contains 578 amino acids with a molecular size of 90-110 kDa, and it shares 60% homology with mouse IL-10Rα. IL-10Rα is primarily expressed by hematopoietic cells, such as B cells, T cells, NK cells, natural Killer T (NKT) cells, monocytes and macrophages, although generally at levels of only a few hundred per cell (Donnelly, et al., J Interferon Cytokine Res 19:563 (1999)). IL-10Rα expression on T cells is downregulated by activation, while it is upregulated on monocytes upon activation, consistent with the idea that IL-10 inhibits the function of those cells after the onset of an immune response (Moore, et al., Annu Rev Immunol 19:683 (2001)). This also supports the observation that naive CD4 T cells are targeted by EL-10, while activated and memory T cells seem to be rather
insensitive toward this cytokine. IL-1 ORa expression has also been observed on nonhematopoietic cells, although it is more often induced rather than constitutive, such as on LPS treated fibroblasts.

IL-1OR \( \beta \) contributes little to IL-10 binding affinity, and it is an accessory subunit of IL-1OR for signaling (Donnelly, et al., J Interferon Cytokine Res 19:563 (1999)). IL-1OR \( \beta \) is part of multiple cytokine receptor complexes, including IL-22 (Asadullah, et al., Curr Drug Targets Inflamm Allergy 3:185 (2004)), IL-28, and IL-29. Human IL-1OR \( \beta \) contains 325 amino acids and is approximately 69% identical to the mouse homologue. In the presence of IL-10, IL-1OR \( \alpha \) associates with IL-1OR \( \beta \) to form tetrameric IL-10R complexes consisting of two of each subunit, which is required for signal transduction. Hence, IL-1OR \( \beta \) knockout mice develop chronic severe enterocolitis, resembling IL-10 knockout animals.

In contrast to cell type restricted expression of IL-1 OR\( \times \), BL-1OR\( \beta \) is constitutively expressed in most cells and tissues examined. Unlike IL-1OR \( \alpha \), IL-1OR \( \beta \) expression in immune cells has not been found to change upon activation. Thus, any stimulus upregulating IL-1OR \( \alpha \) expression is sufficient to render the cell responsive to IL-10.

The combining site of human IL-10 and human IL-1OR \( \alpha \) has been mapped and shown to be discontinuous. One neutralizing anti-IL-10R \( \alpha \) monoclonal antibody (#MAB274, clone 37607, R&D Systems) has been found to recognize discontinuous epitopes that overlap with some of the IL-10/IL-1OR binding regions (Reineke, et al., Protein Sci 7:951 (1998)), suggesting that the natural conformation of IL-1OR might be important for the generation of neutralizing antibody. Commercially available neutralizing anti-IL-10R \( \alpha \) monoclonal antibodies block all known cellular and viral IL-10 activities (Moore, et al., Annu Rev Immunol 19:683 (2001)). Neutralizing anti-IL-1OR \( \beta \) monoclonal antibodies can also abrogate DL-10 response with perhaps additional effects through other receptor complexes using IL-1OR\( \beta \).

**Summary**

The invention is based, at least in part, on the generation of anti-human Interleukin-10 receptor alpha (IL1 ORa) antibodies. Anti-human IL-1OR \( \alpha \) antibodies disclosed herein specifically bind to human IL-1OR \( \alpha \). In particular, for example, exemplary IL-1OR \( \alpha \) monoclonal antibodies, denoted 136C5 (antibody producing hybridoma deposited on April 8, 2008, with deposit designation of PTA-9113, ATCC 10801 University Blvd., Manassas, VA 201 10-2229), 136C8 (antibody producing hybridoma deposited on April 8, 2008, with deposit designation of PTA-9132, ATCC 10801 University Blvd., Manassas, VA 201 10-2229), and 136D29 (antibody producing hybridoma deposited on April 8, 2008, with deposit designation of PTA-9133, ATCC 10801 University Blvd., Manassas, VA 201 10-2229), bind to IL-1OR expressing monocytes and lymphocytes. The exemplary IL-1OR \( \alpha \) monoclonal antibodies also bind to human IL-1OR \( \alpha \) stably transfected cell lines, EL4-hIL-10R \( \alpha \) and CHO-hIL-1ORa, but not to non-transfected parental cell lines. Moreover, the antibodies are blocked from binding to endogenous IL-1OR \( \alpha \) by pre-bound human IL-10. Incubation of IL-1OR \( \alpha \) antibodies with human peripheral blood mononuclear cells (PBMC) treated with lipopolysaccharide and IL-10 neutralizes (i.e.,
inhibits, reduces, antagonizes, prevents or blocks) both exogenous and endogenous IL-10 inhibition of
LPS-induced TNF-α secretion (i.e., modulate human IL-1OR/IL-10 signaling activity).

[0010] Exemplary IL-1ORα antibodies recognize two "epitopes" on IL-1ORα, as determined by
cross-blocking studies, human IL-1ORα single nucleotide polymorphism (SNP) variant binding, and
cross-reactivity with macaque IL-1ORα. Two of the exemplary antibodies, namely 136C5 and 136C8
bind to all known IL-1ORα SNP variants (e.g., SEQ ID NOs: 6, 63, 65, 67, 69 and 71). All exemplary IL-
10Ra antibodies bind to chimpanzee IL-1ORα (e.g. SEQ ID NO: 6) and inhibit IL-1OR/IL-10 signaling
activity, and therefore functionally modulate chimpanzee IL-1ORα activity (i.e., modulate IL-1OR/IL-10
signaling activity). Two of the exemplary IL-1ORα antibodies, namely 136C5 and 136C8, bind to
chimpanzee IL-1ORα and cynomolgus macaque IL-1ORα (SEQ ID NOs: 8 and 10), and inhibit IL-
1OR/IL-10 signaling activity, and therefore functionally modulate chimpanzee IL-1ORα and cynomolgus
macaque IL-1ORα activity (i.e., modulate IL-1OR/IL-10 signaling activity). In comparison to
commercially available antibodies 3F9 (#308806, Biolegend), SPM466 (#E8574, Spring Biosciences)
and 37607 (#MAB274, R&D Systems), the IL-1ORα antibodies disclosed herein are unique in the ability
to functionally modulate both chimpanzee and macaque IL-1ORα activity and to recognize all known IL-
1ORα extracellular SNP variants (e.g., SEQ ID NOs: 6, 63, 65, 67, 69 and 71).

[0011] In accordance with the invention, there are provided antibodies and subsequences thereof
that specifically bind to IL-10 Receptor alpha protein (IL-1ORα). In one embodiment, an antibody or
subsequence thereof specifically binds to IL-10 Receptor alpha protein, and reduces, inhibits or competes
for binding of an antibody designated 136C5, 136C8, or 136D29 to the IL-10 Receptor alpha protein. In
another embodiment, an antibody or subsequence thereof specifically binds to IL-10 Receptor alpha
protein, and reduces, inhibits or competes for binding of an antibody or subsequence thereof comprising a
heavy chain variable region sequence of any of SEQ ID NOs: 31 or 33, and a light chain variable
region sequence of any of SEQ ID NOs: 30, 32, or 34, to the IL-10 Receptor alpha protein. In a further
embodiment, an antibody or subsequence thereof specifically binds to IL-10 Receptor alpha protein, and
does not detectably reduce, inhibit or compete for binding of antibody designated 3F9, SPM466, or
37607 to the IL-10 Receptor alpha protein. In an additional embodiment, an antibody or subsequence
thereof specifically binds to IL-10 Receptor alpha protein, and binds to an epitope distinct from the
epitope to which antibody designated 3F9, SPM466, or 37607 binds. In still a further embodiment, an
antibody or subsequence thereof binds to or recognizes a conformational epitope (e.g., of IL-1ORα), and
not a linear epitope (e.g., of IL-1ORα).

[0012] In accordance with the invention, there are also provided antibodies and subsequences
thereof that specifically bind to IL-10 Receptor alpha protein (IL-1ORα) and modulate an IL-1OR/IL-10
signaling activity. In one embodiment, an antibody or subsequence thereof specifically binds to IL-1ORα,
and reduces, inhibits, decreases, suppresses or limits an IL-1OR/IL-10 signaling activity. In particular
aspects, an antibody or subsequence thereof specifically binds to a human IL-1ORα and a chimpanzee or
cynomolgus macaque IL-1 ORa, and reduces, inhibits, decreases, suppresses or limits an IL-1OR/IL-10 signaling activity. In further particular aspects, an antibody or subsequence thereof specifically binds to a human IL-1 ORa, a chimpanzee IL-1 ORa and a cynomolgus macaque IL-1 ORa, and reduces, inhibits, decreases, suppresses or limits an EL-1OR/IL-10 signaling activity. Exemplary EL-1OR/IL-10 signaling activities include reducing, decreasing or suppressing TNF-alpha, IL-6, IL-1β or IFN-gamma expression or secretion by peripheral blood mononuclear cells PBMC treated with LPS. In particular, TNF-alpha, IL-6, IL-1β or IFN-gamma expression or secretion by PBMCs (e.g., human, chimpanzee or macaque) increases when PBMCs are treated with LPS in vitro - addition of IL-10 reduces, decreases or suppresses TNF-alpha, IL-6, IL-1β or IFN-gamma expression or secretion by PBMCs (e.g., human, chimpanzee or macaque) treated with LPS. Thus, an invention antibody or subsequence thereof that reverses or limits IL-10 suppression, inhibition or reduction of TNF-alpha, IL-6, IL-1β or IFN-gamma expression or secretion by PBMCs will in turn increase, stimulate or induce TNF-alpha, IL-6, IL-1β or IFN-gamma expression or secretion by PBMCs (e.g., human, chimpanzee or macaque) treated with LPS in vitro. Such antibodies and subsequences thereof are considered to functionally modulate IL-1OR/IL-10 signaling. Additional Exemplary IL-1OR/IL-10 signaling activities include reducing, decreasing or suppressing TNF-alpha or IFN-gamma expression or secretion by a human natural killer T (NKT) cell line stimulated with the antigen α-galactosylceramide. In particular, TNF-alpha or IFN-gamma expression or secretion by NKT cells increases when they are treated with a synthetic α-galactosylceramide, KRN7000, in vitro - addition of IL-10 reduces, decreases or suppresses TNF-alpha or IFN-gamma expression or secretion by NKT cells with KRN7000 (Kawano, et al., Science, 278: 1626 (1997); Kobayashi, et al., Oncol Res 7:259 (1995)). Thus, an invention antibody or subsequence thereof that reverses or limits IL-10 suppression, inhibition or reduction of TNF-alpha or IFN-gamma expression or secretion by NKT cells will in turn increase, stimulate or induce TNF-alpha or IFN-gamma expression or secretion by NKT cells treated with KRN7000 in vitro. Such antibodies and subsequences thereof are considered to functionally modulate IL-1OR/IL-10 signaling.

[0013] IL-10 receptor alpha (IL-1OR alpha) antibodies and subsequences (monoclonal or polyclonal) thereof bind to IL-10 receptor (IL-1OR). Antibodies include mammalian, primatized, humanized and fully human antibody. Antibodies can be monoclonal (a single monoclonal or pool of two or more monoclonal) or polyclonal immunoglobulins that belong to any class such as IgM, IgG, IgA, IgE, IgD, and any subclass thereof. Exemplary subclasses for IgG are IgG₁, IgG₂, IgG₃ and IgG₄.

[0014] Specific non-limiting examples of IL-10 antibody include antibodies set forth herein as 136C5 (antibody producing hybridoma deposited on April 8, 2008, with deposit designation of PTA-9131, ATCC 10801 University Blvd., Manassas, VA 20110-2209), 136C8 (antibody producing hybridoma deposited on April 8, 2008, with deposit designation of PTA-9132, ATCC 10801 University Blvd., Manassas, VA 20110-2209), and 136D29 (antibody producing hybridoma deposited on April 8, 2008, with deposit designation of PTA-9133, ATCC 10801 University Blvd., Manassas, VA 20110-2209), subsequences and variants thereof. Specific non-limiting examples of IL-10 antibody
include antibodies with a heavy and light chain variable region amino acid sequences of each of 136C5, 136C8 and 136D29 as set forth herein in Example 2, as SEQ ID NOs:29, 31, and 33; and SEQ ID NOs:30, 32, and 34.

[0015] IL-I ORα antibodies also include antibodies that specifically bind to more than one species type of IL-I ORα such as human BL-IORα, chimpanzee EL-IORα and cynomolgus macaque IL-I ORα. Exemplary invention antibodies include DL-IORα antibodies that bind to human EL-IORα, and chimpanzee IL-IOR α and/or macaque EL-IORα. In contrast, a commercially available antibody that binds to human DL-IORα, 37607, fails to detectably bind to chimpanzee EL-IORα or macaque DL-IORα.

[0016] DL-IORα antibodies further include antibodies that specifically bind to one or more human IL-I ORα SNP variants. In particular embodiments, an antibody or subsequence thereof specifically binds to one or more of EL-IORα variants set forth as SEQ ID NOs.:6, 63, 65, 67, 69 or 71. In further particular embodiments, an antibody or subsequence thereof specifically binds with greater affinity to one or more of EL-IORα variant set forth as SEQ ID NOs.:6, 63, 65, 67, 69 or 71 than binding of 136D29, 3F9, SPM466 or 37607 antibody to EL-IORα variant set forth as SEQ E NOs.:6, 63, 65, 67, 69 or 71.

[0017] EL-Io receptor (EL-IOR) antibody subsequences (antibody fragments) include functional subsequences, which exhibit at least partial HL-IOR binding. Such "functional" subsequences or fragments include but are not limited to Fab, Fab', F(ab')_2, Fv, Fd, single-chain Fv (scFv), disulfide-linked Fvs (sdFv), light chain variable region V_L, heavy chain variable region V_H, trispecific (Fab)_3, bispecific (Fab)_2, diabody ((V_L-V_H)_2 or (V_H-V_L)_2), triabody (trivalent), tetrabody (tetravalent), minibody ((scFv-C_H3)_2), bispecific single-chain Fv (Bis-scFv), IgGdeltaCH2, scFv-Fc and (scFv)_2-Fc. Functional fragments and subsequences also include all or a portion of a full length antibody heavy or light chain, or a heavy or light chain variable region, which includes one or more CDRs of a heavy or light chain variable region sequence (e.g., 1, 2 or all 3 of each of the heavy and light chain variable region CDRs optionally including flanking framework regions, FRs). In various aspects, a functional fragment or a subsequence of a full length antibody heavy or light chain, or a heavy or light chain variable region, has a length from about 20-30, 30-50, 50-100, 100-150, 150-200, 200-250, 250-300, 300-400, or 400-500, amino acid residues.

[0018] EL-Io receptor (EL-IOR) antibody variants include functional variants, which exhibit at least partial EL-IOR binding. In various embodiments, an antibody variant includes one or more amino acid substitutions, deletions or insertions of an antibody constant or variable region sequence set forth herein asl36C5, 136C8 or 136D29, or a heavy or light chain variable regions sequence of 136C5, 136C8 or 136D29, e.g., SEQ ID NOs:29, 31 or 33, or e.g., SEQ ID NOs:30, 32, or 34.

[0019] In accordance with the invention, there are also provided methods of treating a subject for a pathogen infection (chronic or acute). In one embodiment, a method includes administering to a subject in need thereof an amount of an EL-Io receptor alpha (EL-IOR alpha) antibody or subsequence thereof sufficient to treat the subject for the pathogen infection.
In accordance with the invention, there are also provided prophylactic methods including methods of vaccinating and immunizing a subject against a pathogen infection (chronic or acute), for example, to protect the subject from a pathogen infection (e.g., provide the subject with some protection against pathogen infection), to decrease or reduce the probability of a pathogen infection in a subject, to decrease or reduce susceptibility of a subject to a pathogen infection, or to inhibit or prevent a pathogen infection in a subject. In one embodiment, a method includes administering to a subject an amount of an EL-10 receptor alpha (IL-10R alpha) antibody or subsequence thereof prior to, substantially contemporaneously with or following administration of a pathogen antigen, live or attenuated pathogen, or a nucleic acid encoding all or a portion (e.g., an epitope) of any protein or proteinaceous pathogen antigen sufficient to vaccinate or immunize the subject against the pathogen infection (chronic or acute). In various aspects, a method is sufficient to protect the subject from the pathogen infection (e.g., provide the subject with some protection against pathogen infection), to decrease or reduce the probability of pathogen infection in the subject, to decrease or reduce susceptibility of a subject to a pathogen infection, or to inhibit or prevent a pathogen infection, or to decrease, reduce, inhibit or prevent pathogen reactivation in a subject. Methods of the invention include administering the IL-10 receptor (IL-10R) antibody or subsequence thereof at various times and in various quantities. In particular embodiments, IL-10 receptor (IL-10R) antibody or subsequence thereof is administered prior to, substantially contemporaneously with or following contact, exposure to or infection with a pathogen. In other embodiments, IL-10 receptor (IL-10R) antibody or subsequence thereof is administered prior to, substantially contemporaneously with or following exposure to, contact with or infection (chronic or acute) of the subject with a pathogen. In additional embodiments, EL-10 receptor (EL-10R) antibody or subsequence thereof is administered prior to, substantially contemporaneously with or following pathogen infection, development of a symptom associated with or caused by a pathogen (e.g., inflammation), pathogen replication or proliferation, or pathogen reactivation from latency. In further embodiments, an EL-10 receptor alpha (EL-10R alpha) antibody or subsequence thereof, and a pathogen antigen, live or attenuated pathogen, or nucleic acid encoding all or a portion (e.g., an epitope) of any protein or proteinaceous pathogen antigen are administered as a combination composition, or are administered separately, such as concurrently or sequentially, to a subject in order to effect vaccination or immunization, prior to, substantially contemporaneously with or following pathogen infection, development of a symptom associated with or caused by a pathogen (e.g., inflammation), pathogen replication or proliferation, or pathogen reactivation from latency.

Pathogens treated, or vaccinated or immunized against include any pathogen which may respond to an EL-10 receptor antibody or subsequence thereof. In various embodiments, a pathogen is a virus, bacterium, parasite or a fungus.

Exemplary viruses include poxvirus, herpesvirus, hepatitis virus, immunodeficiency virus, flavivirus, papilloma virus (PV), polyoma virus, rhabdovirus, a myxovirus, an arenavirus, a coronavirus, adenovirus, reovirus, picornavirus, togavirus, bunyavirus, parvovirus or retrovirus.
Poxviruses include a vaccinia virus, *Molluscum contagiosum*, variola major smallpox virus, variola minor smallpox virus, cow pox, camel pox, sheep pox, and monkey pox. Herpesviruses include alpha-herpesvirus, beta-herpesvirus, gamma-herpesvirus, Epstein Bar Virus (EBV), Cytomegalovirus (CMV), varicella zoster virus (VZV/HHV-3), and human herpes virus 1, 2, 4, 5, 6, 7, and 8 (HHV-8, Kaposi's sarcoma-associated virus). Hepatitis viruses include hepatitis A, B, C, D, E and G. Immunodeficiency viruses include human immunodeficiency virus (HIV), such as HIV-1, HIV-2 and HIV-3. Flaviviruses include Hepatitis C virus, Yellow Fever virus, Dengue virus, and Japanese Encephalitis and West Nile viruses. Papilloma viruses include human papilloma virus (HPV), such as HPV strain 1, 6, 11, 16, 18, 30, 31, 42, 43, 44, 45, 51, 52, and 54. Polyoma viruses include BK virus (BKV) and JC virus (JCV). Rhabdoviruses include rabies virus and vesiculovirus. Myxoviruses include paramyxovirus (e.g., measles, mumps, pneumovirus and respiratory syncytial virus (RSV) and orthomyxovirus (e.g., influenza virus, such as influenza A, influenza B and influenza C). Arenaviruses include lymphocytic choriomeningitis virus (LCMV), Junin virus, Lassa virus, Guanarito virus, Sabia virus and Machupo virus. Coronaviruses include viruses that cause a common cold or severe acute respiratory syndrome (SARS). Adenoviruses include viral infections of the bronchii, lung, stomach, intestine (gastroenteritis), eye (conjunctivitis), bladder (cystitis) and skin. Reoviruses include a rotavirus, cytopivirus and orbivirus. Picornaviruses include rhinovirus (e.g., causing a common cold), aphthovirus, hepatovirus, enterovirus, coxsackie B virus and cardiovirus. Togaviruses include alphavirus, sindbis virus, and rubellavirus. Bunyaviruses include hantavirus, phlebovirus and nairovirus. Retroviruses include alpha, beta, delta, gamma, epsilon, lentivirus, spumavirus and human T-cell leukemia virus, such as human T-cell leukemia virus 1 and 2 (HTLV-I and HTLV-2). Lentiviruses include immunodeficiency virus, such as bovine, porcine, canine, feline and primate virus.

Exemplary bacteria include a mycobacterium (e.g., tuberculosis and atypical mycobacterium), listeria monocytogenes, helicobacter, bordetella, streptococcus, salmonella and chlamydia. Exemplary parasites include a protozoa or nematode. Exemplary protozoa include a *Toxoplasma gondii*, *Leishmania*, *Plasmodium*, or *Trypanosoma cruzi*. Exemplary nematodes include a *Schistosoma mansoni*, or a *Heligmosomoides polygyrus*. Exemplary fungus includes *Candida albicans*.

Pathogen antigens useful in accordance with the invention can be any antigen, live or attenuated pathogen, or nucleic acid encoding all or a portion (e.g., an epitope) of any protein or proteinaceous pathogen antigen. Particular non-limiting types of pathogen antigens, live or attenuated pathogen, and nucleic acid encoding all or a portion of a pathogen antigen include viral, bacterial, parasite and fungal antigens. Such antigens can be from any pathogen set forth herein or known to one of skill in the art, and can include an antigen that increases, stimulates, enhances, promotes, augments or induces a proinflammatory or adaptive immune response, numbers or activation of an immune cell (e.g., T cell, natural killer T (NKT) cell, dendritic cell (DC), macrophage, neutrophil, eosinophil, mast cell, CD4+ or a CD8+ cell, CD14+, CD1 lb+ or CD1 lc+ cells), an anti-pathogen CD4+ or CD8+ T cell response, production of a Th1 cytokine, or a T cell mediated immune response.
In additional various methods embodiments, an antibody or subsequence thereof and a second active, such as a different antibody, an agent or a drug are administered to a subject, one or more times, as a combination (e.g., an IL-10R antibody or subsequence thereof is administered as a combination composition with another antibody, agent or drug to a subject). In further various methods embodiments, an antibody or subsequence thereof and a second active, such as a different antibody, an agent or a drug are administered to a subject, one or more times, sequentially (e.g., an IL-10R antibody or subsequence thereof and an agent or drug are administered separately to a subject, in a sequence). Additional method embodiments include, for example, second actives such as type I interferons, toll receptor ligands, T cell costimulatory molecules such as OX40, 4-IBB and antagonists to inhibitory receptors or ligands such as antibodies that bind to CTLA4, PD-I, PD-L1, CD160 and LAG3.

Methods of the invention also include increasing numbers or activation of an immune cell in a subject with or at risk of a pathogen infection. In one embodiment, a method includes administering to a subject an amount of IL-10 receptor (IL-10R) antibody or subsequence thereof sufficient to increase numbers or activation of the immune cell in the subject. In another embodiment, a method includes administering to a subject an amount of an IL-10 receptor alpha (IL-10R alpha) antibody or subsequence thereof and administering a pathogen antigen, live or attenuated pathogen, or a nucleic acid encoding all or a portion (e.g., an epitope) of any protein or proteinaceous pathogen antigen sufficient to increase numbers or activation of the immune cell in the subject. In particular aspects, the immune cell is a T cell, NKT cell, dendritic cell (DC), macrophage, neutrophil, eosinophil, mast cell, CD4+ or a CD8+ cell, CD 14+, CD1 Ib+ or CD1 lc+ cells.

Methods of the invention further include, among other things, increasing or inducing an anti-pathogen CD8+ or CD4+ T cell response in a subject with or at risk of a pathogen infection. In one embodiment, a method includes administering to a subject in need thereof an amount of IL-10 receptor (IL-10R) antibody or subsequence thereof sufficient to increase or induce an anti-pathogen CD8+ or CD4+ T cell response, including proliferation, cytokine secretion or cytotoxicity, or chemokine expression or production in the subject. In another embodiment, a method includes administering to a subject an amount of an IL-10 receptor alpha (IL-10R alpha) antibody or subsequence thereof and administering a pathogen antigen, live or attenuated pathogen, or a nucleic acid encoding all or a portion (e.g., an epitope) of any protein or proteinaceous pathogen antigen sufficient to increase or induce an anti-pathogen CD8+ or CD4+ T cell response, including proliferation, cytokine secretion or cytotoxicity, or chemokine expression or production in the subject.

Methods of the invention additionally include, among other things, increasing production of a Th1 cytokine (e.g., interferon gamma, IL-1 alpha, IL-1 beta, IL-2, TNF-alpha, IL-6, IL-9, IL-12, IL-18, GM-CSF, etc.) or a chemokine (e.g., MCP1, MCP5, RANTES, IL-8, IP-10, MIP-2, etc.). In one embodiment, a method includes administering to a subject in need thereof an amount of IL-10 receptor (IL-10R) antibody or subsequence thereof sufficient to increase production of a Th1 cytokine (e.g., interferon gamma, IL-1 alpha, IL-1 beta, IL-2, TNF-alpha, IL-6, IL-12, GM-CSF, etc.) or a chemokine (e.g., MCP1, MCP5, RANTES, IL-8, IP-10, MIP-2, etc.) in the subject. In another embodiment, a
method includes administering to a subject an amount of an IL-10 receptor alpha (IL-10R alpha) antibody or subsequence thereof and administering a pathogen antigen, live or attenuated pathogen, or a nucleic acid encoding all or a portion (e.g., an epitope) of any protein or proteinaceous pathogen antigen sufficient to increase production of a Th1 cytokine (e.g., interferon gamma, IL-1 alpha, IL-1 beta, IL-2, TNF-alpha, IL-6, IL-12, GM-CSF, etc.) or a chemokine (e.g., MCPl, MCP5, RANTES, IL-8, IP-10, MIP-2, etc.) in the subject.

[0032] Methods of the invention include, among other things, methods that provide a therapeutic or beneficial effect to a subject. In various non-limiting embodiments, a method decreases, reduces, inhibits, suppresses, controls or limits pathogen numbers or titer; decreases, reduces, inhibits, suppresses, prevents, controls or limits pathogen proliferation or replication; decreases, reduces, inhibits, suppresses, prevents, controls or limits the amount of a pathogen protein; or decreases, reduces, inhibits, suppresses, prevents, controls or limits the amount of a pathogen nucleic acid. In additional embodiments, a method increases, stimulates, enhances, promotes, augments or induces pathogen clearance or removal; increases, induces, enhances, augments, promotes or stimulates an immune response against a pathogen; decreases, reduces, inhibits, suppresses, prevents, controls or limits pathogen pathology; decreases, reduces, inhibits, suppresses, prevents, controls or limits increases in pathogen numbers or titer; decreases, reduces, inhibits, suppresses, prevents, controls or limits increases in pathogen proliferation or replication, a pathogen protein, or a pathogen nucleic acid. In further embodiments, a method decreases, reduces, inhibits, suppresses, prevents, controls or limits pathogen reactivation from latency, or decreases, reduces, inhibits, suppresses, prevents, controls or limits transmission of pathogen to a host (e.g., transmission of a pathogen from an infected subject to an uninfected subject or susceptible subject). In yet additional embodiments, a method decreases, reduces, inhibits, suppresses, prevents, controls, limits or improves one or more adverse (e.g., physical or physiological) symptoms, disorders, illnesses, diseases or complications associated with or caused by pathogen infection, reactivation from latency or pathology. In still further embodiments, a method provides a subject with protection against a pathogen infection, reactivation from latency, or pathology, or decreases, reduces, inhibits, or limits susceptibility or probability of a subject to a pathogen infection, reactivation from latency, or pathology.

[0033] In various additional non-limiting embodiments, pathogen infection, proliferation or pathogenesis or reactivation from latency, is reduced, decreased, inhibited, limited, delayed or prevented, or a method decreases, reduces, inhibits, suppresses, prevents, controls or limits one or more adverse (e.g., physical or physiological) symptoms, disorders, illnesses, diseases or complications caused by or associated with chronic or acute pathogen infection, proliferation or replication, pathology or reactivation from latency. In additional various non-limiting embodiments, a method reduces, decreases, inhibits, delays or prevents onset, progression, frequency, duration, severity, probability or susceptibility of one or more adverse symptoms, disorders, illnesses, diseases or complications caused by or associated with chronic or acute pathogen infection, proliferation or replication, pathology or reactivation from latency. In further various non-limiting embodiments, a method accelerates, facilitates, enhances, augments, or hastens recovery of a subject from a pathogen infection, reactivation from latency or pathogenesis, or one
or more adverse symptoms, disorders, illnesses, diseases or complications caused by or associated with chronic or acute pathogen infection, proliferation or replication, pathology or reactivation from latency. In yet additional non-limiting embodiments, a method stabilizes a pathogen infection, proliferation, replication, pathogenesis, or an adverse symptom, disorder, illness, disease or complication caused by or associated with chronic or acute pathogen infection, proliferation or replication, pathology or reactivation from latency, or decreases, reduces, inhibits, suppresses, prevents, limits or controls transmission of a pathogen from an infected host to an uninfected host.

[0034] The invention also provides kits that include an IL-10 receptor (IL-1OR) antibody or subsequence thereof. Such kits optionally include a pathogen antigen, live or attenuated pathogen, and further optionally include instructions for treating (prophylactic or therapeutic), vaccinating or immunizing a subject against a pathogen infection, or treating (prophylactic or therapeutic) a subject having or at risk of having a pathogen infection, proliferation, reactivation or pathogenesis. Exemplary non-limiting BL-10 receptor (IL-10R) antibody or subsequence thereof for inclusion in kits include antibody (polyclonal or monoclonal), as set forth herein.

Description of Drawings

[0035] Figures IA-IB show flow cytometric analysis with human anti-human IL-1OR α antibodies. Total human PBMC were stained with biotinylated anti-human DL-1ORa antibodies in the presence (shaded histograms) or absence (filled histograms) of soluble human IL-1OR α protein. Binding of antibodies was detected with streptavidin-PE. The open histogram represents staining with isotype control antibodies. The lymphocyte (A) and monocyte (B) gates were set based on the forward and side scatter profile.

[0036] Figures 2A-2B show relative binding affinity of anti-human DL-1OR antibodies for human IL-1OR α. A) Titration of anti-DL-10Rα antibodies binding to coated human IL-10R α-hFc. Binding was detected with anti-human kappa-HRP, anti-mouse IgG-HRP, or anti-rat IgG-HRP. These data were used to determine the KD and BMAX (Tables 3 and 5); B) Binding to human IL-1 ORα on the surface of the B cell line RPMI-8226 by the human anti-human DL-1ORa monoclonal antibodies. RPMI-8226 cells were labeled with anti-human IL-1OR α antibodies at various concentrations and detected with anti-human IgG-PE. The commercial rat anti-human IL-1OR α antibody 3F9 was detected with anti-rat IgG-PE. The geometric mean fluorescence intensity (geo mean) data are shown.

[0037] Figures 3A-3B show data indicating that binding of anti-human IL-1OR α cells to human IL-1ORα is blocked by pre-binding of human DL-10 to the DL-10 receptor. RPMI-8226 cells were stained with human IL-10 biotin followed by anti-human IL-1OR α antibodies, and binding of antibodies was detected with anti-human IgG-PE or anti-rat IgG-PE. A) Filled histograms represent the maximum binding of the antibodies in the absence of DL-10, the open histograms are in the presence of human IL-10; B) Maximum DL-10 binding (closed histogram) is inhibited by anti-DL-10 (open histogram.) The shaded
histogram represents binding of a negative control protein. Binding of IL-10 was detected with streptavidin-FITC.

[0038] Figure 4 shows data indicating that three human antibodies can be divided into two groups based on competition for binding to IL-10Rα. Individual antibodies were coated in the wells of a 96 well plate. Biotinylated hIL-10Rα:hFc was pre-incubated with soluble anti-IL-10Rα antibodies and then added to coated wells. Binding of hDL-10Rα:hFc to the coated antibody was detected with streptavidin-HRP. Percent inhibition (y-axis) was determined using the following formula (100 - (OD study sample/OD maximum binding sample)) * 100.

[0039] Figures 5A-5B show data indicating that neutralization of IL-10 enhances TNF-α secretion by human PBMC. A) LPS treatment induces TNF-α secretion by human PBMC (open circle), IL-10 blocks TNF-α secretion (gray circle). Addition of anti-human IL-10Rα antibodies increases TNF-α secretion in a dose dependent manner. Panel representative of 10 studies with six donors. B) Effectiveness of human anti-human IL-10Rα antibody 136C8 compared with commercial antibodies. Antibody 136C8 neutralizes IL-10 blockade of TNF-α more robustly than commercial IL-10Rα antibodies 3F9 and 37607.

[0040] Figures 6A-6C show data indicating cross-reactivity with non-human primate IL-10Rα. PBMC from humans (A), chimpanzees (B), and cynomolgus macaques (C) were stained with anti-human IL-10Rα antibodies in the absence (filled) or presence (shaded) of recombinant human IL-10Rα, or with an isotype control antibody (open histograms). Histograms represent staining of IL-10Rα on cells in the lymphocyte gate based on forward and side scatter properties. Staining on monocytes was similar.

[0041] Figure 7A-7B shows data indicating relative binding affinity of anti-human IL-10Rα antibodies for chimp IL-10Rα:hFc (panel A) and cynomolgus macaque IL-10Rα:hFc (panel B), as determined by ELISA. Titration of anti-IL-10Rα antibodies binding to coated chimp or cynomolgus IL-10Rα:hFc. Binding was detected with anti-human kappa-HRP, anti-mouse IgG-HRP, or anti-rat IgG-HRP. Different anti-rat-IgG HRP and anti-mouse IgG antibodies were used to generate the data in panels A and B. The nature of these secondary antibodies can affect the results and lead to different maximum binding (BMAX). The BMAX from these studies are described in Table 5.

[0042] Figure 8 shows data indicating that anti-IL-10Rα antibodies cross-react with chimpanzee IL-10Rα. LPS induction of TNF-α secretion by chimpanzee PBMC was inhibited by human IL-10. Addition of anti-IL-10Rα neutralized IL-10 suppression of TNF-α secretion. This study was repeated twice with two different donors.

[0043] Figures 9A-9B show data indicating a subset of IL-10Rα antibodies functionally cross-react with cynomolgus macaque IL-10Rα. LPS induction of TNF-a by cynomolgus PBMC (open circle) was inhibited by human IL-10 (gray circle). Addition of 136C5 and 136C8 neutralized IL-10 and enhanced TNF-α secretion, while 136D29, 3F9, SPM466, and 37607 did not detectably block IL-10
suppression. Panels A and B represent results from two different animals. Similar results have been observed with 5 different donors.

[0044] Figures 10A - 10B show data demonstrating the ability of the anti-IL-10R α antibodies to restore antigen-induced cytokine secretion from a human NKT cell line treated with IL-10. KRN7000 (Ag only, X symbol) induction of IFN-γ (A) and TNF-α (B) was inhibited by human IL-10 (gray circle). Addition of 136C5, 136C8, 136D29 and 3F9 neutralized IL-10 and restored IFN-γ and TNF-α secretion in a dose dependent manner. Data representative of responses by two human NKT cell lines.

[0045] Figures HA - HC show the additional antagonist activity of the human anti-human IL-10Ra antibodies and the lack of agonist activity. A. HLA-DR expression on human PBMC (solid black bar) is decreased by treatment with IL-10 (solid gray bar). Anti-human IL-10Ra antibodies restore HLA-DR expression on PBMC treated with IL-10. Levels of expression are represented as Geometric mean fluorescence intensity. B-C. STAT3 is phosphorylated in human PBMC following treatment with IL-10. B. Left and middle panels. Incubation with 136C8 or a negative control antibody in the presence of IL-10 prevents STAT3 phosphorylation in a dose dependent manner. Doses were 3, 1.5, 0.75, and 0.38 µg/ml. Right panel. Incubation with 3 µg/ml 136C8, 37607, or SPM466 in the presence of IL-10 show different levels of inhibition of STAT3 phosphorylation. C. Incubation with 136C8 in the presence of a crosslinking antibody, anti-hlgGl, and in the absence of IL-10 does not induce STAT3 phosphorylation.

[0046] Figures 12A-12G show binding of the anti-human IL-10Ra antibodies, 136C8 (A), 136D29 ( ), 3F9 ( ), 37607 ( ) and SPM466 (D, last panel only), to EL-IO itself (A) or single nucleotide polymorphism variants of human EL-10Rα, namely B, L611, C, V1131, D, S159G, E, R212E, F, V233M, and G, R212E.

[0047] Figure 13A - 13B show data demonstrating the neutralizing activity of anti-human IL-10Ra antibodies for cytomegalovirus IL-10. Addition of 136C5, 136C8, 136D29, 3F9, SPM466, or 37607 to human PBMC treated with LPS + CMV IL-10 restored TNF-α production from two different healthy donors. Panels A and B are data from two donors.

**Detailed Description**

[0048] The invention is based at least in part on antibodies and subsequences thereof that specifically bind to IL-10 Receptor alpha protein (IL-10Ra). Invention antibodies and subsequences, including human monoclonal antibodies, are useful in treatment, detection and diagnostic methods. For example, invention antibodies and subsequences are useful in methods of treating a subject for a pathogen infection (chronic or acute). Such treatment methods include therapeutic (following pathogen infection) and prophylactic (prior to pathogen infection) methods including, for example, methods of treating a subject with a pathogen infection, and methods of protecting a subject from a pathogen infection (e.g., provide the subject with protection against pathogen infection), to decrease or reduce the probability of a pathogen infection in a subject, to decrease or reduce susceptibility of a subject to a
pathogen infection, or to inhibit or prevent a pathogen infection in a subject, and to decrease, reduce, inhibit or suppress transmission of the pathogen from one subject to another subject.

[0049] In accordance with the invention, there are provided antibodies and subsequences thereof that specifically bind to IL-10 Receptor alpha protein (DL-IORα). In one embodiment, an antibody or subsequence thereof specifically binds to IL-10 Receptor alpha protein, and reduces, inhibits or competes for binding of an antibody designated 136C5, 136C8, or 136D29 to the IL-10 Receptor alpha protein. In another embodiment, an antibody or subsequence thereof specifically binds to IL-10 Receptor alpha protein, and reduces, inhibits or competes for binding of an antibody or subsequence thereof comprising a heavy chain variable region sequence of any of SEQ ID NOs:29, 31 or 33, and a light chain variable region sequence of any of SEQ ID NOs:30, 32, or 34, to the IL-10 Receptor alpha protein. In a further embodiment, an antibody or subsequence thereof specifically binds to IL-10 Receptor alpha protein, and does not detectably reduce, inhibit or compete for binding of antibody designated 3F9, SPM466, or 37607 to the IL-10 Receptor alpha protein.

[0050] In accordance with the invention, there are provided antibodies and subsequences thereof that specifically bind to more than one species type of IL-1 ORα. In one embodiment, an antibody or subsequence thereof specifically binds to human IL-1 ORα, and optionally also binds to chimpanzee IL-10Ra or binds to cynomolgus macaque IL-1 ORα. In another embodiment, an antibody or subsequence thereof specifically binds to human IL-1 ORα, and binds to chimpanzee IL-1 ORα or binds to cynomolgus macaque DL-10Ra. In a further embodiment, an antibody or subsequence thereof specifically binds to human IL-1 ORα, to chimpanzee IL-1 ORα and to cynomolgus macaque IL-1 ORα.

[0051] In further embodiments, antibodies and subsequences thereof specifically bind to IL-10 Receptor alpha protein (D_-10Ra) and modulate an IL-1OR/IL-10 signaling activity. In one aspect, an antibody or subsequence thereof reduces, inhibits, decreases, suppresses or limits an IL-1OR/IL-10 signaling activity. In another aspect an antibody or subsequence thereof reduces, inhibits, decreases, suppresses or limits an IL-1OR/IL-10 signaling activity greater than the reduction or inhibition of IL-10 signaling activity by any of 3F9, SPM466 or 37607 antibodies.

[0052] In further aspects, an antibody or subsequence thereof specifically binds to a human, a chimpanzee or cynomolgus macaque IL-1 ORα, and reduces, inhibits, decreases, suppresses or limits an activity of human, chimpanzee or cynomolgus macaque IL-1 ORα (e.g., IL-1OR/IL-10 signaling). In still further particular aspects, an antibody or subsequence thereof specifically binds to a human IL-1 ORα, and one or both of a chimpanzee IL-1 ORα and cynomolgus macaque IL-1 ORα, and reduces, inhibits, decreases, suppresses or limits an activity of human, chimpanzee or cynomolgus macaque IL-1 ORα (e.g., IL-1OR/IL-10 signaling). In another embodiment, an invention antibody or subsequence thereof reverses or limits IL-10 suppression, inhibition, or reduction of TNF-alpha, IL-6, IL-1β or IFN gamma expression or secretion by PBMCs, which is reflected by an increase in TNF-alpha, IL-6, IL-1β or IFN gamma expression or secretion by PBMCs (e.g., human, chimpanzee or macaque) treated with LPS in vitro in the presence of IL-10. In still another embodiment, an invention antibody or subsequence thereof increases
or induces TNF-alpha or IFN-gamma expression by NKT cells in the presence of IL-10 and the antigen KRN7000, at least partially restores expression of the HLA-DR MHC class II molecule in the presence of IL-10, or inhibits or reduces IL-10 induced phosphorylation of STAT3. In particular aspects, an invention antibody or subsequence thereof increases or induces TNF-alpha or IFN-gamma expression by PBMC or NKT cells in the presence of EL-10, restores expression of the HLA-DR MHC class II molecule in the presence of IL-10, or inhibits or reduces EL-10 induced phosphorylation of STAT3 greater than another reference antibody, such as any of 3F9, SPM466 or 37607 antibodies.

[0053] In an additional embodiment, an antibody or subsequence thereof specifically binds to EL-10 Receptor alpha protein, and binds to an epitope distinct from the epitope to which antibody designated 3F9, SPM466, or 37607 binds. In particular aspects, antibodies and subsequences thereof may reduce or inhibit binding of a reference antibody to EL-10R by less than 50%, by about 50% or more, e.g., 50-70% or, by about 70% or more.

[0054] In accordance with the invention, there are also provided antibodies and subsequences thereof that specifically bind to EL-10 Receptor alpha protein (EL-10Ra), and that exhibit sequence identity to a heavy or light chain variable region sequence of antibody designated 136C5, 136C8, or 136D29, or a heavy chain variable region sequence of any of SEQ ID NOs:29, 31 or 33, or a light chain variable region sequence of any of SEQ ID NOs:30, 32, or 34. In one embodiment, an antibody or subsequence thereof that specifically binds to EL-10Ra includes a sequence at least 60% or more (e.g., 65%, 70%, 75%, 80%, 85%, 90%, 95%, etc.) identical to any heavy chain variable region sequence set forth as SEQ ID NOs:29, 31 or 33, and a sequence at least 60% or more (e.g., 65%, 70%, 75%, 80%, 85%, 90%, 95%, etc.) identical to any light chain variable region sequence set forth as SEQ ID NOs:30, 32, or 34.

[0055] In another embodiment, an antibody or subsequence thereof that specifically binds to EL-10Ra includes any heavy chain variable region sequence set forth as SEQ ID NOs:29, 31 or 33 and any light chain variable region sequence set forth as SEQ ID NOs:30, 32, or 34, wherein the antibody or subsequence has one or more amino acid additions, deletions or substitutions of SEQ ID NOs:29, 31 or 33, or SEQ ID NOs:30, 32, or 34. In particular aspects, a sequence is at least 80% or more, e.g., 80-85%, 85-90%, 90-95%, 95-100% identical to any heavy chain variable region sequence set forth as SEQ ID NOs:29, 31 or 33, or any light chain variable region sequence set forth as SEQ ID NOs:30, 32, or 34. In further aspects, an antibody that specifically binds to EL-10Ra includes or consists of any one of a heavy chain variable region sequence set forth as SEQ ID NOs:29, 31 or 33, or a light chain variable region sequence set forth as SEQ ID NOs:30, 32, or 34. In particular aspects, antibodies and subsequences thereof may reduce or inhibit binding of a reference antibody to EL-10R by about 50% or more, e.g., 70% or more.

[0056] The term "antibody" refers to a protein that binds to other molecules (antigens) via heavy and light chain variable domains, V_H and V_L, respectively. Antibodies include full-length antibodies that include two heavy and two light chain sequences. Antibodies can have kappa or lambda light chain
sequences, either full length as in naturally occurring antibodies, mixtures thereof (i.e., fusions of kappa and lambda chain sequences), and subsequences/fragments thereof. Naturally occurring antibody molecules contain two kappa or two lambda light chains.

[0057] Antibodies include monoclonal or polyclonal immunoglobulin molecules that belong to any class such as IgM, IgG, IgA, IgE, IgD, and any subclass thereof. Exemplary subclasses for IgG are IgG1, IgG2, IgG3 and IgG4. A "monoclonal" antibody refers to an antibody that is based upon, obtained from or derived from a single clone, including any eukaryotic, prokaryotic, or phage clone. A "monoclonal" antibody is therefore defined structurally, and not the method by which it is produced.

[0058] An IL-10 receptor (IL-10R) antibody or subsequence thereof, which can also be referred to as "IL-10R antibody," "anti- IL-10R" and "anti-IL-10R antibody" refers to a polyclonal or monoclonal antibody that specifically binds to IL-10 receptor (IL-10R). The term "bind," or "binding," when used in reference to an antibody, means that the antibody or subsequence thereof interacts at the molecular level with a corresponding epitope (antigenic determinant) present on an antigen. Thus, an antibody specifically binds to all or a part of sequence or an antigenic epitope present on IL-10R. Specific binding is that which is selective for an epitope present in IL-10R. Antibodies and subsequences thereof include specific or selective binding to IL-10R alpha or beta subunits, or an epitope comprising both alpha and beta subunits of IL-10R. Specific and selective binding can be distinguished from non-specific binding using assays known in the art (e.g., immunoprecipitation, ELISA, flow cytometry, Western blotting).

[0059] Epitopes typically are short amino acid sequences, e.g. about five to 15 amino acids in length. Epitopes can be contiguous or non-contiguous. A non-contiguous amino acid sequence epitope forms due to protein folding. For example, an epitope can include a non-contiguous amino acid sequence, such as a 5 amino acid sequence and an 8 amino acid sequence, which are not contiguous with each other, but form an epitope due to protein folding. Techniques for identifying epitopes are known to the skilled artisan and include screening overlapping oligopeptides for binding to antibody (for example, U.S. Patent No. 4,708,871), phage display peptide library kits, which are commercially available for epitope mapping (New England BioLabs). Epitopes may also be identified by inference when epitope length peptide sequences are used to immunize animals from which antibodies that bind to the peptide sequence are obtained and can be predicted using computer programs, such as BEPTOPE (Odorico et al., /. Mol. Recognt. 16:20 (2003)).

[0060] IL-10 receptor (IL-10R) antibodies and subsequences thereof bind to IL-10R in solution or in solid phase, on cells in vitro or in vivo or in situ. BL-10R can also be present in vivo, such as on one or more cells in vivo, in vitro, in primary cell isolates, passaged cells, cultured cells, immortalized cells and cells ex vivo. Antibody binding to wild type EL-10R expressed by cells typically bind to IL-10R extracellular domain (see, e.g., SEQ ID NO:3). Specific non-limiting cell types that can express IL-10R include activated and other T cells (e.g., naive, effector, memory or regulatory T cells, CD4+ and CD8+ T cells, NKT cells) and non-T cells. Examples of non-T cells include natural killer (NK) cells, granulocytes (neutrophils), eosinophils, monocytes, macrophages, mast cells and dendritic cells (DC). Cells that do not naturally express BL-10R can be made to express EL-10R, for example, by transfecting
or transforming cells with an IL-10R encoding nucleic acid. IL-10 receptor (IL-IOR) antibodies and subsequences thereof can bind to one or more transfected or transformed cells that express or produce DL-10R.

[0061] IL-10 may, but need not, reduce, decrease or inhibit binding of antibodies to IL-10R. In certain embodiments, binding of an antibody or subsequence thereof to IL-10R is reduced, decreased or inhibited by binding of IL-10 to IL-10R. In other embodiments, binding of antibody or subsequence thereof to DL-10R is not detectably blocked, prevented, reduced, decreased or inhibited by binding of IL-10 to IL-10R.

[0062] IL-10 receptor (IL-IOR) antibodies and subsequences thereof bind to IL-10 receptor (DL-10R), including mammalian (e.g., primate, such as chimp, macaque and human) forms of IL-10 receptor (DL-10R). IL-10 receptor (IL-IOR) antibodies and subsequences thereof may bind to primate IL-IOR, such as human IL-10R, but may not detectably bind to chimp IL-10R, or macaque DL-10R. A non-limiting example of IL-10 receptor alpha chain (IL-I ORα) is a human sequence set forth as:

MLPCWLALLA ALSSLRLGSD AHGTELPSPP SVWFEAEFFH HILHWTPPQN QSESTCYEVA
LLRYGIESWN SISNCSQTLS YDLTAVTDLV YHSGNYRARV RAVDGSRHSSN WTVTTNFSTV
DEVTLVGSV NLEHINGFIL GKIQLPRPKM APANDTYESI FSHFREYEIA IRKVGPNFTF
THKKVKHENF SLLTSEGVEG FCVQVKPSVA SRSNKGMSK EECISLTRQY FTVTNVIIFF
AFVLLLSGL AYCLALQLYV RRRKKLPSVL LFKKTPSPFF ISQRPSPETQ DTHIPLDEEA
FKVSPKKN LDLHGSTDSD FGSTKPSQTL EEQFOLLQDP HPQADRTLGNE GEPPVLDGSD
SSGSSNSTDS GICQEPSLS PSTQPTWEOQ VGSNSRGQDD SGDVLQVQNSRE GRADTQGGS
ALGHHSPPEP EVPGEDEPA VAFQGYLRQT RCAAQATKGT GCLEEESPIL DGLGPKFGRC
LVDEAGLHPP ALARKYLKDQ PLEMTLASSG APTGQWKNQPT EEWSLLALSS CSDLGSDWS
FAHDLAPLGC VAAPGGILLS FNSDLVTLPL ISSLQSSSE, **SEQ ID NO:2**

[0063] The term "isolated," when used as a modifier of a composition (e.g., antibodies, subsequences, modified forms, nucleic acids encoding same, etc.), means that the compositions are made by the hand of man or are separated, completely or at least in part, from their naturally occurring *in vivo* environment. Generally, isolated compositions are substantially free of one or more materials with which they normally associate with in nature, for example, one or more protein, nucleic acid, lipid, carbohydrate, cell membrane. The term "isolated" does not exclude alternative physical forms of the composition, such as fusions/chimeras, multimers/oligomers, modifications (e.g., phosphorylation, glycosylation, lipidation) or derivatized forms, or forms expressed in host cells produced by the hand of man.

[0064] An "isolated" composition (e.g., an antibody) can also be "substantially pure" or "purified" when free of most or all of the materials with which it typically associates with in nature. Thus, an isolated antibody that also is substantially pure or purified does not include polypeptides or polynucleotides present among millions of other sequences, such as antibodies of an antibody library or nucleic acids in a genomic or cDNA library, for example. A "substantially pure" or "purified" composition can be obtained from one or more other molecules. Thus, "substantially pure" or "purified"...
does not exclude combinations of compositions, such as combinations of IL-1OR antibodies or subsequences, and other antibodies, agents, drugs or therapies.

[0065] Antibodies include mammalian, primatized, humanized, fully human antibodies and chimeras. A mammalian antibody is an antibody which is produced by a mammal, transgenic or non-transgenic, or a non-mammalian organism engineered to produce a mammalian antibody, such as a non-mammalian cell (bacteria, yeast, insect cell), animal or plant.

[0066] The term "human" when used in reference to an antibody, means that the amino acid sequence of the antibody is fully human, i.e., human heavy and human light chain variable and human constant regions. Thus, all of the amino acids are human or exist in a human antibody. An antibody that is non-human may be made fully human by substituting the non-human amino acid residues with amino acid residues that exist in a human antibody. Amino acid residues present in human antibodies, CDR region maps and human antibody consensus residues are known in the art (see, e.g., Kabat, Sequences of Proteins of Immunological Interest, 4th Ed.US Department of Health and Human Services. Public Health Service (1987); Chothia and Lesk (1987). A consensus sequence of human $V_H$ subgroup III, based on a survey of 22 known human $V_H$ III sequences, and a consensus sequence of human $V_L$ kappa-chain subgroup I, based on a survey of 30 known human kappa I sequences is described in Padlan Mol. Immunol. 31:169 (1994); and Padlan Mol. Immunol. 28:489 (1991). Human antibodies therefore include antibodies in which one or more amino acid residues have been substituted with one or more amino acids present in any other human antibody.

[0067] The term "humanized" when used in reference to an antibody, means that the amino acid sequence of the antibody has non-human amino acid residues (e.g., mouse, rat, goat, rabbit, etc.) of one or more complementarity determining regions (CDRs) that specifically bind to the desired antigen in an acceptor human immunoglobulin molecule, and one or more human amino acid residues in the Fv framework region (FR), which are amino acid residues that flank the CDRs. Such antibodies typically have reduced immunogenicity and therefore a longer half-life in humans as compared to the non-human parent antibody from which one or more CDRs were obtained or are based upon.

[0068] Antibodies referred to as "primatized" are "humanized" except that the acceptor human immunoglobulin molecule and framework region amino acid residues may be any primate amino acid residue (e.g., ape, gibbon, gorilla, chimpanzees orangutan, macaque), in addition to any human residue. Human FR residues of the immunoglobulin can be replaced with corresponding non-human residues. Residues in the CDR or human framework regions can therefore be substituted with a corresponding residue from the non-human CDR or framework region donor antibody to alter, generally to improve, antigen affinity or specificity, for example. A humanized antibody may include residues, which are found neither in the human antibody nor in the donor CDR or framework sequences. For example, a FR substitution at a particular position that is not found in a human antibody or the donor non-human antibody may be predicted to improve binding affinity or specificity human antibody at that position. Antibody framework and CDR substitutions based upon molecular modeling are well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues.
important for antigen binding and sequence comparison to identify unusual framework residues at
particular positions (see, e.g., U.S. Patent No. 5,855,089; and Riechmann et al., *Nature* 332:323 (1988)).

The term "chimeric" and grammatical variations thereof, when used in reference to an
antibody, means that the amino acid sequence of the antibody contains one or more portions that are
derived from, obtained or isolated from, or based upon two or more different species. For example, a
portion of the antibody may be human (e.g., a constant region) and another portion of the antibody may
be non-human (e.g., a murine heavy or murine light chain variable region). Thus, an example of a
chimeric antibody is an antibody in which different portions of the antibody are of different species
origins. Unlike a humanized or primatized antibody, a chimeric antibody can have the different species
sequences in any region of the antibody.

IL-10R antibodies and subsequences of the invention include those having at least partial
sequence identity to a heavy or light chain constant or variable region sequence of 136C5, 136C8 or
136D29, or a heavy chain variable region sequence of any of SEQ ID NOs:29, 31 or 33, or a light chain
variable region sequence of any of SEQ ID NOs:30, 32, or 34. The percent identity of such antibodies
and subsequences thereof can be as little as 60%, or can be greater (e.g., 65%, 70%, 75%, 80%, 85%,
90%, 95%, 96%, 97%, 98%, 99%, etc.). The percent identity can extend over the entire sequence length
of a heavy or light chain constant or variable region sequence of 136C5, 136C8 or 136D29, or a heavy
chain variable region sequence of any of SEQ ID NOs:29, 31 or 33, or a light chain variable region
sequence of any of SEQ ID NOs:30, 32, or 34. In particular aspects, the length of the sequence sharing
the percent identity is 5 or more contiguous amino acids, e.g., 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18,
19, 20, etc. contiguous amino acids. In additional particular aspects, the length of the sequence sharing
the percent identity is 20 or more contiguous amino acids, e.g., 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30,
31, 32, 33, 34, 35, etc. contiguous amino acids. In further particular aspects, the length of the sequence
sharing the percent identity is 35 or more contiguous amino acids, e.g., 35, 36, 37, 38, 39, 40, 41, 42, 43,
44, 45, 47, 48, 49, 50, etc., contiguous amino acids. In yet further particular aspects, the length of the
sequence sharing the percent identity is 50 or more contiguous amino acids, e.g., 50-55, 55-60, 60-65,
65-70, 70-75, 75-80, 80-85, 85-90, 90-95, 95-100, 100-110, etc. contiguous amino acids.

The term "identity" and grammatical variations thereof, mean that two or more referenced
entities are the same. Thus, where two antibody sequences are identical, such as heavy or light chain
variable region sequences, they have the same amino acid sequence. The identity can be over a defined
area (region or domain) of the sequence. "Areas, regions or domains" of homology or identity mean that
a portion of two or more referenced entities share homology or are the same.

The extent of identity between two sequences can be ascertained using a computer program
and mathematical algorithm known in the art. Such algorithms that calculate percent sequence identity
(homology) generally account for sequence gaps and mismatches over the comparison region or area.
For example, a BLAST (e.g., BLAST 2.0) search algorithm (see, e.g., Altschul et al., *J. Mol. Biol.*
215:403 (1990), publicly available through NCBI) has exemplary search parameters as follows:
Mismatch -2; gap open 5; gap extension 2. For polypeptide sequence comparisons, a BLASTP algorithm
is typically used in combination with a scoring matrix, such as PAM100, PAM 250, BLOSUM 62 or BLOSUM 50. FASTA (e.g., FASTA2 and FASTA3) and SSEARCH sequence comparison programs are also used to quantitate the extent of identity (Pearson et al., Proc. Natl. Acad. Sci. USA 85:2444 (1988); Pearson, Methods Mol Biol. 132:185 (2000); and Smith et al., J. Mol. Biol. 147:195 (1981)). Programs for quantitating protein structural similarity using Delaunay-based topological mapping have also been developed (Bostick et al., Biochem Biophys Res Commun. 304:320 (2003)).

[0073] In accordance with the invention, there are provided antibodies and subsequences thereof that specifically bind IL-10R alpha protein and include one, two or all three CDRs of a heavy or a light chain variable region sequence of antibody designated 136C5, 136C8 or 136D29; one, two or all three CDRs of a heavy chain variable region sequence of any of SEQ ID NOs:29, 31 or 33; or one, two or all three CDRs of a light chain variable region sequence of any of SEQ ID NOs:30, 32, or 34. Exemplary heavy chain variable region CDR sequences (SEQ ID NOs:49-55) are as follows: SYSMN; YISTGSSTIYADVSKG; ENYYGSGSYEDYFDY; YISTRSTIYADVSKG; ELSMH; GFDPDDGETIYAQKFQG; and GGYYGPVGMDV. Exemplary light chain variable region CDR sequences (SEQ ID NOs:56-61) are as follows: RASQSVSSYLA; DASN creat; QQRSNWPIFT; RASQGISWLA; AASSLQS; and QQNSYPLT. In particular aspects, an antibody or a subsequence that specifically binds IL-10R alpha protein includes all three CDRs of a heavy chain variable region of 136C5, 136C8 or 136D29; or any of SEQ ID NOs:29, 31 or 33, and all three CDRs of a light chain variable region of 136C5, 136C8 or 136D29; or any of SEQ ID NOs:30, 32, or 34. For example, any of a heavy chain variable region sequence with CDR1 (SYSMN), CDR2 (YISTGSSTIYADVSKG), and/or CDR3 (ENYYGSGSYEDYFDY) and a light chain variable region sequence with CDR1 (RASQSVSSYLA), CDR2 (DASN creat), and/or CDR3 (QQRSNWPIFT); a heavy chain variable region sequence with CDR1 (SYSMN), CDR2 (YISTRSTIYADVSKG), and/or CDR3 (ENYYGSGSYEDYFDY) and a light chain variable region sequence with CDR1 (RASQSVSSYLA), CDR2 (DASN creat), and/or CDR3 (QQRSNWPIFT); and heavy chain variable region sequence with CDR1 (ELSMH), CDR2 (GFDPDDGETIYAQKFQG), and/or CDR3 (GGYYGPVGMDV) and a light chain variable region sequence with CDR1 (RASQGISWLA), CDR2 (AASSLQS), and/or CDR3 (QQNSYPLT).

[0074] IL-10R antibodies and functional fragments can have substantially the same, greater or less relative activity or function than a reference antibody (e.g., 136C5, 136C8 or 136D29, or an antibody or subsequence thereof that includes a heavy chain variable region sequence of any of SEQ ID NOs:29, 31 or 33, and a light chain variable region sequence of any of SEQ ID NOs:30, 32, or 34). For example, an IL-10R antibody can have substantially the same, greater or less relative binding affinity or avidity for IL-10R than a reference antibody (e.g., 136C5, 136C8 or 136D29, or an antibody or subsequence thereof that includes a heavy chain variable region sequence of any of SEQ ID NOs:29, 31 or 33, and a light chain variable region sequence of any of SEQ ID NOs:30, 32, or 34). Such antibodies having measurable binding affinity for IL-10R compete for binding of the reference antibody (e.g., 136C5, 136C8 or 136D29, or an antibody or subsequence thereof that includes a heavy chain variable region sequence of
any of SEQ ID NOs: 29, 31 or 33, and a light chain variable region sequence of any of SEQ ID NOs: 30, 32, or 34) to IL-1OR. IL-10R antibodies and functional fragments therefore include those that compete with any of 136C5, 136C8 or 136D29 antibody, or an antibody or subsequence thereof that includes a heavy chain variable region sequence of any of SEQ ID NOs: 29, 31 or 33, and a light chain variable region sequence of any of SEQ ID NOs: 30, 32, or 34, for binding to IL-1OR, and have substantially the same, greater or less relative binding affinity or avidity for binding to IL-1OR as compared to a reference antibody (e.g., 136C5, 136C8 or 136D29, or an antibody or subsequence thereof that includes a heavy chain variable region sequence of any of SEQ ID NOs: 29, 31 or 33, and a light chain variable region sequence of any of SEQ ID NOs: 30, 32, or 34).  

[0075] IL-1OR antibodies and functional fragments can have a greater or less than 2-5, 5-10, 10-100, 100-1000 or 1000-10,000-fold binding affinity, KD, for binding to IL-1OR, or any numerical value or range within or encompassing such values, than a reference antibody (e.g., within 2-5, 5-10, 10-100, 100-1000 or 1000-10,000-fold of the binding affinity, KD, of 136C5, 136C8 or 136D29, or an antibody or subsequence thereof that includes a heavy chain variable region sequence of any of SEQ ID NOs: 29, 31 or 33, and a light chain variable region sequence of any of SEQ ID NOs: 30, 32, or 34, for binding to EL-10R). In one embodiment, an antibody or a functional thereof has a binding affinity, KD, within about 1-1000 fold (more or less than) of a reference antibody (e.g., 136C5, 136C8 or 136D29, or an antibody or subsequence thereof that includes a heavy chain variable region sequence of any of SEQ ID NOs: 29, 31 or 33, and a light chain variable region sequence of any of SEQ ID NOs: 30, 32, or 34), for binding to IL-10R.  

[0076] BL-1OR antibodies and functional fragments can have substantially the same binding affinity, KD, for binding to IL-10R as a reference antibody. In particular embodiments, an IL-1OR antibody has substantially the same binding affinity, KD, or avidity for IL-1OR as 136C5, 136C8 or 136D29, or an antibody or subsequence thereof that includes a heavy chain variable region sequence of any of SEQ ID NOs: 29, 31 or 33, and a light chain variable region sequence of any of SEQ ID NOs: 30, 32, or 34, for binding to IL-1OR.  

[0077] The term "substantially the same" when used in reference to antibody or functional fragment binding affinity or avidity for antigen, means that the binding is within 100 fold (greater or less than) of the binding affinity of a reference antibody for the antigen (e.g., EL-1OR). Binding affinity can be determined by association (KJ and dissociation (KD or Kd) rate. Equilibrium affinity constant, K, is the ratio of K/Kd. Association (Kd) and dissociation (KD or Kd) rates can be measured using surface plasmon resonance (SPR) (Rich and Myszka, Curr. Opin. Biotechnol. 11:54 (2000); Englebienne, Analyst. 123:1599 (1998)). Instrumentation and methods for real time detection and monitoring of binding rates are known and are commercially available (BiaCore 2000, Biacore AB, Upsala, Sweden; and Malmqvist, Biochem. Soc. Trans. 27:335 (1999)). Thus, for example, if binding of a reference antibody to EL-10R has a KD of 10^-9 M, than an antibody which has substantially the same binding affinity as the reference antibody will have a KD within the range of 10^-7 M to KD 10^-11 M for binding to EL-10R.
IL-10R antibodies and functional fragments can have a binding affinity, KD, for binding to IL-10R within about KD $10^{-2}$ M to about KD $10^{-15}$ M, or within about KD $10^{-6}$ M to about KD $10^{-12}$ M. In particular embodiments, binding affinity, KD, for binding to IL-10R is less than $5 \times 10^{-2}$ M, $10^{-2}$ M, $5 \times 10^{-3}$ M, $10^{-3}$ M, $5 \times 10^{-5}$ M, $10^{-5}$ M, $5 \times 10^{-7}$ M, $10^{-7}$ M, $5 \times 10^{-8}$ M, $10^{-8}$ M, $5 \times 10^{-9}$ M, $10^{-9}$ M, $5 \times 10^{-10}$ M, $10^{-10}$ M, $5 \times 10^{-11}$ M, $10^{-11}$ M, $5 \times 10^{-12}$ M, $10^{-12}$ M, $5 \times 10^{-13}$ M, $10^{-13}$ M, $5 \times 10^{-14}$ M, $10^{-14}$ M, $5 \times 10^{-15}$ M, and $10^{-15}$ M.

In accordance with the invention, there are provided antibodies and subsequences that include modified and variant forms. As used herein, the terms "modify" or "variant" and grammatical variations thereof, mean that an antibody or subsequence thereof deviates from a reference antibody or subsequence thereof (e.g., 136C5, 136C8 or 136D29 antibody, or an antibody or subsequence thereof that includes a heavy chain variable region sequence of any of SEQ ID NOs:29, 31 or 33, or a light chain variable region sequence of any of SEQ ID NOs:30, 32, or 34). Modified and variant antibodies and subsequences thereof may have greater or less activity or function than a reference antibody or an activity or function distinct from a reference antibody, but at least retain partial activity or function of the reference antibody (e.g., 136C5, 136C8 or 136D29 antibody, or an antibody or subsequence thereof that includes a heavy chain variable region sequence of any of SEQ ID NOs:29, 31 or 33, and a light chain variable region sequence of any of SEQ ID NOs:30, 32, or 34).

Non-limiting examples of modifications include one or more amino acid substitutions (e.g., 1-3, 3-5, 5-10, 10-15, 15-20, 20-25, or more residues), additions (e.g., insertions) and deletions (e.g., subsequences or fragments) of antibody constant or variable region sequences. In particular embodiments, a modified or variant antibody retains at least part of a function or an activity of unmodified antibody, e.g., binding affinity (e.g., KD or $K_D$) or binding specificity to IL-10R in vitro or a cell expressing IL-10R, or an activity, such as an antagonist activity of IL-10R, IL-10 or EL-10/IL-10R signaling pathway. Such modified forms and variants can have less than, the same, or greater, but at least a part of, a function or activity of a reference antibody or subsequence thereof, for example, binding to IL-10R, to reduce, decrease inhibit, suppress, limit, prevent or abrogate an activity or function of IL-10 or IL-10R, or the IL-10/IL-10R signaling pathway.

Specific non-limiting examples of substitutions include conservative and non-conservative amino acid substitutions. Substitutions can be within or outside of a constant region, a complementary determining region (CDR) or a framework region (FR) of the antibody. In particular embodiments, a heavy or light chain CDR (CDR1, CDR2 or CDR3) or FR will have 1-8, 1-5, 1-3 or fewer (e.g., 1 or 2) amino acid substitutions. In an additional embodiment, a substitution within a variable region sequence is not within a CDR. In another embodiment, a substitution within a variable region sequence is not within an FR. A particular non-limiting example of an amino acid substitution is a conservative substitution within or outside of a constant region, a complementary determining region (CDR) or a framework region (FR), for example, a substitution of one or more amino acid residues of a constant region, or any heavy or light chain variable region sequence of 136C5, 136C8 or 136D29 antibodies, or any heavy chain
variable region sequence of SEQ ID NOs: 29, 31 or 33, or any light chain variable region sequence of SEQ ID NOs: 30, 32, or 34.

[0082] A "conservative substitution" is the replacement of one amino acid by a biologically, chemically or structurally similar residue. Biologically similar means that the substitution does not destroy a biological activity. Structurally similar means that the amino acids have side chains with similar length, such as alanine, glycine and serine, or a similar size. Chemical similarity means that the residues have the same charge or are both hydrophilic or hydrophobic. Particular examples include the substitution of one hydrophobic residue, such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acids, or glutamine for asparagine, serine for threonine, and the like.

[0083] The structural determinants that contribute to antigen binding, such as complementarity determining regions (CDR) and framework regions (FR) within hypervariable regions are known in the art. The location of additional regions, such as D- and J-regions are also known. Antibodies and subsequences thereof that include one or more CDR sequences, optionally with flanking FR sequences, will typically have sufficient sequence identity to a heavy or light chain variable region sequence exemplified herein so as to retain at least partial function or activity of an antibody that includes a heavy and a light chain sequence exemplified herein, e.g., binding affinity (e.g., KD), avidity or binding specificity or selectivity to IL-10.

[0084] One or a few amino acid substitutions (e.g., 2, 3, 4 or 5) in heavy or light chain variable regions, within or outside a CDR, are likely to be tolerated. Non-conservative substitution of many amino acids in hypervariable regions is likely to affect binding activity, specificity or antibody function or activity. Regional mutability analysis can be used to predict the effect of particular substitutions in complementarity determining regions (CDR) and framework regions (FR) (Shapiro et al., J Immunol. 163:259 (1999)). In brief, sequence comparison indicates a hierarchy of mutability among di- and trinucleotide sequences located within Ig intronic DNA, which predicts regions that are more or less mutable. Quantitative structure-activity relationship (QSAR) can be used to identify the nature of the antibody recognition domain and, therefore, amino acids that participate in ligand binding. Predictive models based upon OSAR can in turn be used to predict the effect of substitutions (mutations). For example, the effect of mutations on the association and dissociation rate of an antibody interacting with its antigen has been used to construct quantitative predictive models for both kinetic (Kₐ and Kᵩ) constants, which can in turn be used to predict the effect of other mutations on the antibody (De Genst et al., J Biol Chem. 277:29897 (2002)). The skilled artisan can therefore use such analysis to predict amino acid substitutions of antibodies and subsequences that are likely to result in an antibody or subsequence that retains at least partial activity or function of non-substituted antibody or subsequence.

[0085] An addition can be the covalent or non-covalent attachment of any type of molecule to the antibody. Specific examples of antibody additions include glycosylation, acetylation, phosphorylation, amidation, formylation, ubiquitination, and derivatization by protecting/blocking groups and any of numerous chemical modifications. Additional specific non-limiting examples of an addition is another
amino acid sequence. In particular embodiments, an addition is a fusion (chimeric) sequence, an amino acid sequence having one or more molecules not normally present in a reference native (wild type) sequence covalently attached to the sequence. A particular example is an amino acid sequence of another antibody to produce an antibody multimer, such as a multispecific antibody.

[0086] Another particular example of a modified antibody having an amino acid addition is one in which a second heterologous sequence, i.e., heterologous functional domain is attached (covalent or non-covalent binding) that confers a distinct or complementary function upon the antibody. Heterologous functional domains are not restricted to amino acid residues. Thus, a heterologous functional domain can consist of any of a variety of different types of small or large functional moieties. Such moieties include nucleic acid, peptide, carbohydrate, lipid or small organic compounds, such as a drug (e.g., an antiviral), a metal (gold, silver), radioisotope. For example, a tag such as T7 or polyhistidine can be attached to the antibody in order to facilitate purification or detection of antigen. Thus, in other embodiments the invention provides antibodies and a heterologous domain, wherein the domain confers a distinct function, i.e. a heterologous functional domain, on the antibody.

[0087] Linkers, such as amino acid or peptidimimetic sequences may be inserted between the antibody sequence and the addition (e.g., heterologous functional domain) so that the two entities maintain, at least in part, a distinct function or activity. Linkers may have one or more properties that include a flexible conformation, an inability to form an ordered secondary structure or a hydrophobic or charged character which could promote or interact with either domain. Amino acids typically found in flexible protein regions include Gly, Asn and Ser. Other near neutral amino acids, such as Thr and Ala, may also be used in the linker sequence. The length of the linker sequence may vary without significantly affecting a function or activity of the fusion protein (see, e.g., U.S. Patent No. 6,087,329). Linkers further include chemical moieties and conjugating agents, such as sulfo-succinimidyl derivatives (sulfo-SMCC, sulfo-SMPB), disuccinimidyl suberate (DSS), disuccinimidyl glutarate (DSG) and disuccinimidyl tartrate (DST).

[0088] Additional non-limiting examples of additions are detectable labels. Thus, in another embodiment, the invention provides IL-10R antibodies and subsequences thereof that are detectably labeled. Specific examples of detectable labels include fluorophores, chromophores, radioactive isotopes (e.g., S³⁵, P³², I¹³¹), electron-dense reagents, enzymes, ligands and receptors. Enzymes are typically detected by their activity. For example, horseradish peroxidase is usually detected by its ability to convert a substrate such as 3,3′,5,5′-tetramethylbenzidine (TMB) to a blue pigment, which can be quantified. Ligands may bind other molecules such as biotin, which may bind avidin or streptavidin, and IgG, which can bind protein A.

[0089] Another non-limiting example of an addition is an insertion of an amino acid within any sequence of 136C5, 136C8 or 136D29 antibodies, or in an antibody that includes a heavy chain variable region sequence of any of SEQ ID NOs:29, 31 or 33, and a light chain variable region sequence of any of SEQ ID NOs:30, 32, or 34. Insertions can occur within a constant or variable region, such as heavy or light chain variable region sequences, within or outside of a CDR or FR. Insertions within CDRs, such
as CDR3, occur naturally during antibody affinity maturation. Amino acid insertions within CDRs, such as CDR3, of invention antibodies and subsequences thereof therefore need not destroy IL-10R binding affinity. In particular embodiments, an insertion is of one or more amino acid residues in any of 136C5, 136C8 or 136D29 antibodies, or in an antibody that includes a heavy chain variable region sequence of any of SEQ ID NOs:29, 31 or 33, and a light chain variable region sequence of any of SEQ ID NOs:30, 32, or 34.

[0090] Additional specific non-limiting examples of modifications and variants include antibody subsequences and fragments. The terms "functional subsequence" and "functional fragment" when referring to an antibody means a portion that retains at least a part of one or more functions or activities as full length or native antibody, e.g., a function or activity of IL-10R antibody, such as binding to IL-10R. Thus, for example, an antibody subsequence or fragment that binds to IL-10R, or a fragment of IL-10R is considered a functional subsequence. Antibody subsequences or fragments retain, at least a part of, a function or activity of an unmodified or a reference full length, native or intact antibody. Subsequences and fragments can have less than, the same, or greater binding affinity or avidity as full length native antibody, the binding specificity as full length native antibody, or one or more activities or functions of as a full length native antibody, e.g., a function or activity of an IL-10R antibody.

[0091] Exemplary subsequences and fragments include antibody subsequences and fragments that bind to IL-10R, such as an antibody with at least one fewer amino acid than a full length IL-10R antibody (e.g., one or more internal or terminal amino acid deletions from either amino or carboxy-termini of IL-10R antibody having two heavy chains and two light chains that bind to EL-10R). Antibody subsequences and fragments, including single-chain antibodies, can include all or a portion of heavy or light chain variable region sequences (e.g., CDR1, CDR2 or CDR3 in any of 136C5, 136C8 or 136D29 antibodies, or in a heavy chain variable region sequence of any of SEQ ID NOs:29, 31 or 33, or a light chain variable region sequence of any of SEQ ID NOs:30, 32, or 34, is an example) alone or in combination with all or a portion of one or more of the following: hinge region, CH1, CH2, and CH3 domains. Non-limiting representative fragments and subsequences of a full length antibody include but are not limited to Fab, Fab', F(ab')2, Fv, Fd, single-chain Fv (scFv), disulfide-linked Fvs (sdFv), V_L, V_H, trisspecific (Fab3), bispecific (Fab2), diabody ((V_L-V_H)2 or (V_H-V_L)2), triabody (trivalent), tetrabody (tetravalent), minibody ((scFv-C_H3)2), bispecific single-chain Fv (Bis-scFv), IgGdeltaCH2, scFv-Fc, (ScFv)2-Fc and IgG4PE.

[0092] Antibody subsequences and fragments can be combined. For example, V_L or V_H subsequences can be joined by a linker sequence thereby forming a V_L-V_H chimera. A combination of single-chain Fvs (scFv) subsequences can be joined by a linker sequence thereby forming an scFv - scFv chimera. Antibody subsequences and fragments include single-chain antibodies or variable region(s) alone or in combination with all or a portion of other antibody subsequences.

[0093] Functional fragments and subsequences also include all or a portion of a full length antibody heavy or light chain, or a heavy or light chain variable region, which includes one, two or three CDRs of a heavy or light chain variable region sequence, optionally with or without a flanking FR. In various
aspects, a functional fragment or a subsequence of a full length antibody heavy or light chain, or a heavy or light chain variable region, has a length from about 20-30, 30-50, 50-100, 100-150, 150-200, 200-250, 250-300, 300-400, or 400-500, amino acid residues.

[0094] Another particular non-limiting example of a modification is where an antibody is altered to have a different isotype or subclass by, for example, substitution of the heavy chain constant region. An alteration of Ig subclass can result in a change or an improvement in a function or activity (e.g., an anti-IL-10R activity). Thus, modifications include deleting small and large regions of amino acid sequences from an antibody and substituting the deleted region with another amino acid sequence, whether the sequence is greater or shorter in length than the deleted region.

[0095] Modified polypeptides also include one or more D-amino acids substituted for L-amino acids (and mixtures thereof), structural and functional analogues, for example, peptidomimetics having synthetic or non-natural amino acids or amino acid analogues and derivatized forms. Modifications include cyclic structures such as an end-to-end amide bond between the amino and carboxy-terminus of the molecule or intra- or inter-molecular disulfide bond. Polypeptides may be modified in vitro or in vivo, e.g., post-translationally modified to include, for example, sugar residues, phosphate groups, ubiquitin, fatty acids, lipids, etc.

[0096] IL-10 receptor (IL-10R) antibodies and subsequences thereof include an antibody or subsequence thereof which functions as an antagonist of EL-10 or IL-10 receptor (IL-10R) signaling pathway. The term "antagonist" and grammatical variations thereof, when used in reference to IL-10 and IL-10 receptor (IL-10R), is an antibody or a subsequence thereof that directly or indirectly reduces, decreases, inhibits, suppresses, prevents, limits, blocks or abrogates an activity or function of IL-10, EL-10R, or EL-10 or EL-10R signaling activity or signaling pathway. Such invention antibodies and subsequences thereof detectably reduce, decrease, inhibit, suppress, prevent, limit, block or abrogate an activity or function of IL-10 or EL-10 receptor (EL-10R) signaling activity or signaling pathway. Thus, an EL-10 receptor (EL-10R) antibody or subsequence thereof antagonist detectably reduces, decreases, inhibits, suppresses, prevents, limits, blocks or abrogates one or more EL-10 or EL-10 receptor (EL-10R) activities or functions, which can include, for example, binding of IL-10 to EL-10R, EL-10 or EL-10R mediated signaling or expression, or an EL-10 or EL-10R-mediated or EL-10 or EL-10R-modulatable cell response, or another EL-10 or EL-10 receptor (EL-10R) activity or function as set forth herein or otherwise one that one skilled in the art would be apprised. Various assays for measuring activity or function of EL-10, EL-10R, or EL-10R or EL-10R signaling activity or signaling pathway, such as measuring TNF-alpha expression or secretion by PBMCs (e.g., human, chimpanzee or macaque) treated with LPS in vitro in the presence of IL-10, and determining an increase in TNF-alpha expression or secretion by the PBMCs, are disclosed herein and known to one of ordinary skill in the art.

[0097] In further embodiments, antibodies and subsequences thereof specifically bind to EL-10 Receptor alpha protein (EL-10Rα) and modulate an EL-10R/EL-10 signaling activity. In one aspect, an antibody or subsequence thereof reduces, inhibits, decreases, suppresses or limits an EL-10R/EL-10 signaling activity. In further aspects, an antibody or subsequence thereof specifically binds to a human, a
chimpanzee or a cynomolgus macaque IL-1 ORa, and reduces, inhibits, decreases, suppresses or limits an activity of human, chimpanzee or cynomolgus macaque IL-1 ORa (e.g., IL-1OR/IL-10 signaling). In still further particular aspects, an antibody or subsequence thereof specifically binds to a human IL-1 ORa, and one or both of a chimpanzee IL-1 ORa and cynomolgus macaque IL-1 ORa, and reduces, inhibits, decreases, suppresses or limits an activity of human, chimpanzee or cynomolgus macaque IL-1 ORa (e.g., IL-1WRJTL-10 signaling).

[0098] Various non-limiting examples of IL-10 and IL-1OR activities and functions which, when contacted with an invention antibody or subsequence thereof, can result in: stimulating, inducing, increasing, enhancing, augmenting, or promoting a proinflammatory (e.g., IL-2, IFN-gamma, IL-4, IL-5, TNF-alpha) or adaptive immune response, production or expression of a cytokine (e.g., IL-1 alpha, EL-lbeta, TNF-alpha, IL-6, IL-9, IL-12, IL-18, GM-CSF, etc.) or a chemokine (e.g., MCI, MCP5, RANTES, IL-8, IP-10, MIP-2, etc.); stimulating, inducing, increasing, enhancing, augmenting, or promoting expression of MHC class II or costimulatory molecules (e.g., OX40L) or anti-pathogen cytokines or chemokines by antigen presenting cells; stimulating, inducing, increasing, enhancing, augmenting, or promoting proliferation, differentiation or expression of CD4 or CD8 T cells or CD4 or CD8 T cell effector responses; stimulating, inducing, increasing, enhancing, augmenting, or promoting macrophage activation or proliferation; reducing, decreasing, inhibiting or suppressing expression or activity of Jak/Stat pathway genes, MAPK or p38 pathways; and reducing, decreasing, inhibiting, suppressing, controlling or limiting pathogen proliferation, replication, pathology, adverse symptoms caused by or associated with the pathogen, reactivation of pathogen from latency and transmission of pathogen from one subject to another subject. Thus, an EL-10R antibody or subsequence thereof that reduces, decreases, suppresses, inhibits, prevents, limits, blocks or abrogates an IL-10 and EL-1OR activity or function disclosed herein or known to the skilled artisan, can result in, for example, inducing, increasing, promoting, enhancing, augmenting, or stimulating cell proliferation, expansion or activation (e.g., CD4+ or CD8+ T cells, NKT cells, dendritic cells, neutrophils, eosinophils, monocytes, or macrophages), cell survival or apoptosis (e.g., lymphocytes such as naive, activated, effector, or memory T cells), cytokines and interferon expression or production (in vivo or in vitro), proinflammatory or adaptive immune response against an pathogen, anti-apoptotic or pro-apoptotic protein expression or production (e.g., Bcl-xL, Bcl-2, Bad or Bim), and treatment, inhibition, reduction, decreasing, prevention, control, limiting or ameliorating one or more disorders, diseases, illnesses, physiological conditions, pathologies or adverse symptoms or complications associated with or caused by pathogen infection, reactivation from latency or transmission of pathogen from one subject to another subject.

[0099] The invention also provides heavy and light chain variable region sequences, which may be optionally isolated or purified as set forth herein. In particular embodiments, a heavy or light chain variable region sequence is a sequence identical to a heavy or light sequence of any of 136C5, 136C8 or 136D29 antibodies, a heavy chain variable region sequence of any of SEQ ID NOs:29, 31 or 33, or a light chain variable region sequence of any of SEQ ID NOs:30, 32, or 34. Such heavy and light chain sequences include variants, such as substitutions, additions and deletions of any of 136C5, 136C8 or
136D29 antibodies, SEQ ID NOs:29, 31 or 33, or SEQ ID NOs:30, 32, or 34, as well as sequences with less than 100% identity to the heavy and light chain variable regions sequences of 136C5, 136C8 or 136D29 antibodies, SEQ ID NOs:29, 31 or 33, and SEQ ID NOs:30, 32, or 34 (e.g., 60% or more, such as 65%, 70%, 75%, 80%, 85%, 90%, 95%, etc., identical to any heavy chain variable region sequence set forth as SEQ ID NOs:29, 31 or 33, or 60% or more, such as 65%, 70%, 75%, 80%, 85%, 90%, 95%, etc., identical to any light chain variable region sequence set forth as SEQ ID NOs:30, 32, or 34).

Methods of producing polyclonal and monoclonal antibodies are known in the art. For example, IL-10R or an immunogenic fragment thereof, optionally conjugated to a carrier such as keyhole limpet hemocyanin (KLH) or ovalbumin (e.g., BSA), or mixed with an adjuvant such as Freund's complete or incomplete adjuvant, and used to immunize an animal. Using hybridoma technology, splenocytes from immunized animals that respond to IL-10R can be isolated and fused with myeloma cells. Monoclonal antibodies produced by hybridomas can be screened for reactivity with IL-10R, or an immunogenic fragment thereof. Hybridoma, recombinant, and phage display methods are known in the art (see, for example, U.S. Patent Nos. 4,902,614, 4,543,439, and 4,41 1,993; see, also Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses, Plenum Press, Kenneth, McKearn, and Bechtol (eds.), 1980, and Harlow et al., Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 2nd ed. 1988).

Animals that may be immunized include primates, mice, rats, rabbits, goats, sheep, cattle, or guinea pigs. Initial and any optional subsequent immunization may be through intravenous, intraperitoneal, intramuscular, or subcutaneous routes. Additionally, to increase the immune response, antigen can be coupled to another protein such as ovalbumin or keyhole limpet hemocyanin (KLH), thyroglobulin and tetanus toxoid, or mixed with an adjuvant such as Freund's complete or incomplete adjuvant. Initial and any optional subsequent immunization may be through intraperitoneal, intramuscular, intraocular, or subcutaneous routes. Subsequent immunizations may be at the same or at different concentrations of antigen, and may be at regular or irregular intervals.

Animals include mammals genetically modified to include human gene loci, such as human immunoglobulin lambda or kappa light chain, which can be used to produce human antibodies. Transgenic (e.g., transchromosomic) animals with one or more human immunoglobulin genes are described, for example, in U.S. Patent No. 5,939,598, WO 02/43478, and WO 02/092812. Human transchromosomic mice (KM mice™) are described, for example, in WO 02/43478, WO 02/092812, and Ishida, et al., IBCs 11th Antibody Engineering Meeting. Abstract (2000)). Such animals include, for example, mice, rat, guinea pig, rabbit, sheep, cow pig and horse.


IL-IOR protein suitable for generating antibodies can be produced by any of a variety of standard protein purification or recombinant expression techniques. Forms of IL-IOR suitable for generating an immune response include IL-IOR subsequences, such as an immunogenic fragment. Additional forms of IL-IOR include IL-10R expressing cells, IL-10R containing preparations or cell extracts or fractions, partially purified IL-IOR. For example, an IL-IOR sequence can be produced by standard peptide synthesis techniques, such as solid-phase synthesis. A portion of the protein may contain an amino acid sequence such as a T7 tag or polyhistidine sequence to facilitate purification of expressed or synthesized protein. The protein may be expressed in a cell and purified. The protein may be expressed as a part of a larger protein (e.g., a fusion or chimera) by recombinant methods.

Suitable techniques that additionally may be employed in antibody methods include IL-IOR-based affinity purification, non-denaturing gel purification, HPLC or RP-HPLC, size exclusion, purification on protein A column, or any combination of these techniques. Antibody isotype can be determined using an ELISA assay, for example, a human Ig can be identified using mouse Ig-absorbed anti-human Ig.

Polypeptide sequences including modified forms can be made using recombinant DNA technology via cell expression or in vitro translation. Polypeptide sequences including modified forms can also be produced by chemical synthesis using methods known in the art, for example, an automated peptide synthesis apparatus (see, e.g., Applied Biosystems, Foster City, CA).

Antibody subsequences and fragments can be prepared by proteolytic hydrolysis of antibody, for example, by pepsin or papain digestion of whole antibodies. Antibody subsequences and fragments produced by enzymatic cleavage with pepsin provide a 5S fragment denoted F(ab')2. This fragment can be further cleaved using a thiol reducing agent to produce 3.5S Fab' monovalent fragments. Alternatively, an enzymatic cleavage using pepsin produces two monovalent Fab' fragments and the Fc fragment directly (see, e.g., U.S. Patent Nos. 4,036,945 and 4,331,647; and Edelman et al., Methods Enzymol. 1:422 (1967)). Other methods of cleaving antibodies, such as separation of heavy chains to
form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic or chemical may also be used.

[0109] The invention also provides nucleic acids encoding heavy and light chain variable region sequences of IL-10R antibodies and subsequences thereof, optionally further encoding a constant region. In one embodiment, a nucleic acid encodes a sequence at least 60 % or more (e.g., 65%, 70%, 75%, 80%, 85%, 90%, 95%, etc.) identical to a heavy chain variable region sequence of any of 136C5, 136C8 or 136D29 antibodies, or a heavy chain variable region sequence set forth as SEQ ID NOs: 29, 31 or 33. In another embodiment, a nucleic acid encodes a sequence at least 60% or more (e.g., 65%, 70%, 75%, 80%, 85%, 90%, 95%, etc.) identical to a light chain variable region sequence of any of 136C5, 136C8 or 136D29 antibodies, or a light chain variable region sequence set forth as SEQ ID NOs: 30, 32, or 34. In an additional embodiment, a nucleic acid encodes a sequence having one or more amino acid additions (insertions), deletions or substitutions of a constant region, or a heavy or light chain variable region sequence of any of 136C5, 136C8 or 136D29 antibodies, SEQ ID NOs: 29, 31 or 33, or SEQ ID NOs: 30, 32, or 34. In particular aspects, the nucleic acid encodes a constant region of an antibody (e.g., a mammalian constant region such as a primate or human).

[0110] The terms "nucleic acid" and "polynucleotide" and the like refer to at least two or more ribo- or deoxy-ribonucleic acid base pairs (nucleotides) that are linked through a phosphoester bond or equivalent. Nucleic acids include polynucleotides and polynucleosides. Nucleic acids include single, double or triplex, circular or linear, molecules. Exemplary nucleic acids include but are not limited to: RNA, DNA, cDNA, genomic nucleic acid, naturally occurring and non naturally occurring nucleic acid, e.g., synthetic nucleic acid.

[0111] Nucleic acids can be of various lengths. Nucleic acid lengths typically range from about 20 nucleotides to 20 Kb, or any numerical value or range within or encompassing such lengths, 10 nucleotides to 10Kb, 1 to 5 Kb or less, 1000 to about 500 nucleotides or less in length. Nucleic acids can also be shorter, for example, 100 to about 500 nucleotides, or from about 12 to 25, 25 to 50, 50 to 100, 100 to 250, or about 250 to 500 nucleotides in length, or any numerical value or range or value within or encompassing such lengths. In particular aspects, a nucleic acid sequence has a length from about 10-20, 20-30, 30-50, 50-100, 100-150, 150-200, 200-250, 250-300, 300-400, 400-500, 500-1000, 1000-2000, nucleotides, or any numerical value or range within or encompassing such lengths. Shorter polynucleotides are commonly referred to as "oligonucleotides" or "probes" of single- or double-stranded DNA. However, there is no upper limit to the length of such oligonucleotides.

[0112] The invention also provides nucleic acid sequences that are complementary to all or a portion of a sequence that encodes a heavy or light chain variable region sequence of any of 136C5, 136C8 or 136D29 antibodies, SEQ ID NOs: 29, 31 or 33, or SEQ ID NOs: 30, 32, or 34, and nucleic acid sequences that specifically hybridize to all or a portion of a heavy or light chain variable region sequence of any of 136C5, 136C8 or 136D29 antibodies, SEQ ID NOs: 29, 31 or 33, or SEQ ID NOs: 30, 32, or 34, or a complementary or antisense sequence thereof.
The term "complementary" or "antisense" refers to a polynucleotide or peptide nucleic acid capable of binding to a specific DNA or RNA sequence. Antisense includes single, double, triple or greater stranded RNA and DNA polynucleotides and peptide nucleic acids (PNAs) that bind RNA transcript or DNA. Particular examples include RNA and DNA antisense that binds to sense RNA. For example, a single stranded nucleic acid can target a protein transcript that participates in metabolism, catabolism, removal or degradation of glycogen from a cell (e.g., mRNA). Antisense molecules are typically 95-100% complementary to the sense strand but can be "partially" complementary, in which only some of the nucleotides bind to the sense molecule (less than 100% complementary, e.g., 95%, 90%, 80%, 70% and sometimes less), or any numerical value or range within or encompassing such percent values.

The term "hybridize" and grammatical variations thereof refer to the binding between nucleic acid sequences. Hybridizing sequences will generally be more than about 50% complementary to a nucleic acid that encodes an amino acid sequence of a reference antibody or subsequence (e.g., an antibody heavy or light chain variable region sequence). The hybridization region between hybridizing sequences typically is at least about 12-15 nucleotides, 15-20 nucleotides, 20-30 nucleotides, 30-50 nucleotides, 50-100 nucleotides, 100 to 200 nucleotides or more, or any numerical value or range within or encompassing such lengths.

Nucleic acid sequences further include nucleotide and nucleoside substitutions, additions and deletions, as well as derivatized forms and fusion/chimeric sequences (e.g., encoding recombinant polypeptide). For example, due to the degeneracy of the genetic code, nucleic acids include sequences and subsequences degenerate with respect to nucleic acids that encode a sequence of any of 136C5, 136C8 or 136D29 antibodies, SEQ ID NOs: 29, 31, or 33, or SEQ ID NOs: 30, 32, or 34, and subsequences thereof, as well as variants and modifications thereof (e.g., substitutions, additions insertions and deletions).

Nucleic acids can be produced using various standard cloning and chemical synthesis techniques. Techniques include, but are not limited to nucleic acid amplification, e.g., polymerase chain reaction (PCR), with genomic DNA or cDNA targets using primers (e.g., a degenerate primer mixture) capable of annealing to antibody encoding sequence. Nucleic acids can also be produced by chemical synthesis (e.g., solid phase phosphoramidite synthesis) or transcription from a gene. The sequences produced can then be translated in vitro, or cloned into a plasmid and propagated and then expressed in a cell (e.g., a host cell such as eukaryote or mammalian cell, yeast or bacteria, in an animal or in a plant).

Nucleic acid may be inserted into a nucleic acid construct in which expression of the nucleic acid is influenced or regulated by an "expression control element." An "expression control element" refers to a nucleic acid sequence element that regulates or influences expression of a nucleic acid sequence to which it is operatively linked. Expression control elements include, as appropriate, promoters, enhancers, transcription terminators, gene silencers, a start codon (e.g., ATG) in front of a protein-encoding gene, etc.
An expression control element operatively linked to a nucleic acid sequence controls transcription and, as appropriate, translation of the nucleic acid sequence. Expression control elements include elements that activate transcription constitutively, that are inducible (i.e., require an external signal for activation), or derepressible (i.e., require a signal to turn transcription off; when the signal is no longer present, transcription is activated or "derepressed"), or specific for cell-types or tissues (i.e., tissue-specific control elements).

Nucleic acid may be inserted into a plasmid for propagation into a host cell and for subsequent genetic manipulation. A plasmid is a nucleic acid that can be propagated in a host cell, plasmids may optionally contain expression control elements in order to drive expression of the nucleic acid encoding IL-10R binding antibody, subsequence thereof or antigen (e.g., IL-10R alpha or beta chain) in the host cell. A vector is used herein synonymously with a plasmid and may also include an expression control element for expression in a host cell (e.g., expression vector). Plasmids and vectors generally contain at least an origin of replication for propagation in a cell and a promoter. Plasmids and vectors are therefore useful for genetic manipulation and expression of IL-10R binding antibodies and subsequences, as well as antibody constant, heavy and light chain variable regions as well as antigen (e.g., IL-1OR). Accordingly, vectors that include nucleic acids encoding or complementary to IL-1OR binding antibodies and subsequences thereof, as well as antibody constant, heavy and light chain variable regions are provided.

Nucleic acids encoding variable regions of IL-10R antibody heavy and light chains or subsequences thereof, or encoding full length IL-1OR antibody heavy and light chains or subsequences thereof, can be produced synthetically or using recombinant methods, or isolated from a cell such as a hybridoma. Isolated nucleic acids may be inserted into a suitable expression vector, and introduced into suitable host cells (e.g., CHO, plant and other cells) which can be cultured for the production of recombinant IL-1OR antibodies, heavy and light chains or subsequences thereof.

In accordance with the invention, there are provided host cells that express or are transformed with a nucleic acid that encodes a IL-1OR antibodies and subsequences of the invention. Host cells include but are not limited to prokaryotic and eukaryotic cells such as bacteria, fungi (yeast), plant, insect, and animal (e.g., mammalian, including primate and human, CHO cells and hybridomas) cells. For example, bacteria transformed with recombinant bacteriophage nucleic acid, plasmid nucleic acid or cosmid nucleic acid expression vectors; yeast transformed with recombinant yeast expression vectors; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid); insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus); and animal cell systems infected with recombinant virus expression vectors (e.g., retroviruses, adenovirus, vaccinia virus), or transformed animal cell systems engineered for stable expression.
The cells may be a primary cell isolate, cell culture (e.g., passaged, established or immortalized cell line), or part of a plurality of cells, or a tissue or organ ex vivo or in a subject (in vivo). In particular embodiments, a host cell is a CHO cell, a hybridoma cell or a HEK293F cell.

The term "transformed" or "transfected" when used in reference to a cell (e.g., a host cell) or organism, means a genetic change in a cell following incorporation of an exogenous molecule, for example, a protein or nucleic acid (e.g., a transgene) into the cell. Thus, a "transfected" or "transformed" cell is a cell into which, or a progeny thereof in which an exogenous molecule has been introduced by the hand of man, for example, by recombinant DNA techniques.

The nucleic acid or protein can be stably or transiently transfected or transformed (expressed) in the cell and progeny thereof. The cell(s) can be propagated and the introduced protein expressed, or nucleic acid transcribed. A progeny of a transfected or transformed cell may not be identical to the parent cell, since there may be mutations that occur during replication.

Introduction of protein and nucleic acid into target cells (e.g., host cells) can also be carried out by methods known in the art such as osmotic shock (e.g., calcium phosphate), electroporation, microinjection, cell fusion, etc. Introduction of nucleic acid and polypeptide in vitro, ex vivo and in vivo can also be accomplished using other techniques. For example, a polymeric substance, such as polyesters, polyamine acids, hydrogel, polyvinyl pyrrolidone, ethylene-vinylacetate, methylcellulose, carboxymethylcellulose, protamine sulfate, or lactide/glycolide copolymers, polylactide/glycolide copolymers, or ethylenevinylacetate copolymers. A nucleic acid can be entrapped in microcapsules prepared by coacervation techniques or by interfacial polymerization, for example, by the use of hydroxymethylcellulose or gelatin-microcapsules, or poly (methylmethacrolate) microcapsules, respectively, or in a colloid system. Colloidal dispersion systems include macromolecule complexes, nano-capsules, microspheres, beads, and lipid-based systems, including oil-in-water emulsions, micelles, mixed micelles, and liposomes.

Liposomes for introducing various compositions into cells are known in the art and include, for example, phosphatidylcholine, phosphatidylserine, lipofectin and DOTAP (e.g., U.S. Patent Nos. 4,844,904, 5,000,959, 4,863,740, and 4,975,282; and GIBCO-BRL, Gaithersburg, MD). Piperazine based amphilic cationic lipids useful for gene therapy also are known (see, e.g., U.S. Patent No. 5,861,397). Cationic lipid systems also are known (see, e.g., U.S. Patent No. 5,459,127). Polymeric substances, microcapsules and colloidal dispersion systems such as liposomes are collectively referred to herein as "vesicles." Accordingly, viral and non-viral vector means delivery into cells, tissue or organs, in vitro, in vivo and ex vivo are included.

The invention is also based at least in part on the role of the IL-10/IL-IOR signaling in decreasing, reducing, inhibiting, preventing, blocking or suppressing anti-pathogen immune responses. In particular, IL-10 signaling during exposure to or contact with a pathogen, infection with a pathogen, or reactivation of a latent pathogen infection, appears to decrease, reduce, inhibit, prevent, block or suppress immune responses against the pathogen. Thus, a decrease, inhibition, reduction, suppression, or blockade of IL-10 or IL-IOR signaling by an IL-10 receptor (IL-IOR) antibody or subsequence thereof
can be used to decrease, reduce, inhibit, prevent, block or suppress IL-10 or IL-10R signaling thereby providing therapeutic treatment or prophylactic (preventative) treatment of a pathogen infection. Binding IL-10R antibodies to IL-10R can therefore enhance, promote, stimulate, augment, induce or increase an immune response, such as a proinflammatory or adaptive response against a pathogen; decrease, reduce, inhibit, suppress, prevent, limit or control pathogen replication or proliferation; ameliorate (e.g., prevent, decrease, reduce, inhibit, suppress, control or limit) one or more pathologies or adverse symptoms associated with or caused by pathogen infection or reactivation from latency; enhance, promote, stimulate, augment, induce or increase pathogen clearance or removal; or decrease, reduce, inhibit, suppress, control or limit transmission of pathogen from one subject to another subject (e.g., to a susceptible host).

[0128] In accordance with the invention, there are provided methods of treating a subject for a pathogen infection (chronic or acute). In one embodiment, a method includes administering to a subject an amount of an invention IL-10R antibody or subsequence thereof sufficient to treat the subject for the pathogen infection (chronic or acute). In another embodiment, a method includes administering to a subject an amount of an IL-10R antibody or subsequence thereof and a pathogen antigen, live or attenuated pathogen, or a nucleic acid encoding all or a portion (e.g., an epitope) of any protein or proteinaceous pathogen antigen sufficient to treat the subject for a pathogen infection.

[0129] Pathogens are typically microorganisms that cause or are associated with adverse symptoms, pathologies, illnesses, complications or undesirable effects in a subject. Non-limiting examples of pathogens include viruses, bacteria, parasites and fungi.

[0130] Particular non-limiting examples of viruses include poxvirus, herpesvirus, hepatitis virus, immunodeficiency virus, flavivirus, papilloma virus (PV), polyoma virus, rhabdovirus, a myxovirus, an arenavirus, a coronavirus, adenovirus, reovirus, picornavirus, togavirus, bunyavirus, parvovirus and retrovirus.

[0131] Non-limiting examples of poxvirus include a vaccinia virus, Molluscum contagiosum, variola major or variola minor smallpox virus, cow pox, camel pox, sheep pox, and monkey pox.

[0132] Non-limiting examples of herpesvirus include an alpha-herpesvirus, beta-herpesvirus, gamma-herpesvirus, Epstein Bar Virus (EBV), Cytomegalovirus (CMV), varicella zoster virus (VZV/HHV-3), and human herpes virus 1, 2, 4, 5, 6, 7, and 8 (HHV-8, Kaposi's sarcoma-associated virus).

[0133] Non-limiting examples of hepatitis virus include hepatitis A, B, C, D, E and G.

[0134] Non-limiting examples of immunodeficiency virus include human immunodeficiency virus (HIV), such as HIV-1, HIV-2 and HIV-3.

[0135] Non-limiting examples of flavivirus include Hepatitis C virus, Yellow Fever virus, Dengue virus, Japanese Encephalitis and West Nile viruses.

[0136] Non-limiting examples of papilloma virus include a human papilloma virus (HPV), such as HPV strain 1, 6, 11, 16, 18, 30, 31, 42, 43, 44, 45, 51, 52, and 54.

[0137] Non-limiting examples of polyoma virus include BK virus (BKV) and JC virus (JCV).
Non-limiting examples of rhabdovirus include rabies virus and vesiculovirus.

Non-limiting examples of myxovirus include paramyxovirus and orthomyxovirus. Non-limiting examples of paramyxovirus include measles, mumps, pneumovirus and respiratory syncytial virus (RSV).

Non-limiting examples of orthomyxovirus include influenza virus, such as influenza A, influenza B and influenza C.

Non-limiting examples of arenavirus include lymphocytic choriomeningitis virus (LCMV), Junin virus, Lassa virus, Guanarito virus, Sabia virus and Machupo virus.

Non-limiting examples of coronavirus include a virus that causes a common cold, and severe acute respiratory syndrome (SARS).

Non-limiting examples of adenovirus include viral infections of bronchii, lung, stomach, intestine (gastroenteritis), eye (conjunctivitis), bladder (cystitis) and skin.

Non-limiting examples of reovirus include a rotavirus, cytopivirus and orbivirus.

Non-limiting examples of picornavirus include a rhinovirus, aphthovirus, hepatovirus, enterovirus, coxsackie B virus and cardiovirus. Rhinovirus can cause the common cold.

Non-limiting examples of togavirus include alphavirus, sindbis virus, and rubellavirus.

Non-limiting examples of bunyavirus include hantavirus, phlebovirus and nairovirus.

Non-limiting examples of retrovirus include an alpha, beta, delta, gamma, epsilon, lentivirus, spumavirus and human T-cell leukemia virus.

Non-limiting examples of lentivirus include an immunodeficiency virus, such as immunodeficiency virus (e.g., a bovine, porcine, equine, canine, feline or primate virus).

Non-limiting examples of human T-cell leukemia viruses include human T-cell leukemia virus 1 and 2 (HTLV-I and HTLV-2).

Non-limiting examples of bacteria include a mycobacterium (e.g., tuberculosis and atypical mycobacterium), listeria monocytogenes, helicobacter, bordetella, streptococcus, salmonella and chlamydia.

Non-limiting examples of parasites include a protozoa or nematode. Non-limiting examples of protozoa include Toxoplasma gondii, Leishmania, Plasmodium, or Trypanosoma cruzi. Non-limiting examples of nematodes include Schistosoma mansoni, or a Heligmosomoides polygyrus.

Non-limiting examples of fungus include Candida albicans.

In accordance with the invention, there are further provided therapeutic and prophylactic methods of treating a subject for a pathogen infection, for example, a subject at risk of a pathogen infection. Such methods include administering an IL-10 receptor (IL-10R) antibody or subsequence thereof to therapeutically or prophylactically (vaccinating or immunizing) treat a subject having or at risk of having a pathogen infection. Such methods can treat the infection or provide the subject with protection from a pathogen infection (e.g., prophylactic protection). In one embodiment, a method includes administering an amount of an EL-IO receptor (IL-10R) antibody or subsequence thereof to a subject in need thereof, sufficient to provide the subject with protection against a pathogen infection.
Pathogen antigens (e.g., protein or an epitope thereof), live or attenuated pathogen, inactivated pathogen, pathogen extract, nucleic acid encoding all or a portion (e.g., an epitope) of any protein or proteinaceous pathogen antigen are useful in the methods of the invention. Thus, in another embodiment, a method includes administering an amount of an IL-10 receptor (IL-10R) antibody or subsequence thereof to a subject in need thereof and a pathogen antigen, a live or attenuated pathogen or a nucleic acid encoding all or a portion (e.g., an epitope) of a pathogen antigen sufficient to vaccinate or immunize the subject against the pathogen infection (chronic or acute). IL-10 receptor (IL-10R) antibody or subsequence thereof can be administered as a combination composition with a pathogen antigen, a live or attenuated pathogen or a nucleic acid encoding a pathogen antigen or a portion of an antigen (e.g., an epitope), or administered separately, such as concurrently or sequentially (prior to or following) administering a pathogen antigen, a live or attenuated pathogen or a nucleic acid encoding a pathogen antigen or a portion of an antigen (e.g., an epitope), to a subject.

Particular non-limiting examples of pathogen antigens, live or attenuated pathogen, or a nucleic acid encoding pathogen antigens or a portion of an antigen (e.g., an epitope) are virus, bacteria, parasite, or fungal antigen, live or attenuated virus, bacteria, parasite, or fungus, or a nucleic acid encoding a virus, bacteria, parasite, or fungal antigen or a portion of a virus, bacteria, parasite, or fungal antigen (e.g., an epitope). Such antigens are from any pathogen set forth herein or known to one of skill in the art, and include an antigen that increases, stimulates, enhances, promotes, augments or induces a proinflammatory or adaptive immune response, numbers or activation of an immune cell (e.g., T cell, natural killer T (NKT) cell, dendritic cell (DC), B cell, macrophage, neutrophil, eosinophil, mast cell, CD4+ or a CD8+ cell, B220+ cell, CD14+, CD11b+ or CD11c+ cells), an anti-pathogen CD4+ or CD8+ T cell response, production of a Th1 cytokine, a T cell mediated immune response, etc.

Non-limiting viral antigens include a poxvirus, herpesvirus, hepatitis virus, immunodeficiency virus, flavivirus, papilloma virus (PV), polyoma virus, rhabdovirus, a myxovirus, an arenavirus, a coronavirus, adenovirus, reovirus, picornavirus, togavirus, bunyavirus, parvovirus or a retrovirus antigen.

Poxvirus viral antigen (or nucleic acid encoding all or a portion of the antigen), live or attenuated virus include vaccinia virus (e.g., B8R, L4R, H3L, E9L, F15L, J4R, B5R, HL, A3L, A8R, A23R, B2R and other poxvirus antigens), Molluscum contagiosum, variola major or variola minor smallpox virus, cow pox, camel pox, sheep pox, or monkey pox antigen.

Herpesvirus viral antigen (or nucleic acid encoding all or a portion of the antigen), live or attenuated virus include an alpha-herpesvirus, beta-herpesvirus, gamma-herpesvirus, Epstein Bar Virus
(EBV), Cytomegalovirus (CMV), varicella zoster virus (VZV/HHV-3), or human herpes virus 1, 2, 4, 5, 6, 7, or 8 (HHV-8, Kaposi's sarcoma-associated virus) antigen.

[H0160] Hepatitis viral antigen (or nucleic acid encoding all or a portion of the antigen), live or attenuated virus include a hepatitis A, B, C, D, E or G antigen.

[H0161] Immunodeficiency viral antigen (or nucleic acid encoding all or a portion of the antigen), live or attenuated virus include a human immunodeficiency virus (HIV) antigen. Non-limiting examples of HIV viral antigen, or attenuated virus include HIV-1, HTV-2 or HIV-3 antigen.

[H0162] Flavivirus viral antigen (or nucleic acid encoding all or a portion of the antigen), live or attenuated virus include a Hepatitis C virus (e.g., core, E1, E2, p7, NS2, NS3, NS4, NS5, or other virus antigen), Yellow Fever virus, Dengue virus, Japanese Encephalitis or West Nile virus antigen.

[H0163] Papilloma viral antigen (or nucleic acid encoding all or a portion of the antigen), live or attenuated virus include a human papilloma virus (HPV) antigen. Non-limiting examples of human papilloma viral antigen, live or attenuated virus include a HPV strain 1, 6, 11, 16, 18, 30, 31, 42, 43, 44, 45, 51, 52, or 54 antigen.

[H0164] Polyoma viral antigen (or nucleic acid encoding all or a portion of the antigen), live or attenuated virus include a BK virus (BKV) or JC virus (JCV) antigen.

[H0165] Rhabdovirus viral antigen (or nucleic acid encoding all or a portion of the antigen), live or attenuated virus include a rabies virus or vesiculovirus antigen.

[H0166] Myxovirus viral antigen (or nucleic acid encoding all or a portion of the antigen), live or attenuated virus include a paramyxovirus or orthomyxovirus antigen. Non-limiting examples of paramyxovirus a viral antigen, live or attenuated virus include a measles, mumps, pneumovirus or respiratory syncytial virus (RSV) antigen. Non-limiting examples of orthomyxovirus viral antigen, live or attenuated virus include an influenza virus antigen.

[H0167] Influenza virus viral antigen (or nucleic acid encoding all or a portion of the antigen), live or attenuated virus include a influenza A, influenza B or influenza C antigen.

[H0168] Arenavirus viral antigen (or nucleic acid encoding all or a portion of the antigen), live or attenuated virus include a lymphocytic choriomeningitis virus (LCMV), Junin virus, Lassa virus, Guanarito virus, Sabia virus or Machupo virus antigen.

[H0169] Coronavirus viral antigen (or nucleic acid encoding all or a portion of the antigen), live or attenuated virus include an antigen of a virus that causes a common cold or severe acute respiratory syndrome (SARS).

[H0170] Reovirus viral antigen (or nucleic acid encoding all or a portion of the antigen), live or attenuated virus include a rotavirus, cypovirus or orbivirus antigen.

[H0171] Picornavirus viral antigen (or nucleic acid encoding all or a portion of the antigen), live or attenuated virus include a rhinovirus, aphthovirus, hepatovirus, enterovirus, coxsackie B virus, or cardioivirus antigen.

[H0172] Togavirus viral antigen (or nucleic acid encoding all or a portion of the antigen), live or attenuated virus include alphavirus, sindbus virus, or rubellavirus antigen.
Bunyavirus viral antigen (or nucleic acid encoding all or a portion of the antigen), live or attenuated virus include a hantavirus, phlebovirus or nairovirus antigen.

Retrovirus viral antigen (or nucleic acid encoding all or a portion of the antigen), live or attenuated virus include an alpha, beta, delta, gamma, epsilon, lentivirus, spumavirus or human T-cell leukemia virus antigen. Non-limiting examples of lentivirus viral antigen (or nucleic acid encoding all or a portion of the antigen), live or attenuated virus include an immunodeficiency virus antigen. Non-limiting examples of immunodeficiency viral antigen, live or attenuated virus include a bovine, porcine, equine, canine, feline or primate virus antigen. Non-limiting examples of human T-cell leukemia viral antigen (or nucleic acid encoding all or a portion of the antigen), live or attenuated virus include a human T-cell leukemia virus 1 or 2 (HTLV-1 and HTLV-2) antigen.

Bacteria antigens (or nucleic acid encoding all or a portion of the antigen), live or attenuated bacteria include Mycobacterium, listeria monocytogenes, Helicobacter, bordetella, streptococcus, salmonella or Chlamydia antigen.

Parasite antigens (or nucleic acid encoding all or a portion of the antigen), live or attenuated parasite include a protozoa or nematode antigen. Exemplary protozoa antigens include a Toxoplasma gondii, Leishmania, Plasmodium, or Trypanosoma cruzi antigen.

Nematode pathogen antigens (or nucleic acid encoding all or a portion of the antigen), live or attenuated nematode include a Schistosoma mansoni or a helminth antigen.

Fungal pathogen antigens (or nucleic acid encoding all or a portion of the antigen), live or attenuated fungus include a Candida albicans antigen.

In additional various methods embodiments, an antibody or subsequence thereof and a second active, such as an antibody (agonist or antagonist) that binds to an immune regulatory molecule to modulate activity of an immune regulatory molecule, or an antibody that binds to a pathogen antigen, a pathogen nucleic acid, an agent or a drug are administered to a subject, one or more times, as a combination (e.g., an IL-10R antibody or subsequence thereof is administered as a combination composition with a second active, such as another antibody, agent or drug to a subject). In further various methods embodiments, an IL-10R antibody or subsequence thereof and a second active, such as a different antibody, an agent or a drug are administered to a subject, one or more times, sequentially (e.g., an DL-10R antibody or subsequence thereof and an agent or drug are administered separately to a subject, in a sequence). Additional method embodiments include, for example, second actives such as type I interferons, toll receptor Hgands, T cell costimulatory molecules such as OX40, 4-1BB, agonists to these or other costimulatory molecules and antagonists to inhibitory receptors or ligands such as antibodies that bind to CTLA4, PD-I, PD-L1, CD160 and LAG3.

In particular methods embodiments, one or more disorders, diseases, physiological conditions, pathologies and symptoms associated with or caused by a pathogen infection or reactivation from latency will respond to treatment or therapy with an IL-10R binding antibody or a subsequence thereof. In particular methods embodiments, treatment methods reduce, decrease, suppress, limit, control or inhibit pathogen numbers or titer; reduce, decrease, suppress, limit, control or inhibit pathogen
proliferation or replication; reduce, decrease, suppress, limit, control or inhibit the amount of a pathogen protein; or reduce, decrease, suppress, limit, control or inhibit the amount of a pathogen nucleic acid. In additional particular methods embodiments, treatment methods include an amount of IL-10R binding antibody or a subsequence thereof sufficient to increase, induce, enhance, augment, promote or stimulate an immune response against a pathogen; increase, induce, enhance, augment, promote or stimulate pathogen clearance or removal; decrease, reduce, inhibit, suppress, limit or control pathogen reactivation from latency (e.g., hepatitis or herpesvirus reactivation from latency); or decrease, reduce, inhibit, suppress, prevent, control, or limit transmission to another subject (e.g., transmission of pathogen from an infected subject to an uninfected subject). In further particular methods embodiments, treatment methods include an amount of IL-1OR binding antibody or a subsequence thereof sufficient to protect a subject from a pathogen infection or pathology, or reactivation from latency, or reduce, decrease, limit, control or inhibit susceptibility to pathogen infection or pathology.

[0181] Methods of the invention include treatment methods, which result in any therapeutic or beneficial effect. In various methods embodiments, pathogen infection, proliferation or pathogenesis is reduced, decreased, inhibited, limited, delayed or prevented, or a method decreases, reduces, inhibits, suppresses, prevents, controls or limits one or more adverse (e.g., physical) symptoms, disorders, illnesses, diseases or complications caused by or associated with chronic or acute pathogen infection, proliferation or replication, pathology or reactivation from latency. In additional various particular embodiments, treatment methods include reducing, decreasing, inhibiting, delaying or preventing onset, progression, frequency, duration, severity, probability or susceptibility of one or more adverse symptoms, disorders, illnesses, diseases or complications caused by or associated with chronic or acute pathogen infection, proliferation or replication, pathology or reactivation from latency. In further various particular embodiments, treatment methods include accelerating, facilitating, enhancing, augmenting, or hastening recovery of a subject from a pathogen infection, reactivation from latency or pathogenesis, or one or more adverse symptoms, disorders, illnesses, diseases or complications caused by or associated with chronic or acute pathogen infection, proliferation or replication, pathology or reactivation from latency. In yet additional various embodiments, treatment methods include stabilizing infection, proliferation, replication, pathogenesis, or an adverse symptom, disorder, illness, disease or complication caused by or associated with chronic or acute pathogen infection, proliferation or replication, pathology or reactivation from latency, or decreasing, reducing, inhibiting, suppressing, limiting or controlling transmission of a pathogen from an infected host to an uninfected host.

[0182] A therapeutic or beneficial effect of treatment is therefore any objective or subjective measurable or detectable improvement or benefit provided to a particular subject. A therapeutic or beneficial effect can but need not be complete ablation of all or any particular adverse symptom, disorder, illness, disease or complication caused by or associated with chronic or acute pathogen infection, proliferation or replication, pathology or reactivation from latency. Thus, a satisfactory clinical endpoint is achieved when there is an incremental improvement or a partial reduction in an adverse symptom, disorder, illness, disease or complication caused by or associated with chronic or acute
pathogen infection, proliferation or replication, pathology or reactivation from latency, or an inhibition, decrease, reduction, suppression, prevention, limit or control of worsening or progression of one or more adverse symptoms, disorders, illnesses, diseases or complications caused by or associated with chronic or acute pathogen infection, pathogen numbers, titers, proliferation or replication, pathogen protein or nucleic acid, or pathogen pathology or reactivation from latency, over a short or long duration (hours, days, weeks, months, etc.).

[0183] A therapeutic or beneficial effect also includes reducing or eliminating the need, dosage frequency or amount of a second active such as another drug or other agent (e.g., small molecule, protein, antibody) used for treating a subject having or at risk of having a pathogen infection or pathogenesis. For example, reducing an amount of an adjunct therapy, for example, a reduction or decrease of a treatment for a pathogen infection, or reactivation from latency, or a vaccination or immunization protocol is considered a beneficial effect. In addition, reducing or decreasing an amount of a pathogen antigen used for vaccination or immunization of a subject to provide protection to the subject from a pathogen infection, or reactivation from latency, is considered a beneficial effect.

[0184] Adverse symptoms, conditions, side effects, pathologies and complications associated with pathogen infection, such as virus, bacteria, parasites and fungus, are known to the skilled artisan. Accordingly, one skilled in the art will be apprised of a variety of clinical indicia by which to ascertain treatment efficacy as well as a therapeutic or beneficial effect.

[0185] Adverse symptoms and complications associated with poxvirus (vaccinia virus) infection and pathogenesis include, for example, high fever, fatigue, headache, backache, malaise, rash (maculopapular, vesicular or pustular) or lesions, delirium, vomiting, diarrhea, and excess bleeding. Other symptoms of poxvirus infection or pathogenesis, including variola major and variola minor smallpox virus, monkeypox, cowpox, Molluscum Contagiosum and camelpox, are known in the art and treatment thereof in accordance with the invention is provided.

[0186] Adverse symptoms and complications associated with herpesvirus infection and pathogenesis include, for example, red skin, blisters, pustules, bumps, healing with skin regeneration, pain, burning or itching in affected area, swollen lymph glands, headache, muscle ache, fever, burning sensation during urination, lower back pain, pox (e.g., chickenpox). Other symptoms of herpesvirus infection or pathogenesis are known in the art and treatment thereof in accordance with the invention is provided.

[0187] Adverse symptoms and complications associated with hepatitis infection and pathogenesis include, for example, abdominal pain, jaundice, flu-like illness, nausea, vomiting, diarrhea, loss of appetite, weight loss, joint pain, fatigue, itchy skin, cirrhosis, liver failure and hepatocellular carcinoma. Other symptoms of hepatitis infection or pathogenesis are known in the art and treatment thereof in accordance with the invention is provided.

[0188] Adverse symptoms and complications associated with immunodeficiency virus (e.g., HIV) infection and pathogenesis include, for example, abdominal cramps, nausea, vomiting, diarrhea, enlarged lymph nodes, fever, headache, muscle ache or pain, skin rash, sore throat, weight loss, loss of T cells (CD4+), increased frequency of opportunistic infections, such as yeast and bacterial infections. Other
symptoms of immunodeficiency virus infection or pathogenesis are known in the art and treatment thereof in accordance with the invention is provided.

[0189] Adverse symptoms and complications associated with flavivirus (e.g., West Nile virus) infection and pathogenesis include, for example, acute febrile illness, malaise, headache, flushing, and diarrhea. Other symptoms of flavivirus infection or pathogenesis are known in the art and treatment thereof in accordance with the invention is provided.

[0190] Adverse symptoms and complications associated with papillomavirus (PPV) infection and pathogenesis include, for example, warts (e.g., genital warts). Other symptoms of papillomavirus infection or pathogenesis are known in the art and treatment thereof in accordance with the invention is provided.

[0191] Adverse symptoms and complications associated with bacteria infection include, for example, inflammation, swelling, fever, lethargy, fatigue, sore muscles, aches, puss, discharge, redness or soreness, coughing, wheezing, nasal congestion discharge or drip, among others. Such adverse symptoms or conditions can affect a variety of cells, tissue or organs, such as skin, reproductive system (e.g., vagina, cervix, uterus, fallopian tubes) or urinary tract, mucosa (e.g., mouth), nervous system, digestive system, cardio-pulmonary system (lung or cardiac tissue), muscles or bone, kidney, liver, for example.

[0192] Adverse symptoms and complications associated with mycobacteria tuberculosis infection include, for example, cough lasting three or more weeks that may produce discolored or bloody sputum, weight loss, fatigue, fever, night sweats, chills, loss of appetite and pleurisy. Adverse symptoms and complications associated with atypical mycobacteria infection include, for example, abscesses, septic arthritis, and osteomyelitis (bone infection). Symptoms of mycobacterium avium, which frequently affects AIDS patients, includes lung disease. Symptoms of mycobacterium marinum are skin infections and swimming pool granuloma. Symptoms of mycobacterium ulcerans include skin infections. Symptoms of mycobacterium kanssii include lung disease.

[0193] For listeria monocytogenes, adverse symptoms include fever, muscle aches, gastrointestinal symptoms such as nausea or diarrhea, headache, stiff neck, confusion, loss of balance, or convulsions. Pregnant women can experience mild, flu-like illness, but during pregnancy can lead to miscarriage or stillbirth, premature delivery, or infection of the newborn.

[0194] For helicobacter pylori, adverse symptoms include heartburn, bloating, nausea, abdominal pain, gastritis (inflammation of the stomach), and ulcers in stomach or duodenum.

[0195] Adverse symptoms and complications associated with symptoms of Bordetella pertussis and parapertussis, which cause whooping cough, include paroxysmal coughing, whooping and vomiting, nocturnal coughing and contact anamnesis.

[0196] Adverse symptoms and complications associated with Streptococcus pyogenes, which causes strep throat include fever, pain, redness, and swelling of the throat or tonsil.

[0197] Adverse symptoms and complications associated with salmonella include nausea, vomiting, diarrhea, fever, and abdominal cramps.
For Chlamydia, three quarters of infected women and half of infected men have no apparent symptoms. When adverse symptoms and complications associated with chlamydia do appear, they include abnormal vaginal discharge or a burning when urinating. After infection spreads from cervix to fallopian tubes, there may still be no signs or symptoms, but there may be lower abdominal pain, low back pain, nausea, fever, pain during intercourse, and bleeding between menstrual periods. Symptoms may not be apparent until complications develop.

Adverse symptoms and complications associated with Toxoplasma gondii resemble a mild case of mononucleosis, such as lack of energy, headache, fatigue, loss of appetite or chills.

Adverse symptoms and complications associated with cutaneous Leishmania include skin sores, which can change in size and appearance over time, and may be covered by a scab. The sores can be painless or painful. Swollen glands may be near the sores (for example, under the arm if the sores are on the arm or hand). Adverse symptoms and complications associated with visceral Leishmania include fever, weight loss, an enlarged spleen or liver, swollen glands, low blood counts, such as a low red blood cell count (anemia), low white blood cell count, or low platelet count.

Adverse symptoms and complications associated with Plasmodium, which can cause malaria, include shaking chills, high fever, sweating, fatigue, headache, dizziness, nausea, vomiting, abdominal cramps, dry cough, muscle or joint pain, back ache and cerebral malaria death.

Adverse symptoms and complications associated with Trypanosoma cruzi, which causes Chagas' disease, include, in the acute phase, typically inflammation, swelling or chagoma, as well as fever, hepatosplenomegaly, adenopathy and myocarditis sinus tachycardia and cardiomegaly; and in the intermediate phase or chronic phase, lesions of internal organs such as the heart, esophagus and colon as well as the peripheral nervous system and in severe cases heart failure.

Adverse symptoms and complications associated with Schistosoma mansoni, which can cause Schistosomiasis include an initial rash following infection that mimics scabies or other types of rashes, followed within two to ten weeks later by symptoms that include fever, aching, cough, diarrhea, or gland enlargement. Katayama fever may also develop from infection, as well as fever, lethargy, the eruption of pale temporary bumps associated with severe itching (urticarial) rash, liver and spleen enlargement, and bronchospasm, which if left untreated is followed by intestinal schistosomiasis, leading to an immune system reaction called a granulomatous reaction, which can lead to obstruction of the colon and blood loss. Eggs can also become lodged in the liver, leading to high blood pressure through liver, enlarged spleen, fluid buildup in the abdomen, and dilations or swollen areas in the esophagus or gastrointestinal tract that can tear and bleed profusely (esophageal varices).

Adverse symptoms and complications associated with fungal infection include, for example, for Candida albicans, discomfort, swelling, itching, burning, rash or blisters in or around mucosal tissues, vaginal discharge, vaginitis, pelvic pain, cramps and/or menstrual irregularities, premenstrual tension, prostatitis, urinary urgency or frequency, burning on urination, fatigue, lethargy, dry or sore throat, cough, bronchitis, rash or blisters in mouth or tongue, mouth infections/thrush, white coating on
tongue, mucus in stool, rectal itch, muscle weakness or aches, nasal congestion or discharge, nasal itching, sinusitis, pain and swelling in joints, and canker sores.

[0205] Additional adverse symptoms, conditions, complications, disorders, diseases, pathologies, and illnesses associated with or caused by a pathogen infection will of course depend upon the particular type, stage of pathogen, the particular subject infected, etc. Specific adverse symptoms, conditions, complications, disorders, diseases, pathologies, and illnesses associated with or caused by a pathogen infection are known to the skilled artisan.

[0206] Methods and compositions of the invention include administration of an amount of IL-10R antibody or subsequence thereof to a subject with or at risk of a pathogen infection or reactivation from latency. In a particular aspect, a subject is administered an IL-10R antibody or subsequence alone or in combination with pathogen antigen, live or attenuated pathogen, or a nucleic acid encoding all or a portion (e.g., an epitope) of any protein or proteinaceous pathogen antigen, thereby increasing numbers or activation of an immune cell (e.g., natural killer T (NKT) cells, dendritic cells, macrophages, neutrophils, eosinophils, mast cells, CD4+ or CD8+ cells, CD14+, CD1 lb+, CD1 lc+ cells etc.). In another particular aspect, a subject is administered an IL-10R antibody or subsequence alone or in combination with pathogen antigen, live or attenuated pathogen, or a nucleic acid encoding all or a portion (e.g., an epitope) of any protein or proteinaceous pathogen antigen, prior to, substantially contemporaneously with or following vaccination or immunization of the subject against the pathogen infection, as well as administration prior to, substantially contemporaneously with or after a subject has been contacted by, exposed to or infected with a pathogen, acute or chronic, or pathogen reactivation from latency.

[0207] Methods and compositions of the invention also include increasing, stimulating, promoting, enhancing, augmenting or inducing an anti-pathogen CD8+ or CD4+ T cell response in a subject with or at risk of a pathogen infection or reactivation from latency. In one embodiment, a method includes administering to a subject an amount of IL-10R antibody or subsequence thereof sufficient to increase, stimulate, promote, enhance, augment or induce anti-pathogen CD8+ or CD4+ T cell response in the subject. In another embodiment, a method includes administering to a subject an amount of an IL-10 receptor alpha (IL-10R alpha) antibody or subsequence thereof and administering a pathogen antigen, live or attenuated pathogen, or a nucleic acid encoding all or a portion (e.g., an epitope) of any protein or proteinaceous pathogen antigen sufficient to increase, stimulate, promote, enhance, augment or induce anti-pathogen CD8+ or CD4+ T cell response in the subject.

[0208] Methods of the invention additionally include, among other things, increasing production of a Th1 cytokine (e.g., interferon gamma, IL-1 alpha, IL-1 beta, DL-2, TNF-alpha, IL-6, IL-8, IL-12, GM-CSF, etc.). In one embodiment, a method includes administering to a subject in need thereof an amount of IL-10 receptor (IL-10R) antibody or subsequence thereof sufficient to increase production of a Th1 cytokine in the subject (e.g., interferon gamma, IL-1 alpha, IL-1 beta, EL-2, TNF-alpha, IL-6, IL-8, IL-12, GM-CSF, etc.). In another embodiment, a method includes administering to a subject an amount of an EL-10 receptor alpha (IL-10R alpha) antibody or subsequence thereof and administering a pathogen...
antigen, live or attenuated pathogen, or a nucleic acid encoding all or a portion (e.g., an epitope) of any protein or proteinaceous pathogen antigen, sufficient to increase production of a Th1 cytokine in the subject (e.g., interferon gamma, IL-lalpha, IL-lbeta, IL-2, TNF-alpha, EL-6, IL-8, IL-12, GM-CSF, etc.).

[0209] Methods and compositions of the invention further include administration of IL-10R antibody or subsequence thereof to a subject prior to contact, substantially contemporaneously with or following administration of a pathogen antigen, live or attenuated pathogen, or a nucleic acid encoding all or a portion (e.g., an epitope) of any protein or proteinaceous pathogen antigen, to the subject. A subject can be administered IL-10R antibody or subsequence thereof alone or in combination with pathogen antigen, live or attenuated pathogen, or a nucleic acid encoding all or a portion (e.g., an epitope) of any protein or proteinaceous pathogen antigen, prior to contact, substantially contemporaneously with or following contact, exposure or infection by a pathogen. IL-10R antibody or subsequence thereof can therefore be administered to a subject in a combination with a pathogen antigen, live or attenuated pathogen or a nucleic acid encoding all or a portion (e.g., an epitope) of any protein or proteinaceous pathogen antigen, or separately, i.e., the IL-10R antibody or subsequence thereof and antigen, live or attenuated pathogen or nucleic acid encoding all or a portion (e.g., an epitope) of any protein or proteinaceous pathogen antigen are administered sequentially to a subject, i.e. IL-10R antibody or subsequence thereof is administered followed by administering a pathogen antigen, live or attenuated pathogen or a nucleic acid encoding all or a portion (e.g., an epitope) of any protein or proteinaceous pathogen antigen.

[0210] Methods and compositions of the invention include administration of an IL-10R antibody or subsequence thereof to a subject prior to contact, exposure or infection by a pathogen, administration prior to, substantially contemporaneously with or after a subject has been contacted by, exposed to or infected with a pathogen, acute or chronic, and administration prior to, substantially contemporaneously with or after pathogen reactivation from latency. Methods and compositions of the invention also include administration of an IL-10R antibody or subsequence thereof to a subject prior to, substantially contemporaneously with or following a pathology or adverse symptom, disorder, illness or disease caused by or associated with a pathogen infection, or reactivation from latency. A subject infected with a pathogen may have an acute infection or be chronically infected over a period of days, months, or years, or may be chronically affected that may over time be relatively asymptomatic but may suffer from acute incidents of reactivation from latency.

[0211] Invention compositions (e.g., antibodies or subsequences thereof) and methods can be combined with any compound, agent, drug, treatment or other therapeutic regimen or protocol having a desired therapeutic, beneficial, additive, synergistic or complementary activity or effect. Exemplary treatments and therapies include second actives, such as anti-pathogen compounds, agents and drugs, as well as agents that assist, promote, stimulate or enhance efficacy. Such anti-pathogen drugs, agents, treatments and therapies can be administered or performed prior to, substantially contemporaneously with or following any other method of the invention, for example, a therapeutic method of treating a subject...
for a pathogen infection or reactivation from latency, or a method of prophylactic treatment of a subject for a pathogen infection.

[0212] Combination methods embodiments include, for example, second actives such as anti-pathogen drugs, such as protease inhibitors, reverse transcriptase inhibitors, virus fusion inhibitors and virus entry inhibitors, antibodies to pathogen proteins, live or attenuated pathogen, or a nucleic acid encoding all or a portion (e.g., an epitope) of any protein or proteinaceous pathogen antigen, immune stimulating agents, etc., and include contact with, administration in vitro or in vivo, with another compound, agent, treatment or therapeutic regimen appropriate for pathogen infection, vaccination or immunization.


[0214] Specific non-limiting examples of anti-bacterials include antibiotics. Antibiotics can be first, second, third, fourth, fifth or subsequent generations. Antibiotics include, for example, aminoglycosides
(e.g., gentamycin, kanamycin, streptomycin, etc.), Carbapenems (e.g., cilastatin), cephalasporins (e.g., cefalexin, cefoxitin, cefdinir, cefapime, etc.), glycopeptides (e.g., vancomycin), macrolides (erythromycin), monobactams (e.g., aztreonam), penicillins (e.g., ampicillin, amoxicillin, oxacillin, etc.), quinolones (e.g., ciprofloxacin), sulfonamides (e.g., Mafenide, Sulfasalazine, etc.), tetracyclines (e.g., doxycycline, tetracycline, etc.) and others such as chloramphenicol, rifampicin, etc.

[0215] Specific non-limiting examples of anti-parasites include albendazole, mebendazole, thiabendazole, metronidazole, nitazoxanide, niclosamide, oxamniquine, praziquantel, pyrantel, and pyantel pamoate.

[0216] Specific non-limiting examples of anti-fungals include clotrimazole, econazole, fenticonazole, miconazole, sulconazole, tioconazole, amphotericin, nystatin terbinafine, itraconazole, fluconazole, ketoconazole and griseofulvin.

[0217] The invention provides combinations in which a method of the invention is used in a combination with any compound, agent, drug, therapeutic regimen, treatment protocol, process, remedy or composition, such as an anti-pathogen or immune stimulating, enhancing or augmenting protocol, or pathogen vaccination or immunization (e.g., prophylaxis) set forth herein or known in the art. The compound, agent, drug, therapeutic regimen, treatment protocol, process, remedy or composition can be administered or performed prior to, substantially contemporaneously with or following administration of IL-10R antibody or subsequence thereof, pathogen antigen, live or attenuated pathogen, or a nucleic acid encoding all or a portion (e.g., an epitope) of any protein or proteinaceous pathogen antigen, to a subject. Specific non-limiting examples of combination embodiments therefore include the foregoing or other compound, agent, drug, therapeutic regimen, treatment protocol, process, remedy or composition, known to the skilled artisan.

[0218] Treatments such as steroidal and non-steroidal anti-inflammatory drugs such as acetaminophen, ibuprofen, naproxen, indomethacin, piroxicam, ketoprofen and pyrancarboxylic acid (Lodine). Further additional exemplary treatments include pathogen protein, antibody that binds to a pathogen antigen, pathogen nucleic acid, passive immunoglobulin therapy, such as VIG.

[0219] Methods of the invention also include, among other things, methods that result in a reduced need or use of another compound, agent, drug, therapeutic regimen, treatment protocol, process, or remedy. For example, for a pathogen infection, reactivation from latency, vaccination or immunization, a method of the invention has a therapeutic benefit if in a given subject a less frequent or reduced dose or elimination of an anti-pathogen treatment or therapy results. Thus, in accordance with the invention, methods of reducing need or use of a treatment or therapy for a pathogen infection, reactivation from latency, or vaccination or immunization, are provided.

[0220] In invention methods in which there is a desired outcome, such as a therapeutic or prophylactic method that provides a benefit from treatment or vaccination or immunization of a pathogen infection or pathogenesis, an IL-10R antibody or subsequence thereof alone or in combination with each other or another composition or method, such as a pathogen antigen, live or attenuated pathogen, or a nucleic acid encoding all or a portion (e.g., an epitope) of any protein or proteinaceous pathogen antigen,
can be administered in a sufficient or effective amount. As used herein, a "sufficient amount" or "effective amount" or an "amount sufficient" or an "amount effective" refers to an amount that provides, in single or multiple doses, alone or in combination with one or more other compounds, treatments, therapeutic regimens or agents (e.g., a drug), a long term or a short term detectable or measurable improvement in a given subject or any objective or subjective benefit to a given subject of any degree or for any time period or duration (e.g., for minutes, hours, days, months, years, or cured).

An amount sufficient or an amount effective can but need not be provided in a single administration and can but need not be achieved by IL-10R antibody or subsequence thereof alone, in a combination composition or method that includes a pathogen antigen, live or attenuated pathogen, or a nucleic acid encoding all or a portion (e.g., an epitope) of any protein or proteinaceous pathogen antigen. In addition, an amount sufficient or an amount effective need not be sufficient or effective if given in single or multiple doses without a second or additional administration or dosage, since additional doses, amounts or duration above and beyond such doses, or additional antigens, compounds, drugs, agents, treatment or therapeutic regimens may be included in order to provide a given subject with a detectable or measurable improvement or benefit to the subject.

An amount sufficient or an amount effective need not be therapeutically or prophylactically effective in each and every subject treated, nor a majority of subjects treated in a given group or population. An amount sufficient or an amount effective means sufficiency or effectiveness in a particular subject, not a group of subjects or the general population. As is typical for such methods, different subjects will exhibit varied responses to treatment.

The term "subject" refers to an animal, typically a mammalian animal, such as a non human primate (apes, gibbons, gorillas, chimpanzees, orangutans, macaques), a domestic animal (dogs and cats), a farm animal (poultry such as chickens and ducks, horses, cows, goats, sheep, pigs), experimental animal (mouse, rat, rabbit, guinea pig) and humans. Subjects include animal disease models, for example, mouse and other animal models of pathogen infection and reactivation from latency known in the art.

Subjects appropriate for treatment include those having or at risk of having a pathogen infection or pathogenesis, or reactivation from latency. Target subjects therefore include subjects that have been exposed to or contacted with a pathogen, or that have an ongoing infection and have developed one or more adverse symptoms caused by or associated with pathogen infection or pathogenesis, regardless of the type, timing or degree of onset, progression, severity, frequency, duration of the symptoms, or subjects that are chronically infected and may not exhibit apparent adverse symptoms but are at risk of pathogen reactivation from latency.

Target subjects also include those at risk of pathogen exposure, contact, infection or pathogenesis or at risk of having or developing a pathogen infection or pathogenesis. The invention methods are therefore applicable to treating a subject who is at risk of pathogen exposure, contact, infection or pathogenesis, but has not yet been exposed to or contacted with pathogen. Prophylactic methods are therefore included. Target subjects for prophylaxis can be at increased risk (probability or
susceptibility) of pathogen exposure, contact, infection or pathogenesis, as set forth herein and known in the art. Such subjects are considered in need of treatment due to such a risk.

[0226] Target subjects for prophylaxis need not be at increased risk but may be from the general population in which it is desired to vaccinate or immunize a subject against a pathogen infection, for example, an child such as an infant or toddler in which it is desired to vaccinate or immunize against a pathogen can be administered an IL-10R antibody or subsequence thereof and an appropriate antigen. In another non-limiting example, a subject that is not specifically at risk of exposure to or contact with a pathogen, but nevertheless does wish to protect against pathogen infection, such as a measles or mumps virus, or papilloma virus, can be administered an BL-10R antibody or subsequence thereof and an appropriate antigen. Such subjects are also considered in need of treatment.

[0227] At risk subjects appropriate for treatment also include subjects exposed to other subjects having a pathogen infection or having been exposed to another subject having a pathogen infection (e.g., at risk of pathogen infection due to transmission from one subject to another). Subjects appropriate for treatment therefore include human subjects exposed to or at risk of exposure to other humans that may have a pathogen infection, or are at risk of a pathogen infection. At risk subjects appropriate for treatment also include subjects where the risk of pathogen infection or pathogenesis is increased due to changes in pathogen infectivity or cell tropism, environmental factors, or immunological susceptibility (e.g., an immune-suppressed, immunocompromised, or HIV-positive subject). Such subjects are also considered in need of treatment due to such a risk.

[0228] "Prophylaxis" and grammatical variations thereof mean a method in which contact, administration or in vivo delivery to a subject is prior to contact with or exposure to or infection with a pathogen. In certain situations it may not be known that a subject has been contacted with or exposed to pathogen, but administration or in vivo delivery to a subject can be performed prior to pathogen infection or manifestation of pathogenesis (or an associated adverse symptom, condition, complication, etc. caused by or associated with a pathogen). For example, a subject can be immunized or vaccinated with a pathogen antigen, live or attenuated pathogen, or a nucleic acid encoding all or a portion (e.g., an epitope) of any protein or proteinaceous pathogen antigen, and administered an IL-10R antibody or subsequence thereof. In such case, a method can eliminate, prevent, inhibit, suppress, limit, decrease or reduce the probability of or susceptibility towards a pathogen infection or pathogenesis, or an adverse symptom, condition or complication associated with or caused by or associated with a pathogen infection, pathogenesis or reactivation from latency.

[0229] Treatment of an acute or chronic (persistent) infection can be at any time during the infection. A chronic infection may or may not be latent. Non-limiting examples of chronic (persistent) infections that are not considered latent are hepatitis B and C viruses. In such non-latent chronic infections, pathogen continues to proliferate or replicate at reduced levels and to induce adverse events, but evades clearance due to immune suppression or repression, for example, by DL-10 or EL-10 analogs produced by the pathogen. Latency refers to a quiescent phase of an infection in which there is no viral production or symptoms and detection of the pathogen is difficult. Reactivation from latency refers to
reactivation and subsequent proliferation of a pathogen, which is triggered by an event, such as immune suppression, stress, etc. An example of an infection that can become latent is an acute herpesvirus infection that after the initial acute infection is controlled by the immune system, becomes a latent persistent infection.

[0230] Methods of the invention may be practiced by any mode of administration or delivery, or by any route, systemic, regional and local administration or delivery. Exemplary administration and delivery routes include intravenous (i.v.), intraperitoneal (i.p.), intrarterial, intramuscular, parenteral, subcutaneous, intra-pleural, topical, dermal, intradermal, transdermal, transmucosal, intra-cranial, intra-spinal, rectal, oral (alimentary), mucosal, inhalation, respiration, intranasal, intubation, intrapulmonary, intrapulmonary instillation, buccal, sublingual, intravascular, intrathecal, intracavity, iontophoretic, intraocular, ophthalmic, optical, intraglandular, intra-organ, intralymphatic.

[0231] IL-10R antibody or subsequence thereof can be administered as a combination (e.g., with an antigen, live or attenuated pathogen, or a nucleic acid encoding all or a portion (e.g., an epitope) of any protein or proteinaceous pathogen antigen), or separately concurrently or in sequence (sequentially) in accordance with the methods as a single or multiple dose e.g., one or more times hourly, daily, weekly, monthly or annually or between about 1 to 10 weeks, or for as long as appropriate, for example, to achieve a reduction in the onset, progression, severity, frequency, duration of one or more symptoms or complications associated with or caused by pathogen infection, pathology, or an adverse symptom, condition or complication associated with or caused by a pathogen. Thus, a method can be practiced one or more times (e.g., 1-10, 1-5 or 1-3 times) an hour, day, week, month, or year. The skilled artisan will know when it is appropriate to delay or discontinue administration. A non-limiting dosage schedule is 1-7 times per week, for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 or more weeks, and any numerical value or range or value within such ranges.

[0232] Doses can be based upon current existing protocols, empirically determined, using animal disease models or optionally in human clinical trials. Initial study doses can be based upon animal studies set forth herein, for a mouse, which weighs about 30 grams, and the amount of IL-10R antibody or subsequence thereof administered that is determined to be effective. Exemplary non-limiting amounts (doses) are in a range of about 0.1 mg/kg to about 100 mg/kg, and any numerical value or range or value within such ranges. Greater or lesser amounts (doses) can be administered, for example, 0.01-500 mg/kg, and any numerical value or range or value within such ranges. The dose can be adjusted according to the mass of a subject, and will generally be in a range from about 1-10 ug/kg, 10-25 ug/kg, 25-50 ug/kg, 50-100 ug/kg, 100-500 ug/kg, 500-1,000 ug/kg, 1-5 mg/kg, 5-10 mg/kg, 10-20 mg/kg, 20-50 mg/kg, 50-100 mg/kg, 100-250 mg/kg, 250-500 mg/kg, or more, two, three, four, or more times per hour, day, week, month or annually. A typical range will be from about 0.3 mg/kg to about 50 mg/kg, 0-25 mg/kg, or 1-10 mg/kg, or any numerical value or range or value within such ranges.

[0233] Doses can vary and depend upon whether the treatment is prophylactic or therapeutic, the onset, progression, severity, frequency, duration probability of or susceptibility of the symptom, condition, pathology or complication the type of pathogen infection or pathogenesis, reactivation from
latency or vaccination or immunization to which treatment is directed, the clinical endpoint desired, previous or simultaneous treatments, the general health, age, gender, race or immunological competency of the subject and other factors that will be appreciated by the skilled artisan. The skilled artisan will appreciate the factors that may influence the dosage and timing required to provide an amount sufficient for providing a therapeutic or prophylactic benefit.

[0234] Typically, for therapeutic treatment, IL-10R antibody or subsequence thereof will be administered as soon as practical, typically within 1-2, 2-4, 4-12, 12-24 or 24-72 hours after a subject is exposed to or contacted with a pathogen, or within 1-2, 2-4, 4-12, 12-24 or 24-48 hours after onset or development of one or more adverse symptoms, conditions, pathologies, complications, etc., associated with or caused by a pathogen infection or reactivation from latency. For prophylactic treatment in connection with vaccination or immunization, IL-10R antibody or subsequence thereof and an antigen, live or attenuated pathogen, or a nucleic acid encoding a pathogen antigen, can be administered for a duration of 0-4 weeks, e.g., 2-3 weeks, prior to exposure to, contact or infection with pathogen, or at least within 1-2, 2-4, 4-12, 12-24, 24-48 or 48-72 hours prior to exposure to, contact or infection with pathogen. For a chronic infection, such as a latent pathogen infection in a subject that has or is at risk of reactivation from latency, IL-10R antibody or subsequence thereof is administered at any appropriate time.

[0235] The dose amount, number, frequency or duration may be proportionally increased or reduced, as indicated by the status of the subject. For example, whether the subject has a pathogen infection, whether the subject has been exposed to, contacted or infected with pathogen or is merely at risk of pathogen contact, exposure or infection, whether the subject is or is at risk of suffering from reactivation from latency or whether the subject is a candidate for or will be vaccinated or immunized. The dose amount, number, frequency or duration may be proportionally increased or reduced, as indicated by any adverse side effects, complications or other risk factors of the treatment or therapy.

[0236] IL-10R antibodies and subsequences thereof, optionally in combination with an antigen, live or attenuated pathogen, or a nucleic acid encoding a pathogen antigen, can be incorporated into pharmaceutical compositions, e.g., a pharmaceutically acceptable carrier or excipient. Such pharmaceutical compositions are useful for, among other things, administration to a subject in vivo or ex vivo.

[0237] As used herein the term "pharmaceutically acceptable" and "physiologically acceptable" mean a biologically acceptable formulation, gaseous, liquid or solid, or mixture thereof, which is suitable for one or more routes of administration, in vivo delivery or contact. Such formulations include solvents (aqueous or non-aqueous), solutions (aqueous or non-aqueous), emulsions (e.g., oil-in-water or water-in-oil), suspensions, syrups, elixirs, dispersion and suspension media, coatings, isotonic and absorption promoting or delaying agents, compatible with pharmaceutical administration or in vivo contact or delivery. Aqueous and non-aqueous solvents, solutions and suspensions may include suspending agents and thickening agents. Such pharmaceutically acceptable carriers include tablets (coated or uncoated), capsules (hard or soft), microbeads, powder, granules and crystals. Supplementary active compounds
(e.g., preservatives, antibacterial, antiviral and antifungal agents) can also be incorporated into the compositions.

[0238] Pharmaceutical compositions can be formulated to be compatible with a particular route of administration. Thus, pharmaceutical compositions include carriers, diluents, or excipients suitable for administration by various routes. Exemplary routes of administration for contact or in vivo delivery which a composition can optionally be formulated include inhalation, respiration, intranasal, intubation, intrapulmonary instillation, oral, buccal, intrapulmonary, intradermal, topical, dermal, parenteral, sublingual, subcutaneous, intravascular, intraarticular, intracavity, transdermal, iontophoretic, intraocular, opthalmic, optical, intravenous (i.v.), intramuscular, intraglandular, intraorgan, intralymphatic.

[0239] Formulations suitable for parenteral administration comprise aqueous and non-aqueous solutions, suspensions or emulsions of the active compound, which preparations are typically sterile and can be isotonic with the blood of the intended recipient. Non-limiting illustrative examples include water, saline, dextrose, fructose, ethanol, animal, vegetable or synthetic oils.

[0240] For transmucosal or transdermal administration (e.g., topical contact), penetrants can be included in the pharmaceutical composition. Penetrants are known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. For transdermal administration, the active ingredient can be formulated into aerosols, sprays, ointments, salves, gels, or creams as generally known in the art. For contact with skin, pharmaceutical compositions typically include ointments, creams, lotions, pastes, gels, sprays, aerosols, or oils. Carriers which may be used include Vaseline, lanolin, polyethylene glycols, alcohols, transdermal enhancers, and combinations thereof.

[0241] Cosolvents and adjuvants may be added to the formulation. Non-limiting examples of cosolvents contain hydroxyl groups or other polar groups, for example, alcohols, such as isopropyl alcohol; glycols, such as propylene glycol, polyethylene glycol, polypropylene glycol, glycol ether; glycerol; polyoxyethylene alcohols and polyoxyethylene fatty acid esters. Adjuvants include, for example, surfactants such as, soya lecithin and oleic acid; sorbitan esters such as sorbitan trioleate; and polyvinylpyrrolidone.

[0242] Supplementary compounds (e.g., preservatives, antioxidants, antimicrobial agents including biocides and biostats such as antibacterial, antiviral and antifungal agents) can also be incorporated into the compositions. Pharmaceutical compositions may therefore include preservatives, anti-oxidants and antimicrobial agents.

[0243] Preservatives can be used to inhibit microbial growth or increase stability of ingredients thereby prolonging the shelf life of the pharmaceutical formulation. Suitable preservatives are known in the art and include, for example, EDTA, EGTA, benzalkonium chloride or benzoic acid or benzoates, such as sodium benzoate. Antioxidants include, for example, ascorbic acid, vitamin A, vitamin E, tocopherols, and similar vitamins or provitamins.
An antimicrobial agent or compound directly or indirectly inhibits, reduces, delays, halts, eliminates, arrests, suppresses or prevents contamination by or growth, infectivity, replication, proliferation, reproduction, of a pathogenic or non-pathogenic microbial organism. Classes of antimicrobials include, antibacterial, antiviral, antifungal and antiparasitics. Antimicrobials include agents and compounds that kill or destroy (-cidal) or inhibit (-static) contamination by or growth, infectivity, replication, proliferation, reproduction of the microbial organism.

Exemplary antibacterials (antibiotics) include penicillins (e.g., penicillin G, ampicillin, methicillin, oxacillin, and amoxicillin), cephalosporins (e.g., cefadroxil, ceforanid, cefotaxime, and ceftriaxone), tetracyclines (e.g., doxycycline, chlortetracycline, minocycline, and tetracycline), aminoglycosides (e.g., amikacin, gentamycin, kanamycin, neomycin, streptomycin, netilmicin, paromomycin and tobramycin), macrolides (e.g., azithromycin, clarithromycin, and erythromycin), fluoroquinolones (e.g., ciprofloxacin, lomefloxacin, and norfloxacin), and other antibiotics including chloramphenicol, clindamycin, cyclodexirine, isoniazid, rifampin, vancomycin, aztreonam, clavulanic acid, imipenem, polymixin, bacitracin, amphotericin and nystatin.

Particular non-limiting classes of anti-virals include reverse transcriptase inhibitors; protease inhibitors; thymidine kinase inhibitors; sugar or glycoprotein synthesis inhibitors; structural protein synthesis inhibitors; nucleoside analogues; and viral maturation inhibitors. Specific non-limiting examples of anti-virals include nevirapine, delavirdine, efavirenz, saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, zidovudine (AZT), stavudine (d4T), lamivudine (3TC), didanosine (DDI), zalcitabine (ddC), abacavir, acyclovir, penciclovir, ribavirin, valacyclovir, ganciclovir, 1-D-ribofuransol-1,2,4-triazole-3-carboxamide, 9->2-hydroxy-ethoxy methylguanine, adamanantanamine, 5-Iodo-2'-deoxyuridine, trifluorothymidine, interferon and adenine arabinoside.


IL-10R antibody and subsequences thereof, along with any adjunct agent, compound drug, composition, whether active or inactive, etc., can be packaged in unit dosage form (capsules, tablets, troches, cachets, lozenges) for ease of administration and uniformity of dosage. A ”unit dosage form” as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active ingredient optionally in association with a pharmaceutical carrier (excipient, diluent, vehicle or filling agent) which, when administered in one or more doses, is calculated to produce a desired effect (e.g., prophylactic or therapeutic effect). Unit dosage forms also include, for example, ampules and vials, which may include a composition in a freeze-
dried or lyophilized state; a sterile liquid carrier, for example, can be added prior to administration or delivery *in vivo*. Unit dosage forms additionally include, for example, ampules and vials with liquid compositions disposed therein. Individual unit dosage forms can be included in multi-dose kits or containers. Pharmaceutical formulations can be packaged in single or multiple unit dosage form for ease of administration and uniformity of dosage.

[0249] The invention provides kits comprising IL-10R antibodies and subsequences thereof, optionally with a pathogen antigen, live or attenuated pathogen, combination compositions and pharmaceutical formulations thereof, packaged into suitable packaging material. A kit typically includes a label or packaging insert including a description of the components or instructions for use *in vitro, in vivo, or ex vivo*, of the components therein. A kit can contain a collection of such components, e.g., IL-10R antibody or subsequence thereof and optionally a pathogen antigen, live or attenuated pathogen, alone (individual vessel or pack) or in combination (e.g., mixture), or another compound, agent, drug or composition.

[0250] The term "packaging material" refers to a physical structure housing the components of the kit. The packaging material can maintain the components steriley, and can be made of material commonly used for such purposes (e.g., paper, corrugated fiber, glass, plastic, foil, ampules, vials, tubes, etc.).

[0251] Kits of the invention can include labels or inserts. Labels or inserts include "printed matter," e.g., paper or cardboard, or separate or affixed to a component, a kit or packing material (e.g., a box), or attached to an ampule, tube or vial containing a kit component. Labels or inserts can additionally include a computer readable medium, such as a disk (e.g., floppy diskette, hard disk, flash memory), optical disk such as CD- or DVD-ROM/ RAM, DVD, MP3, magnetic tape, or an electrical storage media such as RAM and ROM or hybrids of these such as magnetic/optical storage media, FLASH media or memory type cards.

[0252] Labels or inserts can include identifying information of one or more components therein, dose amounts, clinical pharmacology of the active ingredient(s) including mechanism of action, pharmacokinetics and pharmacodynamics. Labels or inserts can include information identifying manufacturer information, lot numbers, manufacturer location and date.

[0253] Labels or inserts can include information on a condition, disorder or disease (e.g., viral infection, vaccination or immunization) for which a kit component may be used. Labels or inserts can include instructions for the clinician or subject for using one or more of the kit components in a method, or treatment protocol or therapeutic regimen. Instructions can include dosage amounts, frequency or duration, and instructions for practicing any of the methods, treatment protocols or prophylactic or therapeutic regimes described herein. Exemplary instructions include, instructions for treating a pathogen infection or pathology, and instructions for providing a subject with protection against pathogen infection, pathology or reactivation from latency.

[0254] Labels or inserts can include information on any benefit that a component may provide, such as a prophylactic or therapeutic benefit. Labels or inserts can include information on potential adverse
side effects, complications or reactions, such as warnings to the subject or clinician regarding situations where it would not be appropriate to use a particular composition. Adverse side effects or complications could also occur when the subject has, will be or is currently taking one or more other medications that may be incompatible with the composition, or the subject has, will be or is currently undergoing another treatment protocol or therapeutic regimen which would be incompatible with the composition and, therefore, instructions could include information regarding such incompatibilities.

[0255] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described herein.

[0256] All applications, publications, patents and other references, GenBank citations and ATCC citations cited herein are incorporated by reference in their entirety. In case of conflict, the specification, including definitions, will control.

[0257] As used herein, the singular forms "a," "an," and "the" include plural referents unless the context clearly indicates otherwise. Thus, for example, reference to an 'TL-10 antibody" or a "pathogen" includes a plurality of antibodies or pathogens and reference to an "activity or function" such as "an IL-10 activity or function" or "an IL-10R activity or function" can include reference to one or more IL-10R activities or functions, including any activity or function of any component of the IL-10/IL-10R signaling pathway or activity, and so forth.

[0258] As used herein, all numerical values or ranges include fractions of the values and integers within such ranges and fractions of the integers within such ranges unless the context clearly indicates otherwise. Thus, to illustrate, reference to a numerical range, such as a percentage range, 90-100%, includes 91%, 92%, 93%, 94%, 95%, 97%, etc., as well as 91.1%, 91.2%, 91.3%, 91.4%, 91.5%, etc., 92.1%, 92.2%, 92.3%, 92.4%, 92.5%, etc., and so forth. Reference to a range of 1-5 fold therefore includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, fold, etc., as well as 1.1, 1.2, 1.3, 1.4, 1.5, fold, etc., 2.1, 2.2, 2.3, 2.4, 2.5, fold, etc., and so forth.

[0259] The invention is generally disclosed herein using affirmative language to describe the numerous embodiments and aspects. The invention also specifically includes embodiments in which particular subject matter is excluded, in full or in part, such as substances or materials, method steps and conditions, protocols, procedures, assays or analysis. For example, in certain embodiments or aspects of the invention, antibodies or other materials and method steps are excluded. In certain embodiments and aspects of the invention, for example, an IL-10R antibody or pathogen antigen is excluded. Thus, even though the invention is generally not expressed herein in terms of what is not included, embodiments and aspects that expressly exclude compositions (e.g., antibodies or pathogen antigens) or method steps are nevertheless disclosed and included in the invention.

[0260] A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the
invention. Accordingly, the following examples are intended to illustrate but not limit the scope of invention described in the claims.

Examples

Example 1

[0261] This example includes a description of various materials and methods.

[0262] Human IL-I ORa cloning: The full length IL-I ORa MGC clone in pCMVsport δ (Accession number BE272922) was purchased from Invitrogen Corp. (Carlsbad, CA) and the full-length IL-I ORa open reading frame was subcloned by polymerase chain reaction [primers: hIL-I ORa F48 EcoRI and hEL-10Ra R1857 NotI (Table 1)] from the MGC clone. The amplified product was digested with EcoRI and NotI restriction enzymes and subcloned into pCDNA3.1(+) (Invitrogen Corp.) previously digested with EcoRI and NotI. The sequence encoding the human IL-I ORa extracellular domain was amplified from pCMVsport δ vector by polymerase chain reaction [primers: hIL-I OR Forward and hIL-I OR Reverse (Table 1)]. The amplified product was digested with EcoRI and BglII restriction enzymes, which were included in the primers, and the human IgGl Fc sequence was excised from the pVL 1392.fc vector using BgiII and NotI restriction enzymes. The shIL-I OR α (EcoRI-BglII) and hFc (BglII-NotI) fragments were subcloned into the pCDNA3.1(+) expression vector previously digested with EcoRI and NotI to generate a hIL-10R α-hFc expression vector.

[0263] Nucleotide sequence of full-length human DL-I ORa from initiation codon (ATG) through the IL-I ORa stop codon (underlined): SEQ ID NO: 1

```
ATGCTGCCGT GCCTGCTAGT GCTGCTGGCG GCGCTCCTCA GCCTCCGCTCT TGAGTCAGAC  60
GCTCATGGGA CAGAGCTGCC CAGCCCTCCG CTTGCTGAGT TGGAAGCAGA AGTTTTTACAC 120
CACATCCTCC ACTGGACACC CATCCTCAAT CAGTCTGAAA GTACCTGCTA TGAAGTGCGA 180
CTCCTGAGGT ATGAATAGA GTCCTGGAAC TCCATCCTCA ACTGAGCGCA GACCTGCTGC 240
TATGACCTTA CCGCAGTGGC CGGCGGAGTA TACACGACGA AGTGGACCGA 300
CGGCGCTGGG AGCGAGCGAG GCACCTCAAC TGACGGCTCA CCAACACCGC CTTCTGCTG 360
GATGAAGTGA CCTGACAGGT TGGACGGTGG AACCTAGAAG TCCCAAGATG CTTCACTGCTC 420
GGGAAGATTC AGCTACCCAG GCCAAGATG GCCCGCGCAA ATGACACATA TGAAGACATC 480
TTCAGTACTG TCCGAGAGTA TGAGATTGCC ATTCGCAAGG TGCGGGAACA CTTCTGCTTC 540
ACACACAAGA AAGTAAACCA TGAAACTCTC AGGCTCTCAA CCACTGAGA AGTGGAGAGAG 600
TTCTGTGTCG AGGTGAACAC ATCGTCGCTG TCCTGAGAAT ACAAGGGGAT GTGGACCTAA 660
GAGGAGTGCA TCTCCCTCAG CAGGCTAGAT TCCACGCTGA CCAACGCTAT CATCTTCTTT 720
GCTCTTTGTCG TGCTGCTTCT CGGAGCCCTC GCTACTGCCC TGGGCTCTCC GCTGTATGAG 780
GGCGCGCCGA AGAAGCTTAC CAGTGTCTCG CTTCTTGAGA AGGCCAGGCC CTTCTAGTTC 840
ATCAGGACGC GTCTCTTCCC AGAAGCCCAA GACACATACG ACCGCTGTTA TGAGAGGCC 900
TTTTTGAGGG TGCAGCCCGA GCTGAGAGAC TTGAGCTGCG AGCGACAGAC AGACAGAGCC 960
TTGGAGCCGA CCAAGCCTAC CCTGCAAGGT GAAGAGCCCC AGTCTGCTCT CCTGACCGCT 1020
CACCCCCAGG CTGACACAGC GCTGGGAAAC GGGAGGACCCT GTGTCGCTGG GGACAGCTGC 1080
AGTAGTGCGA GCACGACTAG CACAGACAGC GGGATCTGCC TGCAGGAGCC CAGCTGAGC 1140
```
Amino acid sequence of full-length human IL-10Rα from the start Met to the terminal amino acid: SEQ ID NO:2

MLPCLWLLA ALLSLRLGSD AGHTELPSPP SVWFEAEFFH HILHWTPIPN QSESTCYEVA 60
LLRYGIESWN SINSQSTSLS YDLITAVTLDL YHSNGYRARV RAVDGSRHSN WTVTNTFPSV 120
DEVTLVGSV NLEIHNGFIL GKIQLPRPKM APANDTYESI FSHEFREYEA IRKVPGNFTF 180
THKKVKHENF SLLTSGEVGE FCVQVKPSVA SRSNKGMWSK EECISLTRQY FTVTNVIIFF 240
AFVLLSGAL AYCLALQVLV RRRKLPSVL LFKKPSPFIF ISQRPSETPQ DTIHIPDEEA 300
FLKVSPELKN LDLHSOSTSG FGSTKPSLQT EEPQFLLPDP HPQADRTLGN GEPPVLGDS 360
SSGSSNSTD SICLQEPSLS PSTGPTWEQQ VGNSRQGQQ SGIDLVQMSR GRAGTQGQS 420
ALGHHSPPEP EVGGEEDPAVF VAFQYLRQT RCAAEEKATK GCLEEESPLT DGLFGKFRGC 480
LVDEAGLHPP ALAKGYLQKD PLEMTLASSG SRSNKGMWSK EECISLTRQY FTVTNVIIFF 540
FAHDLPLGVC VAAPGGGLGS FNSDLVTPLL ISSLQSE 600

Nucleotide sequence of human IL-10Rα:human IgGl fusion protein from initiation codon (ATG) through human IL-10Rα extracellular domain to end of human Fc sequence (underlined): SEQ ID NO:3

ATGGTGCCGT GCCTCGTAGT GCTGCTGGCG GCGCTCCTCA GCCTCCGTCT TGGCTCAGAC 60
GCTCATGGGA CAGGCTGCC CAGCCCTCCG TCTGTGTGGT TTGAAGCAGA ATTTTTCCAC 120
CACTTTCCCA ACTCCCAAT CAGTCTGAAA GTACCTGCTA TGAAGTGGCG 180
CTCCTAGATG ATGGAATAGA GTCCTGGAAC TCCATCTCCA ACTGTAGCCA GCCCTGTCC 240
TATGACCTTA CCGAGCTGGC TTTGACCTTG TACCAAGAC ATGGCTACCG GGCCAGAGTG 300
CACAGCTAGT TCCTGTTGCA AACTTTAGAGA TCCACAATGG CTTCATCCTC 360
GCTGGAGTCA AGCTGCTTGGC TCTGTGTGGT TTGAAGCAGA ATTTTTCCAC 420
GCGCCATGGA CAGGCTGCC CAGCCCTCCG TCTGTGTGGT TTGAAGCAGA ATTTTTCCAC 480
TTCTAGTCTC TGGAGTCAAGA AGCTGCTTGGC TCTGTGTGGT TTGAAGCAGA ATTTTTCCAC 540
ACACACAGA AGATTTGAAA GGGCTCCTA TGGCTCAGAC 600
TTCTGTGCCT GTGGATGGGC ATCTGCTTGGC TCTGGGAAAA CCTCAAGTCC 660
GAGGAGTGCA TCTTGGCTAGT GGCCAGAGTG CAGTCTGCTA TGGCTCAGAC 720
ACTCACTACT GCACCTGGGT CCCAGCTAGT GACCTTCTGG GCACCTGGGT CCCAGCTAGT 780
TTCTGGGAGT GCAGGCCCCG AACTTTAGAGA TCCACAATGG CTTCATCCTC 840
GTTTGGAACG GCAGGCCCCG AACTTTAGAGA TCCACAATGG CTTCATCCTC 900
Amino acid sequence of human IL-10Rα-extracellular domain fused to the Fc portion of human IgGl (underlined): SEQ ID NO:4

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<th>Amino Acid Sequence</th>
<th>Length</th>
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<tr>
<td>MVPCLWLLA ALLSRLGSD AHGTELPSSPV SVWFEAEFFHH HILHWTIPNP QSESTCYEVA</td>
<td>60</td>
</tr>
<tr>
<td>LLRYGIESWNSNSCQGTSLS YDLTAVTLDD YHNSGYRARAV RAVDGSRHSHN WTVTNRFSV</td>
<td>120</td>
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<td>DEVTLTGSVSHLEIHNGFIL GIQILPPRFKMK APANDTYESI FSHFREYIEA IRKVPGNFTF</td>
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<td>THKKVKBHENFSLLTSGEVGEFCVQVKPSV ASRSNKGMWSK EECISLRTQY FTVTNRSCD</td>
<td>240</td>
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<td>THTCPPCPAP LEILGGSPSVFL FPPKFKTLIM ISRTPETCV WDVSHEDEPD VRKFNYDVG</td>
<td>300</td>
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<tr>
<td>EVHNAKTKPREEQYNSTYVRVFVSUTVLHODW LNKGEYKCKVS NALPAPI EKTISKAGQ</td>
<td>360</td>
</tr>
<tr>
<td>PREPOVYTLPSERDELTKQVSLTCLVKGF YPSDIAVEAE SNQOPENNYK TTPPVLDSDG</td>
<td>420</td>
</tr>
<tr>
<td>SFFLYSKLTV DKSRWQOGNV FSCTSMMEAL HNHYTQKSLSLHSPG</td>
<td>480</td>
</tr>
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</table>

Pan troglodyte (chimpanzee) IL-1QRa cloning: The predicted amino acid sequence of the extracellular region of Pan troglodyte IL-1 ORa contains two amino acid differences from the human amino acid sequence of IL-1ORα: arginine at amino acid position 92 instead of histidine, and valine at amino acid position 224 instead of isoleucine (Accession number NC_006478.2). The amino acid change at position 224 (V224I) is a recognized single nucleotide polymorphism (SNP) in human EL-10Ra sequence. The DNA sequence of human IL-1ORa was mutated within the codons for amino acid His92 [nucleotide 275 (adenine) was changed to guanine (A275G)], and amino acid Val224 [nucleotide 670 (adenine) was changed to guanine (A670G)] using polymerase chain reaction [primers hIL-1OR Forward, IL-1ORa Notl R1857, IL-10R-a275g-F, EL-10R-a275g-R, IL-10R-a670g-F, and EL-10R-a670g-R (Table 1)]. The amplified product was cloned into vector pCR-BluntII-Topo (Invitrogen Corp.) using a Zero Blunt TOPO PCR Cloning Kit (Invitrogen Corp.). Clones were then sequenced and verified to contain the mutations designed. The modified sequence encoding the modified full length human EL-10Ra (referred to as cIL-1ORα-FL) was subcloned into mammalian expression vector pcDNA3.1(+) previously digested with EcoRI and Notl.

A construct coding for a fusion protein consisting of the extracellular region of Pan troglodyte EL-1ORa fused to the Fc portion of human IgGl was constructed as follows. The DNA sequence encoding the extracellular domain of the Pan troglodyte-modified human EL-1ORa (cEL-10Ra-EX) from amino acid Metl through Asp235 was amplified by polymerase chain reaction using cEL-10Ra-FL as template. The restriction site for BamHI was integrated into the 3’ primer and placed
directly after the codon for Asp235. Polymerase chain reaction was performed [primers hIL-1ORa Forward, hIL-1ORa Reverse (Table 1)]. The amplified product was digested with EcoRI and BamHI. The Fc portion of human IgGl was excised from the human IL-10Rα:hFc expression vector construct with restriction enzymes (BglIII at the 5’ end, and NotI at the 3’ end). The cIL-1ORα-EX and human IgGl Fc were subcloned into the mammalian expression vector pcDNA3.1(+) previously digested with EcoRI and NotI.

Nucleotide sequence of Pan troglodyte IL-10Rα:human IgGl fusion protein from initiation codon (ATG) through Pan troglodyte IL-1ORα extracellular domain to end of human Fc sequence (underlined): SEQ ID NO: 5

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ATGTTGCCGCT GCCCTCGTATG GCTGCTGGCG GCGCTCCCTCA GCCTCCCGTCT TGGTCCTGAC  60
GCTCATGGGA CAGAGCTGCC CAGCCCTCCG TCTCTGTGCG TTGAAGGAGA AGTTTTCCAC 120
CACATCCCTCC ACTGACACCC CATCCTAAAT CAGTCTGAAA GTCACCTCGA TGAAGGAGA 180
CTCCTCAGGTG ATGGAATAAG ATCTTGGAAC TCCATCTCAA ACTGTAGCCA AACCTAGAGA 240
TATGACCTTA CCGGAGTCGT CTTGAGACCT TACCCAGAGC ATGGCTACCG GGCAGGAGA 300
GGGGCTCGTG AAGATTGCCG AGCCCTGGCA AGCTACCGTA CAGTCTGAAA GCGCTCCCTCA 360
GATGAAGTGA CTCTGACATG TGGCATGGTG AACCTAGAGA TCCAAATGAG TCTTACCTCA 420
GGGAAGATTCC GCCAAATGGA GCGCCCGGAA ATGACATAAT TGAAGGAGA 480
TTCAGTCACT TCCCTGACATG TGGCATGGTG AACCTAGAGA TCCAAATGAG TCTTACCTCA 540
ACACACAAAG AGTTAAAACCA TGGAAACCTC AGCTCTCAAA GCTGCTGGCG GCTGCTGGCG 600
TTCTGTGCC AGTTGAAACAT CTCGTCCTGC TCCGAAGATC ACAAGGAGGAGCTGGCTAA 660
GAGGAGTGCCT TCTCATCTAAC CAGCCGTATA TCCACGTTGA CCAACAGATC TTGTCACAA 720
ACTCACATCC GCCCACGCGG CCCACCCCTAT GAACCTGGGG GGGCGGCGTG AGCTTCTCCC 780
TTCCCCCAAA AACCCAAGGA CACCCCTCATG ATCTTCCCGG CCCCTGACCT CACATGCCGT 840
GTGGTGCGAC GCAGCCCTAG TCTCTGGGAG GCAAGGAGGAGCTGGCTAA 900
GAGGAGTGCATA ATGCCAAGGA AAAGCCTGCG GAGGAGGACAT ACAAAGCATG TCCACGCTGC 960
GTGACGGTCC TCCACGCTCA GCACCGAGGC TGGGTCGATG GCAAGGAGGAGCTGGCTAA 1020
GTTCACCTCA AACCCCCTAT GACCCCAACT GAAAGAAACAA TCTACGACG CAAAGGCAGC 1080
CCCCGGAGAC CAGGCTGTTA CACCCCGGCC CATCTCGCGG AGAGGATGAC CAGAACCAG 1140
GTGACCGGTCA CAGAGTCTGT CAAAGGCGGT TATCCACAGC ACATCGCGGT GGAAGGGGCG 1200
AGCAAGGCC AGCCCGAGGA CAATACAAAG CACCACCCCT CGGTCTGGGA CTCCGACGCC 1260
TCCTTCTTCA CTTATACGCA GCTCAGCGGT GACAGAGGAG GGTGCGAGCA GGGGGACGTC 1320
TCTCTACTCT CCTGAGTGGG TGGGCGCTGA CACAAACACT CACACAGGAGG GACCTCTCTC 1380
CTGTCCTCCGG GTAATGGA 1440
```

Amino acid sequence of Pan troglodyte IL-10Rα:extracellular domain fused to the Fc portion of human IgGl (underlined), the amino acid corresponding to the human SNP is bold: SEQ ID NO: 6

```
MVFCLWLLA ALLSLRGLSD AGHTELPSPP SVWFEAEFFH HILHWTIPPN QSETECYEVA  60
LLRHYIESWN SISNSCZTLD YDLTAVTLDL YRSNGYARV RAVDGRSRSHN VTVTNRFSV 120
DEVLTVGVS VNLHINFGIL KIQLPRPKM APANDYESI FSHFYRTEIA IRKVPGNTF 180
```
Cynomolgus macaque IL-10Rα cloning: Full length cynomolgus macaque IL-10Rα was cloned from purified cynomolgus T cells activated for 41 hours with 1 ng/ml phorbol myristic acid (PMA) (Sigma, St. Louis, MO) and 500 ng/ml ionomycin (Calbiochem, San Diego, CA). The T cells were purified from peripheral blood mononuclear cells using a Pan T cell negative isolation kit from Miltenyi Biotec (Auburn, CA) and following the manufacturer’s instructions. RNA was isolated from 2x10⁶ cells using an RNAeasy kit (QIAGEN, Frankfurt, Germany) and first strand cDNA was made by reverse transcription using a SuperScriptII kit (Invitrogen Corp.). The predicted sequence for rhesus IL-10Ra (Accession number XM_001092376) was initially used to design primers for amplification of the cynomolgus IL-10Rα, however, amplification with this primer set was unsuccessful. Analysis of the predicted sequence suggested a miscalculation in the splice site that disrupted the forward primer binding sequence. Therefore a forward primer designed from the Pan troglodytes sequence and a rhesus IL-10Rα reverse primer [chDL-10RaFl and rML-10Ra_R2098 (Table I)] were used. The amplified product was put into pCR®-Blunt II-TOPO® using a Zero Blunt® TOPO® PCR Cloning Kit and was sequenced. The full-length cyEL-10Rα was then amplified using polymerase chain reaction [primers hIL-10Ra Forward, M13R (Table I)] and subcloned into pcDNA 3.1 (+) previously digested with EcoRI. The construct was verified by restriction digest and sequencing.

Nucleotide sequence of cynomolgus IL-10Ra full-length protein from initiation codon (ATG) through cynomolgus IL-10Rα stop codon sequence (underlined): SEQ ID NO:7

```
ATGCGTGGCGT GCCTCGTATG GTGCTGGCGG CCAGTTCCTCA TGGGCGGCAC 60
GCTCATGGGA CAGGAGCGCA CAGGCGCCCA TCTGTGTTGT TGAACAGA ATTTCAGAC 120
CACATCCTCC AGTGACACC CTACCAAAAT GACTGTAAG GTACCTGTA A 180
CTCTAGGCTT ATGGAACAGG CCAGGCTGAC TCTACTCCCA ACTGTAAGCC GCCTCGGTCC 240
TGATCACCTTA CCGCGTGGAC CTGGACCTCG TACCGAGACA ATGGCTACCC GCGAGATG 300
CCTGGCTGTGG ACGCAACCG GACACTCCAC TGAGCGGCTA CCAACACCG CTCTCTCTT 360
GATGAAATTG GTGCAAGAGT TGCCAGTGG AGTCAAGAGA AACAGAGGAT CCGGAGATTC 420
ACAGAGAGCT GCCCCCCAG GCCAACAGT GCCTGGCTCA ATGACACTAC TGAAGCATC 480
TTCAGCTACT TCAGACTGGA TTAGATGACG AGGACCGGCA TTTAGTGGC CT 540
ACACACAGGA AAGTAAACCA TGAAACTTCA CGCTTCTCAA CTCAGGAGA ATGGGAGAG 600
TTCTGCATCC AGGTAAACCA ATCTGTCCTC TCCGGAACAA ACAAGGAGT GTGGCTCTG 660
GGAGTGGCG TCTCTTCAC CAGGAGATAT TTAGAGAGG GCAACAGGCA CCGAGAAGG 720
GCCCTGCTCC TGGATGCTCC GAGGAGCGCG GCCCTCTGCC GCTGGATTGT 780
CCGCGCGGA AAGCAGCTCC CAGGAGCTCC TTTAGGTCGG ACAGCAGGCA CCCCC 840
ATCAGCCAGA GTCTCCTCCC AGAGACCCCA GACACCATCC ACCCGTGGTA TGGAGAAGCC 900
TTCCTGAAGG GTCGACAGGA TCGACGCGG ACGAGTGCC 960
```
Amino acid sequence of cynomolgus macaque IL-10Ra full-length protein from the start Met through the terminal amino acid: SEQ ID NO: 8

MLPCLWLLA AFLSRLGLSD AHGTELPSPP SVWFEEAFFH HILHWTPIN QSESTCYEVA 60
LLRYGTGRWN SISNCSQALS YDLTAVTDL YRSNGYRARV RAVDGSRHSN WTVTNRFSL 120
DEVTLTGVS VHLJHIHGFIL GKIJP RRPRFKM APANDTVIESI FSHFREYEIA IRKVPGNFT 180
THKKVKHENF SLSTHSVEGE FCVQVKSPTV SRTNKGWMSK EECSVETRQY FTVTNYIFF 240
AVFLLLSGAL AYCLALQLYLVR RRRKCLKRLV LFKPKPAFIF ISQRPSPETQ DIHTPLDEEA 300
FLKVSPELRR 5LHGSTDGG TCTCGTGGGG GCACAGCTGC AAGCATGGAAC 360
SSGSSNSTDSS GICLQEPSLSS PSTGPTWEQQ VGSDSRGQDD SGGFLYQNSF GGAQDTQGQS 420
ALGDSPPEPP EVPQE0QDPTA WFRGYLRTQ RACEEKTATK GCLHEEFLPT GGGPPEKFRGC 480
LDDEAGLHP SALKYLKQD PLEMTLASSG APAEOQWNQPT EEWSSLALSS CSDLGTSWDS 540
FAHDLAPLGC VAAPDGLLLGS FNSDLVTLPL ISSLHSSSDS 600

A construct expressing the cynomolgus macaque IL-10Ra extracellular region fused to the Fc portion of human IgGl (cyDL-10Rα) was fabricated using the same method described for making the hIL-10Rα:hfC fusion construct.

Nucleotide sequence of cynomolgus macaque IL-10Rα:human IgGl fusion protein from initiation codon (ATG) through cynomolgus macaque IL-10Rα extracellular domain to end of human Fc sequence: SEQ ID NO: 9

ATGGTGCCGT GCTCCTGATT GCTGCTGGGG CGGGTCTCTCA GTGCGGCTGT TGGCTGACAC 60
GCTCATGGGA CAGAGTGGG CAGGCCGCCA TCTGCTGTGT TTAGACGAGA ATITTTCCAC 120
CACATCCTCC ACTGACGAC CATTCCCAAT CAGTCTGCAA GCAGTCTGC TGAAGTGGCA 180
CTCCTGAGGT ATGGAACAGG GCCTGCAAAC ATCTCTCCCA ACTGCTGACG GGGCTGCTGC 240
TATGACCTAA CCAGCGTGAC CTTGACCTGC TACGCAAGCA ATGGCTACCG GGCAGTGGCA 300
CGTCTGTGG ACGGACGGG GCACCTGACA TGGACGCCA CCAACACGC CTTCTCTTGT 360
GATGAAAGTGA CTGTAGACTG TGGAGCTGTG AAGCATGGA AAGCATGGA TCCACATGGC 420
GGGAAGATTC AGCCCCCCAG GCCCAAGATG GCTCCTGCAA ATGACACATA TGAAAGCATC

TTCAGTCACT TCCGAGAATA TGAGATTGCC ATTCGCAAGG

TTTCTGTGGC AGGTGAAACC ATCTGTCACT TCCGCAACCA ACAAGGGGAT GTGCTCTAAA

GAGGTGGCAG TCTCCCTCAG CAGCAGATG TGCAAGTCTA CCAACAGATC TTGGTCAAAAA

ACTCACACAT GCCACCGGTG CCCAGCACTT GAATCTTGTG GGGGACCCTG AGTCTCTCTC

TTCCCCCCAA AACCCCGAAG CACCCCTCATG ATCTCCCGGA CCCCTGAGGT CACAGTCTG

GTGGTGGACG TGAGCCAGCA AGACCCTGAG GTCAAGTTGGT GAGGCGCTG

GAGGTGCATA ATGCAAGAGC AAAGGCGCGG GAGGAGCAGG ACAAGACAC GTACCTCTTG

GTGACGCTTC TACAGCTCTG GCAGCAGACG TGGCTGATGCA TCTATAGCAG TCCGAACGGC

GTCTCCAACA AGGCCCTCCC AGGCCCTGAC GAAGAAACCA TTCCTAAAGC CAAAGGCGAG

CCCGGAAGAC CACCATTTGC CACCCCTCCC CACATCCCGG AGAGATGAC CAAGATCCAG

GTACGGCTGA CCGCTGCTGT CAAGGCTGCT ACTCCAGCTG ACATCCGGGT GAGGTCGAGG

AGCAATGGGC AGCGCGAGAA CAATCAACAG ACCAGCGCTC CCGTCTGGGA CTCCGAGGCC

TCCCTCTGCC TTCTACAGCA GGCACCGCTG GACACAGGCA GTGGGACGAC GGGGACCCTC

TTCTCATGCT CGGATGCTGA TGGCCCTCTG CACAACACT ACACCGAGAA GAGCTTCCCT

CTGCTCAGCG GTAAAATGGA

[0276] Amino acid sequence of cynomolgus macaque IL-10R α-extracellular domain fused to the Fc portion of human IgGl (underlined): SEQ ID NO: 10

MVPCLWLLA AFLSRLGSD AHGETLPSSPV SVWFEAEFFH HILHWPITPN QSSESTCYEVA 60
LLRYGTRMN SISNCSSALS YDLTAVTLDL YRSGYRARV RAVDGSSRHSN WTVTNTEFS 120
DEVILYGVG KLEIHNGFIL GKIQQPRPRKM APANDTYEIS FSHEFREYIE IRKVPQNFPT 180
THKVKVENF SLLTSGEVGE FCQVCQRSVST SRTNKGWMRS EECVSLTRQY FTTVNRSCK 240
THTCPBPCAP ELLGPPSVFL FPPKPKTDLM ISRTPEVTCT VDVSHEDEPE VKFNHYPDGV 300
EVINARTKPR EEQYNSTYRV VSULTVHLHC WNGKEYCCK VSNKALPAIP KRTISKARGQ 360
PREPOVYTLPS RSREMTKNQ VSLTCLVKGF YPSDIAYVENE SNGQPENNYK TTPVLDGSG 420
SFFLYSKLTVC DKSRWQOQGNN FSCWMHEAL HNHYTOKSLS LSPGK 480

[0277] Human IL10Ra SNP Variant Cloning: Five mammalian expression vectors were constructed, each expressing one of five known single nucleotide polymorphism (SNP) variants of the extracellular domain of hIL-10R α (705 nucleotides coding for amino acids 1-235 when including signal peptide) fused with human IgGl Fc. The SNP variants are designated L61V, V1131, S159G, R212E, and V233M where the first letter denotes the consensus amino acid, the number denotes the amino acid number when counting from the start methionine of hIL-10R α, and the second letter denotes the amino acid of the SNP after mutation. The constructs were designed such that the vector, and protein produced thereof, should be identical to the hIL-10R α:hFc vector and protein except at the site of SNP mutation. In general, mutation of L61V, V1131, S159G, and R212E were performed in a two-step PCR reaction followed by cloning of the complete expression construct, which was performed using the same method as the hIL-10R α:hFc fusion construct.
Specifically, L61V will be used as an example for V131, S159G and R212E, which were generated using the same technique with their own unique primers for mutation (Table 1). cDNA coding for hIL-10Rα was used as template for two separate PCR reactions: Rxn1 (primers hIL10R Forward, IL10Ra-R-L 61V) amplified the N-term cDNA from the start ATG to 13 nucleotides 3' of the SNP site; Rxn2 (primers IL10Ra-F-L 61V, hIL10R Reverse) amplified from 13 nucleotides 5' of the SNP site through Asp235 of hBL-10Rα(nucleotide 705). Primer "hIL10R Forward" adds a restriction enzyme recognition site for EcoRI 5' of the sense strand immediately preceding the start methionine, and primer "hIL10R Reverse" adds the restriction enzyme recognition site for BglIII at the 3' end of the sense strand immediately following Asp235. Primers "IL10Ra-F-LoIV" and "IL10Ra-R-L 61V" are 100% complementary for each other and contain the nucleotide mutation responsible for conversion of the consensus amino acid to the SNP variant near the center of the primers. The PCR products were purified by gel electrophoresis, and added at roughly equimolar ratios to a standard PCR mix containing no primers. Three cycles of PCR thermal cycling were performed such that the complementary regions of each PCR product, Rxn1 and Rxn2, "primed" the reverse strand of the other reaction product allowing for elongation of the sense and antisense strands to the full length of the hIL-10Rα extracellular domain. This reaction was used as template in a second PCR reaction with primers "hIL10R Forward" and "hIL10R Reverse." The amplified product was digested with EcoRI and BglIII restriction enzymes, which were included in the primers, and the human IgGl Fc sequence was excised from the pVI 1392.fc vector using BglIII and NotI restriction enzymes. The shIL-10Rα (EcoRI-BglIII) and Fc (BglIII-NotI) fragments were subcloned into the pcDNA3.1(+) expression vector previously digested with EcoRI and NotI to generate a hIL-10Rα-L61V-hFc expression vector.

Construction of the SNP variants V1131, S159G and R212E all used the common primers "hIL10R Forward" and "ML10R Reverse." Unique primers (Table 1) containing the SNP nucleotide mutation are: V1131 (IL10Ra-F-V 1131, IL10Ra-R-V 1131), S159G (IL10Ra-F-S 159G, IL10Ra-R-S 159G) and R212E (IL10Ra-F-R212E, IL10Ra-R-R212E).

Construction of hIL-10Rα variant V233M was done with a single PCR step (primers "hIL10R Forward ", "hIL10_V233M_R"), followed by a restriction cloning method identical to that described for construction of the hIL-10Rα-hFc vector. Primer "hIL10_V233M_R" contains the SNP mutation and the BglIII restriction site for fusion to hFc. Each construct was verified by DNA sequencing.

Nucleotide sequence of SNP variant L61V (codon bolded) of human IL-10Rα:human IgGl 1 fusion protein from initiation codon (ATG) through human IL-10Rα extracellular domain to end of human Fc sequence (underlined): SEQ ID NO:62

| ATG TGG GCG GT | GCT CGT GTG TAG | GCT CGT GCG GCG TCT CTCA GCT CC GTG CTG CTCTAG | 60 |
| GCT CAT GGA | CAC GAC GTC G | TCT GGT GTG TTG AAG CAG A | ATTT TCC AC | 120 |
| CAC AT CTC CCC | ACT GGC ACC | CAT TCC AAT | GAG TCT GAA | TGA AG TGG G | 180 |
| GT CCT GAG GT | ATG GAT GGA | GCT CCT GAC C | TCA TCT CCA | ACT GT GAC CCA | GAC CAG TGG | 240 |
| TAT GAC CTT A | CCG CAG TGA CCG | TTC GAC CTG T | TAC CAG AGC | ATG GCT ACCG | GGC CAG GSTG | 300 |
Amino acid sequence of SNP variant L61V (amino acid bolded) of human IL-10Ra-extracellular domain fused to the Fc portion of human IgGl (underlined): SEQ ID NO:63

```
CGGGCTGTGG ACAGCAGCGG GCACCTCGAAC TGGACCGTCA CCAACACCCG CTTCTCTGTG 360
GATGAAGTGA CTCTGACAGT TGGCAGTGTG AACCTAGAGA TCCACAATGG CTTCATCCTC 420
GGGAAGATTC AGCTACCCAG GCCCAAGATG GCCCCCGCGA ATGACACATA TGAAAGCATC 480
TCTCGTGTCC AGGTTAAAC ATCTGTCGCT TCCGGAGTA ACAAGGGGAT GTGGTCTAAA 540
GAGGAGTGCAC TCTCCTTCAC CAGGAGTATG TTACACGTTG CCAACAGATC TGGTGACAAAA 600
ACTACACACAT GCCCACGGTG CCCACACCT TGAACCTCGG GGGGACGGTG AGTCACACATC 660
TTCCCCCAAC AACCACAGGA CACCCTCATG ATCTCCCCGGAG CCCCCTAGGT CATACGCGTG 720
```

Nucleotide sequence of SNP variant V131 (codon bolded) of human IL-10Ra-human IgGl fusion protein from initiation codon (ATG) through human IL-10Ra extracellular domain to end of human Fc sequence (underlined): SEQ ID NO:64

```
ATGTTGCCGTC GCCTCGTGGTT GCTCGTGGCG GCACCTCGAAC TGGACCGTCA CCAACACCCG CTTCTCTGTG 360
GCTGACGCA CGAAGCTGCC GCACCTCGAAC TGGACCGTCA CCAACACCCG CTTCTCTGTG 420
GATGAACTCT CTCCCTGGAC GCACCTCGAAC TGGACCGTCA CCAACACCCG CTTCTCTGTG 480
TCTGACTCTG ATGTGACGCA GCCACCGGAA ATGACACATA TGAAAGCATC CTTCATCCTC 540
GAGGAGTGCAC CACCTCTCGG TCCACGTCG CTCCGGAGTA ACAAGGGGAT GTGGTCTAAA 600
GAGGAGTGCAC CACCTCTCGG TCCACGTCG CTCCGGAGTA ACAAGGGGAT GTGGTCTAAA 660
GAGGAGTGCAC TCTCCTTCAC CAGGAGTATG TTACACGTTG CCAACAGATC TGGTGACAAAA 720
```

[0282] [0283]
TTCAGTCACT TGGATGAGTA CAGGTCGGTAT CCGGCAAGG TGGTGCAGT 540
ACACACAAGA AAGTAAAACA TGAAAACTTC AGCCTCCTAA ... TGAAAACTTC AGCCTCCTAA CCTCTGGAGA AGTGGGAGAG 600
TTCTGTGCTC AGGTCAGACC AGGACCCTGA ATCAGTGTAA 660
GAGGAGTACA TTTCCCCATC CAGGCAAGTA TTGCGGATG 720
ACTACACAGT GCACCAGCCT GAGCTCCGTC CGGAGGACCT 780
TTCCCCCAAA AACCCCGGGA CACHTCCTGAT ATCTCCCCGGA CCCCTGAGGT CACATGGCTG 840
GTGTTGAGCC TGGAGCCAGG AGACCGTCTG AGTCAAGGTA GTCAAGGCTG GGAGCGGG 900
GAGGAGTACA ATGCAAGAAG AAGCGCGGGA GAGGAGCGT ACAAGGCGAC GTACGGTG 960
GTCAGCCGTCC TACCCGTCTC GCACCAGGAC TGGCTGAAAT GCAGGAGGTA CAGTCAAGT 1020
GTTCACAAAC AGCCCTCCCT AGAGCCACATC GAGAAAACCA TCTCTGAGGT 1080
CCCCCGAGTAC CACAGGCTTG CACCTCCGGG ATGTACCCATG CAAAGACACAG 1140
GTTCACCTCA GCTCCGCTGT CACAGGCGGT TACTCCGCGG ACATCGGCGT GGAGGCGG 1200
AGCAATGGGC AGGCAGCGGA CAACACTACG ACCACACCTG CCGTCTGGGA CTGCCAGGCG 1260
TCTTCCTTCT TCTACCAAGA GCTCCGCTGG GACAGGAGGAA GGTCGACGCA GGGGACGG 1320
TTCATGAC GTGGAGACTAC TGGAGGCCTTG CACAACGCAAG AAACACGGAGA GGCCCTCC 1380
CGTCTCCCGG TCTGAATAGA 1440

[0284] Amino acid sequence of SNP variant V1131 (amino acid bolded) of human IL-10Ra extracellular domain to the Fe portion of human IgGl (underlined): SEQ ID NO:65

MVPCLWLLA ALLLRLGSDL AGHTETLPSSPV SVWFPEAFFHI HILHWTIPPN QSESTCYEVA 60
LLRYGIESSW SSNCQCTLIS YDLTVNLDL YHSNGYRAKV RAVDG2RHSN WTIINRFYSV 120
DEVLTVGSSV NLEIHNGFIL GKIQLPRPKM APANDTVESI FSHFREYEEIA IRKVPGNFT 180
THKKVKHenF SLSTGEGYVE FCQQVGPVSA SSRNKGMAWK EECISLIRQY FTVNRSD 240
THTCPCCPAP ELGGPSVFL FPPKPKDTLM IRSTPEVTG WDVSHEDPE VRFNHWYDGV 300
EVRHKAKPR EEEQYNSTYRV VSVSLTVLHOD WNLNGKEYCK VSNKALPAPI EKTSKAAG 360
PREQVYTLTP PSRDELTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPFNYYK TTPPVLSGD 420
SFFLYSKLTV DKSRWQOQNVF PCSWVMEHNL HNNHTQKSLSL LSPGK 480

[0285] Nucleotide sequence of SNP variant S159G (codon bolded) of human IL-10Ra:human IgGl fusion protein from initiation codon (AGT) through human IL-10Ra extracellular domain to end of human Fe sequence (underlined): SEQ ID NO:66

ATGGTGGCGT GCCGCCGAGT ATGGTGGCGG GCCGCCGCTCA GCCTCCGCTGC TGGCTCAAGC 60
GCTGCCGCTG TGGTGGCGG GCCGCCGCTCA GCCTCCGCTGC TGGCTCAAGC 120
CACATCCTCC ACTGGACACCC CATCCCCAAAT CAGCTGAAAA GTACCTGCTA TGAAGGGCGG 180
ACTCGAGGAGT AGTGGATCGCC GTGCTCCGAGC TCCAGGGCAGC TGGAGGCTGCG 240
TATGACCTCTA CGGCACTGGC CTTGGACCTG TACCAGACCA ATGGTACCCG GCCACAGGTA 300
CCGGGCTGGG AGCGCGCAGCC GACTCCACCA TGGACCTGCC CCAACACGCC CTTCCTCTGC 360
GATAGAAGTG CCGCACTGGG TGGCACTGGG AACCTAGAGA TCCCAATGGG CTTTGTATTCC 420
GGGGAGATTCA CCGGCAGCGA GCCCAAGTGG GCCGGCCGCA ATGGACACAT TGAAGGACATC 480
TTCCATGGCTC TCGGAGCTGTTGAGAGTCA TCGGGCAAA TCTCCGACATG 540
ACACACAGA AAGTAAACA TGGAAAAACTTC AGCCTCCCTAA CCTCTGAGGA AGTGGGAGAG 600
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**[0286]** Amino acid sequence of SNP variant S159G (amino acid bolded) of human IL-10Rα-extracellular domain fused to the Fc portion of human IgGl (underlined): SEQ ID NO:67

**[0287]** Nucleotide sequence of SNP variant R212E (codon bolded) of human IL-10Rα human IgG1 fusion protein from initiation codon (ATG) through human IL-10Rα extracellular domain to end of human Fc (underlined): SEQ ID NO:68
Amino acid sequence of SNP variant R212E (amino acid bolded) of human IL-1ORo-extracellular domain fused to the Fc portion of human IgG1 (underlined): SEQ ID NO:69

MVPCLWLLA ALLSLRLGSD AHTGETLSSP SWVFEAEFFH HILHWTIPN QSESTCYEVA 60
LLRYGIESWN SISNCQTLS YDLTAVTLDL YHSNGYRARV RAVDGSRHSN WTVTNIRFSV 120
DEVTLTVGVS NLEIHNHFGIL KQIQLRPKPM APANDYESTI FSHFREYELIA IRKPGQNTFP 180
THKVKVENFSSL TSTGVYEGFVC FQCVQKPSVA SESNKGMSWS EECISLTRQY FTVTNRS CKD 240
THTCPPCPAP ELLGQPSVFL FPPPKDNLML ISETPEVTCV WDVSHEDEPE VKFNYQVDYG 300
EVINAKTPKRP EEQYNSTYRVS VSVLTVLHQDL NNGKEYKCK VSNRALPAPI EKTISAKGCO 360
PREPOVYTLPSR DELTKNQOVS LTCLVLKGT VPSDIAVEWE SNGQPENNYK TTPPVLODSD 420
SFLYSLKLTV DKSRRQGONNV PSSCVYHELAL HNHYTOKSLS LSPGK 480

Nucleotide sequence of SNP variant V233M (codon bolded) of human IL-1ORchuman IgG1 fusion protein from initiation codon (ATG) through human IL-1ORoc extracellular domain to end of human Fc sequence (underlined): SEQ ID NO:70

ATGTTGGCCGT GCCTCGTAAG TCTGGCTGGCG GCCGCTCTCA GCCCTGCTCT TGGCTCAGAC 60
GCTCATGGGA CAGAGCTGCC CAGCCCTCCGC TCTGTGCTGT TGGAGCAAG ATTTTTCAC 120
CACACTCCCA ACTGGACACC CATCACAATT CAGTGCTGAA TACGCCTGTA TGAAGGCGG 180
CTCTCAGGTT ATGGAATAAG GTCTGGGAAC TCCATCTCCA ACTGGACCCA GACCCCTGCC 240
TATGACCTCTA CCCAGCTGCT CTTGGACCTG TACACAGGCA ATGCCTGACG GCGGAGCTG 300
CGGGCTCTGG ACGCCCGGCG GCACCTACCA TGAGGGCTGA TCGCAATGG TGCAATGGCG 360
GATGAATGAC CTCGACAGCT TGCCAGTGCT AACCTAGAGG TCCATCTGAC TGGTGAGCT 420
GGGAGAGTTCT GCATCGACCG GGGGAGCGGC CTGCAGACAT TGAAGGATCC 480
TTCAGTCACT TCCAGAAGTA TGAGGATCC ATCGCGAGAC TGCCCGGGAA TCCAAGTTCA 540
ACACACAGAA AGAATCAAAC AAGGCTTCA CTGCACCCCG TGGAGGCAAC GTCGACGCTT 600
TTCCTGTCCA AGTGAAACAG ACTCTGTCAT TCCCAATGGA ACAAGGGGAT TGTTGCTAAA 660
GGAGGTTCA CTCCTCCTCA CAGGAGATAG TCCATACGTA CCAAGACATG TGTGACAAA 720
ACTCACACAT GCCACCGCTT GCCACCGCTT GCCACCGCTT GCCACCGCTT GCCACCGCTT 780
TTCCCCCAAA AACCACAGAG CACCCCTCAG ATCTCCCGGA CCCCCGAGGT CACCATGCG 840
GCTGAGAAGCT AGAAGGAGAG GAGGTCCAGG CTAGTGCTGCT TGAAGGCAAC GGGGAGAGG 900
GAGGTCACT ATGCTCAGGAA AGAGCCCGCG GAGGCAGCAG AACAACGACG CTACGTGCTG 960
GTCAGGCTCC TCACTGCTCT GCACCGCTAT TGGGTAGCTG GCAGAGGATG CAACTGCG 1020
GTCTCCAACA AAGCCCTCCC AGCCCCCATC GAGAAAACCA TCTCCAAAGC CAAAGGGCAG 1080
CCCCGAGAAC CACAGGTGTA CACCCTGCCC CCATCCCGGG ATGAGCTGAC CAAGAACCAG 1140
GTCAGCCTGTA CCTGCTGGTG AAGGGTTGCT TATCCAGGG ACACTCCGGG GGGAGGAGAC 1200
AGCAAGGCCC AAGCGGAGAA CAACTACAAG ACCACGCTTC CGTGCTGAGA CTCGACGCGG 1260
TCTCTCTTCC TCTAGCAGG GCTTCCGGCG GACAGAGCGA GGGACAGCTC GGGAGAACGC 1320
TCTCTCATGCT CGGTGATGCA TGGACGCTTG CACAACTCCG ACAGCAGAGAA GACGCTTCCC 1380
CTGTCTCCGG GTCGAGATGG 1440

[0290] Amino acid sequence of SNP variant V233M (amino acid bolded) of human IL-10Rc-extracellular domain fused to the Fc portion of human IgGl (underlined): SEQ ID NO: 71

MVPCLWLIA ALLSLRLGSD AHGTELPSPP SVWFEEAFFF HILHNTP1PN QSESTCYEV 60
LLRYGIESWN SISNCSQTLS YDLTAVTLDD YHNSYRARV RAVDGSSRHSN WTVMTRFSV 120
DEVLTIVGVS NLEIHNFIL GKIQLPPRKM APANDYESI FSHFREYEA IRKVPGNTF 180
THVKYKRNFT LSTGSEVGE FCVQVPSVSA SRSNKMGWKS EECISLTRQY FTMNRSQDK 240
THTCPFPCAP ELLGOPSVEL FPPKPMDLTM ISRTPEVEPE WDVSHEDEPE VKFNYWGVQ 300
EVINAKTPRP EEOYNSTVR VSYLVTLHOD XINGKKG CKVSNKALPAPI EKTISAKGQ 360
PREPOVITICAL PSREDLTQMKY VSLTCLYHFT YPSIATWNC SNGQFENNYK TIPFVLDSG 420
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Table 1: DNA primers for cloning IL-10Rq

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<tr>
<td>13</td>
<td>hIL-10R Forward</td>
<td>AAAACCGGAATTCCTACACATGCTGCAGCTG</td>
<td>41-mer</td>
</tr>
<tr>
<td>14</td>
<td>hIL-10R Reverse</td>
<td>AAGGAAGAATATCGCGTCACGCTAAATCGCCTG</td>
<td>36-mer</td>
</tr>
<tr>
<td>15</td>
<td>IL-10R-a275g-F</td>
<td>CTGGTGACCCTGTCACAGCAATGCTCGCC</td>
<td>31-mer</td>
</tr>
<tr>
<td>16</td>
<td>IL-10R-a275g-R</td>
<td>CGGTAGCATGTCATCGGCTAGCTAGCTCGG</td>
<td>31-mer</td>
</tr>
<tr>
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<td>IL-10R-a670g-F</td>
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<td>31-mer</td>
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<tr>
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<tr>
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<td>chIL-10Ra F1</td>
<td>ATCTGCGCGTCCTGAGTGCCTGC</td>
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<td>21</td>
<td>rhIL-10Ra R2098</td>
<td>ATGGTCCCCCGAGCAATAATC</td>
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<tr>
<td>22</td>
<td>M13R</td>
<td>CAGGAAACAGCTATGAC</td>
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<td>IL10Ra-F-L61V</td>
<td>CTTAGAGATGGGCGGTTCTGGAGGTATTG</td>
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<td>IL10Ra-F-V113I</td>
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<td>hIL10_V233M_R</td>
<td>GTCACAAGATCTGTTGTCATGAGTGAATACTGCTCGG</td>
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Protein Expression: Protein was expressed by transient expression in Freestyle 293F cells (Invitrogen Corp.) transfected using 293fectin (Invitrogen Corp.) following the manufacturer's instructions.

Generation of stable lines: The full-length human and chimpanzee IL-10Rα pCDNA3.1 expression vectors were individually transfected into EL-4 (ATCC TIB-39) cells using lipofectamine 2000 (Invitrogen, Corp.) according to the manufacturer's instructions. Stable transfectants were selected using geneticin (Invitrogen, Corp.). CHO-K1 (ATCC CCL-61) human IL-10Rα stable transfectants were generated using the Amaxa nucleofector system (Amaxa, Gaithersburg, MD) according to the manufacturer's instructions. In all cases after two weeks under selection, the cells expressing a high level of IL-10Rα based on staining with an IL-10Rα antibody were sorted using a FACS Aria (Becton Dickinson Bioscience, Palo Alto, CA).

Mice: Human trans-chromosomal KM mice™ [WO 02/43478; WO 02/0928 12; Ishida and Lonberg, IBCs 11th Antibody Engineering Meeting. Abstract (2000); and Kataoka, S. IBCs 13th Antibody Engineering Meeting. Abstract (2002)] harboring human chromosome fragments encoding the human immunoglobulin region were obtained from Kirin Pharma Co., Ltd. An overview of the technology for producing human antibodies is described in Lonberg and Huszar [Int Rev. Immunol 13:65 (1995)]. Transgenic animals with one or more human immunoglobulin genes (kappa or lambda) that do not express endogenous immunoglobulins are described, for example in, U.S. Patent No. 5,939,598. Additional methods for producing human antibodies and human monoclonal antibodies are described (see, e.g., WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598). Development of bovine carrying human immunoglobulin genes, transchromosomal (TC) cows, is described, for example, in Ishida and Lonberg [IBCs 11th Antibody Engineering Meeting. Abstract (2000)].

Immunization: Soluble human IL-10Rα:hFc recombinant protein was mixed 1:1 with an equal volume of RIBI adjuvant (Corixa, Seattle, WA) and an emulsion was prepared. Mice were immunized with 20 μg of soluble hIL-10Rα:hFc recombinant protein intraperitoneally and were boosted intraperitoneally with 20 μg of protein mixed 1:1 with RIBI adjuvant (Corixa) at 2-week intervals for 3 boosts. A final intraperitoneal injection of 20 μg of soluble hIL-10Rα:hFc without adjuvant was given 3 days prior to fusion. A second group of mice was immunized in a similar manner, but were boosted at 1-week intervals for 4 boosts.

Hybridoma production: The mice with the highest anti-human IL-10Rα IgG specific antibody titer in their serum were selected for the production of monoclonal antibodies. Human anti-human IL-10Rα IgG antibodies were confirmed by flow cytometric analysis. The spleens were harvested and single cell suspensions were fused with a myeloma cell line (SP2/O-Agl4) (ATCC, Rockville, MD) at a ratio of 5:1 with 100% polyethylene glycol (Roche, Basel, Switzerland). The fusions were plated into 96 well flat bottom plates at an optimal density and cultured in DMEM
(Dulbecco’s Modified Engle’s Media, Invitrogen, Corp.) with 10% fetal bovine serum (FBS, Hyclone, Ogden, UT), 100 mg/L sodium pyruvate (Invitrogen, Corp.), 4.5 g/L D-glucose (Invitrogen, Corp.), 2 mM L-glutamine (Sigma), 100 U/ml penicillin (Sigma), 100 µg/ml streptomycin sulfate (Sigma), 55/IM 2-mercaptoethanol (Invitrogen, Corp.), HAT supplement (Sigma), and 5 ng/ml human IL-6 (Kirin Pharma Co., Ltd., Takasaki, Japan) in a 10% CO₂, 37°C incubator. Approximately 2000 wells from 2 fusions were screened by ELISA for human IgG containing human IL-10Rα specific antibodies. Human anti-human IL-10Rα IgG antibodies were confirmed by flow cytometric analysis. Positive wells were expanded and subjected to 2 rounds of limiting dilution cloning to obtain monoclonal antibodies.

Antibody and protein purification: For antibody purification, hybridomas were cultured in 2-liter roller bottles at 300-350 milliliter per bottle with hybridoma-SFM medium (Invitrogen, Corp.). Soluble hIL-10Rα:hFc, chimp IL-10Rα:hFc and cynomolgus IL-10Rα:hFc recombinant proteins were generated by transient expression in FreeStyle™ HEK293F cells following manufacturer’s protocols (Invitrogen, Corp.). Human monoclonal antibodies and the EL-10Rα:hFc recombinant proteins were purified from culture media using HiTrap MAB Select SuRe Protein A resin (Amersham, Piscatway, NJ). In cases when supernatant volume exceeded 1 L, conditioned medium was first concentrated using a Sartorius tangential flow filtration system (Sartorius Stedim, Goettingen, Germany). The conditioned medium was filtered with a 0.22 µm vacuum filter unit (Millipore, Bedford, MA) and loaded onto the Protein A column of a size appropriate for the amount of the target protein in the medium. The column was washed thoroughly with 6 column volumes of PBS and the bound protein was eluted with appropriate buffer (0.1 M Gly-HCl, pH 3.4, 0.15 M NaCl for the antibody or ice cold 50 mM Citrate/NaCitrate pH 3.5, 0.15 M NaCl for the recombinant fusion protein). Eluted fractions were immediately neutralized with IM Tris-HCl, pH 8.0. The fractions with high absorbance at 280 nm were pooled and concentrated with a centrifugal concentrator (Vivaspin, 10,000 MWCO: Sartorius). Concentrated samples were then loaded into 12 mL or 30 mL Slide-A-Lyzer dialysis cassettes (3,500 MWCO: Pierce, Rockford, IL), and dialyzed against 4L PBS, pH 7.4 (Sigma, St. Louis, MO). Following the dialysis the proteins were filter sterilized using 0.22 µm syringe filters and their concentrations were determined by the Lowry method. Pyrogen content was quantitatively determined using the Endosafe Portable Testing System unit (Charles River, Charleston, SC) with high sensitivity Limulus Amebocyte Lysate (LAL) test cartridges. The samples were considered endotoxin-negative if the test result was less than 0.05 EU/mg (the assay limit of detection).

Human IgG Quantitation ELISA: To determine the amount of human antibody present in supernatants and purified stocks the following protocol was used. Goat anti-human Fcγ specific antibody (Jackson Immunoresearch Laboratories, West Grove, PA) was coated onto 96 well plates (Nunc, Denmark) in carbonate buffer (pH9.4) at 0.5 µg/well for 1 hour at 37°C. The plates were then blocked with Superblock (Pierce) for 30 minutes followed by addition of the samples to the plates. Standard curves were generated using total human IgG (Sigma) or purified human IgG1 or IgG4 (Kirin Pharma Co. Ltd.). The plates were incubated for 1 hour at 37°C, washed in PBS/1 %BSA/0.1% Tween20 (Sigma),
and the bound antibody was detected with goat anti-human Fcγ specific antibody conjugated to horseradish peroxidase (HRP, Jackson Immunoresearch) for 1 hour at 37°C. The TMB substrate (Sigma) was added for 10 minutes and the reaction was stopped with H2SO4 (LabChem, Pittsburgh, PA). The optical density (OD) was measured at 450 nm on a microplate reader and the antibody concentration calculations were computed using SoftMax Pro software (Molecular Devices, Sunnyvale, CA).

[0298] IL-10Ra Specific Antibody Detection ELISA. Antibody titers, specificity, and production by hybridomas were determined by ELISA. In brief, 96 well flat bottom Maxisorb plates were coated with 50 µl of hIL-10Rα (R&D Systems, Minneapolis, MN) at 1 µg/ml m carbonate buffer (pH 9.4) overnight at 4°C or at 37°C for 1 hour. After washing three times with PBS/0.05% Tween 20, plates were blocked with Superblock blocking buffer in TBS (Pierce) at room temperature for 30 minutes. The serum, supernatant, or purified antibody was diluted in blocking buffer, added to the wells, and the plates were incubated for 1 hour at room temperature. The plates were washed 3 times with PBS/0.05% Tween 20 and peroxidase-conjugated goat anti-human IgG (Fcγ specific), anti-rat IgG, or anti-mouse IgG detection antibodies (Jackson Immunoresearch) were added at a dilution of 1:5000. Multiple lots of anti-rat and anti-mouse IgG antibodies were used in different assays. These secondary antibodies demonstrated variable binding to 3F9, SPM466 and 37607. Following a 1 hour incubation at room temperature, the plates were washed and the TMB (Sigma) substrate was added and incubated at room temperature for 5 to 10 minutes. The reaction was stopped with H2SO4 (LabChem) and the optical density was measured at 450 nm by a microplate reader. A second ELISA protocol using soluble human, chimpanzee, and cynomolgus IL-10Ra hFc recombinant proteins as the coating antigens was also employed. In this assay, binding of specific human IgG was detected with a peroxidase labeled sheep anti-human kappa antibody (The Binding Site, Birmingham, UK).

[0299] Flow Cytometry. Antibody titers, specificity, and relative binding affinities were determined by flow cytometric analysis using human IL-10Ra stable CHO-K1 transfectants, EL-4 transfectants, RPMI-8226 cells (ATCC, CCL-155) or total human peripheral blood mononuclear cells (PBMC). The cells were washed once in staining buffer PBS + 2% FBS + 0.01% NaN3 + 10 mM EDTA, then blocked with 20 µg/ml rabbit IgG (Jackson Immunoresearch), resuspended in serum, supernatant, purified anti-human IL-10Ra antibodies, or isotype control antibodies in a final volume of 50 µl. The cells were incubated with the antibodies on ice for 20 minutes, washed twice in staining buffer then resuspended in an anti-human IgG secondary antibody for 20 minutes. Two different antibodies were used, goat anti-human IgG biotin (Jackson Immunoresearch) or anti-human IgG PE (Southern Biotech Associates, Birmingham, AL). If the biotinylated antibody was used, antibody binding was detected by labeling with streptavidin-phycocerythrin (SA-PE) for 20 minutes. Binding of rat and mouse anti-hIL-10Ra antibodies were detected with either biotinylated or PE conjugated anti-rat or anti-mouse IgG antibodies from multiple sources (Jackson Immunoresearch, Southern Biotech Associates, and BD Pharmingen, San Diego, CA). The cells were then washed once and fixed 10 minutes with 1% paraformaldehyde. After a final wash the cells were resuspended in staining buffer and the samples were acquired using a FACS flow cytometer.
Calibur flow cytometer (Becton Dickinson Biosciences, Palo Alto, CA) and the data were analyzed using Cellquest (Becton Dickinson Biosciences) or FlowJo (TreeStar, Inc., San Carlos, CA) softwares.

[0300] **IL-10 Blocking Assay:** To determine if the anti-human IL-1 ORa antibodies were blocked by binding of IL-10 to the receptor on the cell surface, a flow cytometric protocol was used. In the flow cytometric assay, RPMI-8226 cells were washed and resuspended in staining buffer and then incubated with biotinylated human IL-10 or a negative control protein (R&D Systems) for 30 minutes on ice. The anti-IL-10 ORa antibodies were then added to the cells for an additional 30 minutes. The cells were washed and incubated with anti-human IgG conjugated to PE (Southern Biotech Associates) for 30 minutes. After another wash, the cells were fixed with 1% paraformaldehyde and analyzed on a FACS Calibur. The fold reduction in antibody binding was determined using the geometric mean fluorescence intensity in the following formula: Fold reduction = geometric mean fluorescence intensity in the absence of EL-10/geometric mean fluorescence intensity binding in presence of IL-10.

[0301] **Anti-IL-10Rq Antibody Competition ELISA:** In order to determine if the antibodies bind the same "epitope" of human IL-1 ORa an ELISA protocol was used. Nunc 96 well flat bottom ELISA plates were coated with the human anti-human IL-1 ORa antibodies 136C5, 136C8, 136D29, mouse anti-human IL-1 ORa 37607 (R&D Systems) or rat anti-human IL-1 ORa 3F9 (Biolegend, San Diego, CA) in carbonate buffer at 2 µg/ml for 1 hour at 37°C. The plates were washed and then blocked with PBS/1 %BSA/Tween 20. Soluble anti-human IL-10R α antibodies were pre-incubated with biotinylated recombinant human IL-10R α:chFc fusion protein for 30 minutes at room temperature. The in-house generated human IL-10R α:chFc recombinant protein was biotinylated using the NHS-PEO4-biotin labeling kit (Pierce, Rockford, IL) according to the manufacturer's instructions. The combinations of antibody-IL-10R α:chFc-biotin were added to the plate and incubated for 1 hour at 37°C. After 3 washes, bound IL-1 ORa:chFc-biotin was detected with streptavidin-horseradish peroxidase (Southern Biotech Associates). The ELISA was completed as described above. The percent inhibition was determined using the OD of each sample in the following formula: percent inhibition = 100 - [(experimental sample OD/maximum binding OD)* 100].

[0302] Methods to detect antibody binding to peptides derived from the human IL-1 ORa sequence: Multiple methods were employed to detect binding of anti-human IL-1 ORa antibodies to peptides derived from the extracellular domain of human IL-10R α that have been reported by Reineke, et al. (Reineke, et al., Protein Sci 7:951 (1998)) to be included in the IL-10 binding site of the receptor. The following amide synthesized peptides were tested: Ac-YHSNGYRARVRA-NH2, Ac-TVTNTRFSVDDNH2, Ac-SIFSHFREYE-NH2, Ac-GNFTFTHKKV-NH2, Ac-SVASRSNKGM-NH2 (SEQ ID NO. 81 - 85). In addition, the following biotinylated peptides were tested: Biotin-SGSTLDL YHSNGYRAR VRAVG-NH2, Biotin-SGSTYISIFSHFREYEAIRKV-NH2 (SEQ ID NO. 86 - 87). The amide peptides were synthesized by A&A Labs, LLC (San Diego, CA) and the biotinylated peptides were synthesized by GenScript (Piscataway, NJ) both at >95% purity and reconstituted at 20 mg/ml in DMSO. In one assay design peptides were spotted at 5 µg/spot in PBS onto PVDF membranes.
or nitrocellulose membranes that had been prepared according to the manufacturer's instructions. The membranes were incubated with anti-IL-10Rα antibodies using standard Western blotting methods (e.g. Towbin, et al., Proc Natl Acad Sci 76:4350 (1979)). Native human IL-10Rα:Fc and heat denatured human IL-10Rα:Fc were also spotted at 1 µg/spot on the membranes as controls. Negative controls included an irrelevant peptide and isotype controls. 136C5, 136C8, 136D29, 3F9, and 37607 only bound to native human IL-10Rα:Fc. None of the antibodies bound to the spotted denatured protein or to any of the peptides derived from the human IL-10Ra extracellular domain or the irrelevant peptide.

[0303] Multiple ELISA methods were also employed to detect binding of anti-IL-10Rα antibodies to IL-10Rα derived peptides. The biotinylated IL-10Rα derived peptides (SEQ ID NO. 86 - 87) were diluted to 1µg/ml in TBS/BSA/0.05% Tween 20 and added to Extravidin or Neutravidin pre-coated ELISA plates (Pierce) for 2 hours at room temperature. After washing three times with TBS/BSA/0.05% Tween 20. The purified anti-human IL-10Rα antibodies were diluted in TBS/BSA/0.05% Tween 20 and added to the wells. The plates were incubated for 1 hour at room temperature. The plates were washed 3 times with TBS/0.05% Tween 20 and peroxidase-conjugated goat anti-human IgG (Fcγ specific), anti-rat IgG, or anti-mouse IgG detection antibodies (Jackson ImmunoResearch) were added. Following 1 hour incubation at room temperature, the plates were washed and the TMB (Sigma) substrate was added and incubated at room temperature for 5 to 10 minutes. The reaction was stopped with H₂SO₄ (LabChem) and the optical density was measured at 450 nm using a microplate reader. An OD reading above background wells with no primary antibody is indicative of antibody binding to the peptides. 136C5, 136C8, 136D29, 3F9, or 37607 did not bind to any of the IL-10Rα derived peptides in this assay system.

The antibodies only bound the positive control biotinylated recombinant human IL-10Rα:Fc protein. In a second ELISA, the IL-10Rα amide peptides (SEQ ID NO. 81-85) were tested for their ability to block the anti-IL10Rα antibodies from binding to coated human IL-10Rα. The peptides were pre-incubated with 136C5, 136C8, 136D29, 3F9 or 37607 at 200 µg/ml peptide: 0.1 µg/ml antibody for 30 minutes. The peptide antibody mixture was then added IL-10Rα coated ELISA plates. Antibody binding was detected with species-specific anti-IgG-HRP secondary antibodies. None of the IL-10Rα derived peptides reduced the binding of the anti-IL-10Rα antibodies to the coated IL-10Rα protein. These data demonstrate that the antibodies disclosed herein do not bind linear epitopes of the sequences described in SEQ ID NO. 81-87. Using the conditions described here, we could not confirm the binding of 37607 that was previously reported by Reineke, et al. (Reineke, et al., Protein Sci 7:951 (1998)).

[0304] Purification of human peripheral blood mononuclear cells (PBMC) from whole blood:
Whole blood was collected from healthy donors between the ages of 18 and 50 by the normal blood donor program at Scripps Green Hospital (La Jolla, CA). Heparin was added to prevent clotting. No race, ethnicity, or gender was specified. The blood was diluted in PBS and then underlayed with Ficoll-Paque Plus (Amersham Biosciences). The mononuclear cells were separated from the serum and platelets by centrifugation at 1800 RPM without the brake. The interface containing the PBMC was collected and washed two times with PBS.
TNF-ct Enhancement Assay: Human, chimpanzee or cynomolgus peripheral blood mononuclear cells (PBMC) were plated at 4x10^5 cells per well in a 96 well flat-bottom plate with and without 10 ng/ml LPS (Sigma) and recombinant human IL-10 (R&D Systems) at 3 ng/ml (human, chimpanzee) and 5ng/ml (cynomolgus), and the anti-human IL-10Rα antibodies at various concentrations in a final volume of 200µl. AIM-V media (Invitrogen) supplemented with 1% human AB serum (MP Biomedicals, Solon, OH) was used as the culture media, and cells were cultured for 20hr and 48hr at 37°C, 5% CO2. Samples from each time point were stored in a -20°C freezer until analyzed. In some experiments with human PBMC recombinant cytomegalovirus (CMV) IL-10 (R&D Systems) was used at 10 ng/ml to suppress LPS induced TNF-α.

NKT Cell Assay: Human natural killer T (NKT) cell lines generated as described in Rogers, et al. (J Immunol Meth 285: 197 (2004)) that are specific for α-galactosylceramide (KRN700, Kirin Pharma Company, Ltd. (Kawano, et al., Science 278: 1626 (1997); Kobayashi, et al., Oncol Res 7:529 (1995)) were plated at 2 x 10^5 cells per well with 1 x 10^6 total allogeneic PBMC and 100 ng/ml KRN7000 in the presence or absence of anti-IL-10Rα antibodies or control antibodies. Following a 1 hr incubation at 37°C, 10 ng/ml of IL-10 was added to the wells and the cells were cultured for 48 to 72 hrs at 37°C with 5% CO2. Supernatants were removed at 48 and 72 hrs and cytokine specific ELISAs were used to measure cytokine production.

Detection of HLA-DR expression: Human PBMC were incubated at 1 x 10^6/ml with 10 µg/ml of anti-IL-10R α antibodies for 30 min prior to the addition of 10 ng/ml IL-10. The culture media was RPMI-1640 (Invitrogen) supplemented with 5% fetal bovine serum (FBS) (Hyclone), 1% L-glutamine, 1% penicillin/streptomycin, 1% HEPES, and 0.1% β-mercaptoethanol. Following an overnight incubation at 37°C with 5% CO2 the cells were labeled with anti-HLA-DR-PE (Immunotech, Marseilles, France) using standard methods. Staining was detected by flow cytometric analysis on a FACS Calibur. The mean fluorescence intensity (MFI) was determined using Cell Quest or Flow Jo softwares.

Detection of STAT3 phosphorylation: Human PBMC were diluted to 1x10^7/ml in media containing 10% FBS, 1% penicillin/streptomycin, 1% L-Glutamine, 1% HEPES in RPMI 1640 media. Cells were treated with IL-10 at 10ng/ml in the presence or absence of titrated anti-IL-10Rα or control antibody starting at 3ug/ml for 15 min at 37°C. Untreated cells were also included as a control. A fraction of the samples were treated with anti-DL-10Rα plus goat anti-human IgG (Jackson ImmunoResearch) to crosslink the antibody in the absence of IL-10 for 0, 15 or 30 min. The stimulation was stopped by transfer of the samples to ice. The cells were then lysed using NP-40 lysis buffer: 150mM NaCl, 50mM Tris-HCL pH8.0, 1% NP-40 (Calbiochem, San Diego, CA), IX Protease Inhibitor Cocktail (Roche Applied Science, Indianapolis, IN) in dH2O, and homogenized. Protein concentrations were determined using the Lowry method with bovine gamma globulin (Pierce) as protein standards and Lowry reagent solutions from BioRad. Approximately 15ug of lysate samples were loaded on 4-20% Tris-Glycine SDS-PAGE gels (Invitrogen), transferred to PVDF membranes (Invitrogen) and probed.
with anti-phospho-STAT3 and anti-STAT3 (Cell Signaling Technology, Danvers, MA). Antibody binding was detected with anti-rabbit-IgG-HRP (Jackson ImmunoResearch) and ECL reagents (Amersham Biosciences, Piscataway, NJ) using standard western blotting protocols.

Cytokine ELISAs: Nunc 96 well flat bottom ELISA plates were coated overnight with anti-human TNF-α (Biolegend) or anti-IFN-γ (eBioscience, San Diego, CA) at 2μg/ml or 1μg/ml respectively, in carbonate buffer. The following day, plates were washed three times in ELISA wash buffer (1×PBS plus 0.05% Tween 20.) The plates were blotted dry and incubated for 30 minutes at room temperature with 250μl of Blocking Buffer (1×PBS plus 1% BSA.) Samples were thawed and 50μl were added to the 96 well plates, in duplicate, along with recombinant human TNF-α (eBioscience) or recombinant human IFN-γ (R&D Systems) standard dilutions. After 3 washes, biotinylated anti-human TNF-α or anti-human IFN-γ (Biolegend) at 1μg/ml or 0.5 μg/ml, respectively, was incubated for 1 hr at RT. Plates were washed 3 times and then streptavidin-horseradish peroxidase (Southern Biotech Associates) at 1:2000 was added to the wells for 30 minutes. Following three washes, 100 μl TMB substrate was added for 3 to 10 minutes. The reaction was stopped with 50 μl H₂SO₄ and the plates were immediately read at 450 nm. The optical density (OD) of the experimental samples was used to calculate the amount of cytokine secreted based on the standard curve using Softmax Pro software.

Example 2

This example includes a description of exemplary antibodies that bind to IL-10Ra.

Isolation of Human Anti-EL-10Rα Antibody Genes: Cultured hybridoma cells (136C5, 136C8 or 136D29), which produce 136C5 (IgGl), 136C8 (IgG2) or 136D29 (IgG4) antibodies respectively, were collected by centrifugation. Total RNA was purified from these cells using RNeasy kit (QIAGEN Inc., Valencia, CA) following the manufacturer's instructions. SMART RACE cDNA Amplification Kit (Clontech Co., Ltd., Palo Alto, CA) and the reverse transcriptase SuperScriptII (Invitrogen Corp.) were used for cloning of cDNA that encodes the variable region of the immunoglobulin genes from total hybridoma cell RNA. Briefly, first strand cDNA was prepared by reverse transcriptase from 2 microgram of RNA. This cDNA was used as a template for polymerase chain reaction (PCR) to amplify the variable region and a part of the constant region of heavy and light chains (VH and VL, respectively). The amplified sequences also contained the leader sequences. The reaction was as follows: 1 U KOD Hot Start DNA polymerase (EMD, Novagen Brand, Madison, WI); 0.2 μM 3’ Primer [for Heavy chain: IgGlP, for Light chain: hK5, (Table 1); IX Universal Primer Mix A for the 5’ end (UMP primer Mix A included in the SMART RACE Kit); 200 μM dNTP mix; 1 mM MgCl₂; KOD Hot Start Buffer (final concentration is IX); and cDNA template. The thermocycling program was 1 cycle of 94°C x 4 min: 35 cycles of: 94°C x 30 sec, 55°C x 30 sec, 68°C x 1.5 min. followed by an extension at 72°C x 7 min.

Amplified DNA fragments were collected by agarose gel electrophoresis, and purified by QIAGen Gel Extraction Kit (Qiagen Co., Ltd., Germany). Purified DNA fragments of VH and VL were integrated into PCR Bluntll-TOPO vector using the Zero Blunt TOPO PCR Cloning Kit, and each construct plasmid was transformed into E. coli, and then cloned. Nucleotide sequences of each insert...
(VH and VL) in the construct plasmids were analyzed using specific primers (M13F, M13R, Table 1). Based on the sequence obtained from VH and VL, oligonucleotide primers (Table 2) were designed to amplify 136C5 VH (136C5H_F, 136C5H_R) and VL (136C5H_R, 12k reverse BsiWI), 136C8 VH (136C8H_F, 136C8H) and VL (136C5K1_F, 12k reverse BsiWI), or 136D29 VH (136D29H_F, 14hl reverse Nhel) and VL #1 (136C5K1_F, 136D29K1_R), and VL #2 (136D29K2_F, 136D29K1_R).

Due to the nature of the KM mice®, multiple kappa chain genes may be rearranged and expressed in a single B cell. This was the case for the 136D29 hybridoma. Two kappa chain genes were cloned. Recombinant antibodies with the two potential kappa chains paired with the single heavy chain were generated and the correct light chain gene, 136D29 light chain #2 was identified as the correct kappa chain and paired with the 136D29 heavy chain to yield an antibody with human IL-1 ORa specificity.

CDRs were defined using the Kabat method. CDR-I and CDR-2 were identified automatically by BLAST (NCBI website, http://www.ncbi.nlm.nih.gov/igblast/), and CDR-3 was identified by manual analysis using the following Kabat rules. The CDR-H3 is 3 to 25 amino acids in length, starts exactly 33 residues after the CDR-H2 and the preceding amino acids are always cysteine followed by two amino acids, typically the sequence will be cysteine-alanine-arginine; the end of CDR-H3 is always followed by the sequence tryptophan, glycine, any amino acid, glycine. CDR-L3 is 7 to 11 residues in length, always starts 33 residues after the end of the CDR-L2, which is always a cysteine; the end of CDR-L3 is always followed by phenylalanine-glycine-any amino acid-glycine. The VH and VL CDR1 and CDR2 sequences of 136C5, 136C8, and 136D29 were compared to the genomic sequence of human VH and VL genes using NCBI Ig BLAST. Changes in the amino acid sequences between the human anti-human IL-1 ORa VH and VL and the germline amino acid sequences were identified and noted in the sequences below. The CDR3-sequences arise from joining of the variable, diversity and joining gene segments in the case of VH and variable and joining gene segments for VL. Therefore this region is prone to insertions and deletions and cannot be compared with germline sequences.

Nucleotide sequence of cDNA of 136C5 heavy chain variable region (VH) (from initiation codon (ATG) to the end of variable region). The CDR sequences are in bold text. SEQ ID NO:23

ATGGACTTGG GCCTGTGCTG GTTTTCTTT GTTTGCTATT TAGAAGGTTG CCACTGTGAG 60
GTCGACGCTGG TGGAGTCTGG GGGAGGCTTG GTACAGCCTG GGGGGTCCCT GAGACTCTCC 120
TGTGCACGCT CCTAGTACC CTTAGATGC TATAGCATGA CTGGATTCAC TGGAGTCTGG 180
GGGAAGGGGC TGGAGTGCTT TTCTACATTT AGTACTGGTA GTGACTACCAT ATACTACCCA 240
GACCTCTGTA AGGGCGGATT CACACCTCCC AGAGAACATG CCAAACACTC ACTGATCTG 300
CAATATGACA CCCTAGAGAA CGAGAGCAAG GCTGTGATT ACTGTGGCAG AGAGAATTAC 360
TATGGTTCCGG GGAGTCTAGA AGACTACTTT GACTACTGGG GCCAGAGAAC CCTGGTGCACC 420
GTCCTCCTCA 480

Nucleotide sequence of cDNA of 136C5 light chain variable region (VL) (from initiation codon (ATG) to the end of variable region). The CDR sequences are in bold text. SEQ ID NO:24

ATGGAAAGCC CAGCTCAGCT TCTCTTCTTC GTGACTCTCT GGCTCCCAGA TACCACGGGA 60
Nucleotide sequence of cDNA of 136C8 heavy chain variable region (VH) [from initiation codon (ATG) to the end of variable region]. The CDR sequences are in bold text. SEQ ID NO:25

ATGGGAGTGG CAGCTGCTGG GGGTTCCTTT GTGCTATTT TAGAAGGTGT CAGCTGTGAG 60
GTGCAGCGTG TGGAGTCTGG GGGGCTTTCA GTACAGCTTG GGGGTCTTGC GAGACTCTCG 120
TGTCACGCTC CTGATGGTAC TGCATGATGA TATGACTGAT ACTGGGCGTG CAGCCGTCGA 180
GGGAGGCGGC TGGAGGGTGT TTCACTATCT AGTACAGAGA TGTACACAT ATACTACAGC 240
GACTTCGCTA AGGCGGATCA CACCAGCTCC AGAGAAATCC CCAAGAAGTC ACTGATCTCG 300
CAATGACACA GGGGCGACAG GCAGAGACAG GTCTGTATTT ACTGTCGAGG AGAAGATCCT 360
TATGGAGTGG CAGCTGCTGG GGGTTCCTTT GTGCTATTT TAGAAGGTGT CAGCTGTGAG 420
GTCTCCCTCA CAAA 480

Nucleotide sequence of cDNA of 136C8 light chain variable region (VL) [from initiation codon (ATG) to the end of variable region]. The CDR sequences are in bold text. SEQ ID NO:26

ATGGGAAGCCC CAGGCGAGGT TCTCTTTCTC CTGCTACTCT GGGCTCCAGG TACCACCGGA 60
GAAATTTGTT GAGCTGCTGG GGGTTCCTTT GTGCTATTT TAGAAGGTGT CAGCTGTGAG 120
CTCTGCTGCA GGGGCGACAG GCAGAGACAG GTCTGTATTT ACTGTCGAGG AGAAGATCCT 180
GGGAGGCGGC TGGAGGGTGT TTCACTATCT AGTACAGAGA TGTACACAT ATACTACAGC 240
GACTTCGCTA AGGCGGATCA CACCAGCTCC AGAGAAATCC CCAAGAAGTC ACTGATCTCG 300
CAATGACACA GGGGCGACAG GCAGAGACAG GTCTGTATTT ACTGTCGAGG AGAAGATCCT 360
TATGGAGTGG CAGCTGCTGG GGGTTCCTTT GTGCTATTT TAGAAGGTGT CAGCTGTGAG 420
GTCTCCCTCA CAAA 480

Nucleotide sequence of cDNA of 136D29 heavy chain variable region (VH) [from initiation codon (ATG) to the end of variable region]. The CDR sequences are in bold text. SEQ ID NO:27

ATGGACGCTCA CCTGGAAGAT CCTCTTTCTG TGTCACGCAG CTACAGCCAC CCGGCAAGCT 60
GTCAGCTGCG TACAATCTCG GGGCGGCTGT AGAGAACCTT GGCGCTGGAG GAAGGTTCGCC 120
TGCCATTGTT CCCGAGGAGA CCTCAGTGCT TATCCACTGC AGGCGGGTCC CAGCCGTCGA 180
GAAATTTGTT GAGCTGCTGG GGGTTCCTTT GTGCTATTT TAGAAGGTGT CAGCTGTGAG 240
CAGAAGGTCC CAGCTGACAG TTCCATGACC GAGGACTATT ACTGTCGACC AGAAGATCCT 300
GAGCTGAGCA CCTGGAAGAT CCTCTTTCTG TGTCACGCAG CTACAGCCAC CCGGCAAGCT 360
TATGGAGTGG CAGCTGCTGG GGGTTCCTTT GTGCTATTT TAGAAGGTGT CAGCTGTGAG 420
GTCTCCCTCA CAAA 480

Nucleotide sequence of cDNA of 136D29 light chain #2 variable region (VL2) [from initiation codon (ATG) to the end of variable region]. The CDR sequences are in bold text. SEQ ID NO:28

ATGGGACATGCA GGGTGCTCTCG TCAGGCTCCTG GGGGCGAGGT TCTCTTTCTG TGTCACGCAG 60
AGATGCA TCGAGTCTGAC CCAGCTCCTCA CTCTCACTGT CGCATCTGTAG AGGAGACAGA 120
GTCACCATCA CAGTTGCGGCA GAGTGCGGATT TTACGCTTGGT TGATGCGAGA CCGGCAAGCT 180
Amino acid sequence of cDNA of 136C5 heavy chain variable region (VH) [leader sequence (italics) and variable region.] The CDR sequences are in bold text and changes from the germline sequence are underlined. SEQ ID NO:29

MDLGLCWFL VAILEGQCE VQLVESGGGL VQPGSGSRLS CAASGTFSS YSMNWVRQAP 60
GKGLEWSYI SITLETIYYA DSVKGRFTIS RDNKNSLYL QMNRLEDST AVYYCARENY 120
YGSGSYEDFY DYWGQGTLVT VSS 180

Amino acid sequence of cDNA of 136C5 kappa light chain variable region (VL) [leader sequence (italics) and variable region]. The CDR sequences are in bold text and changes from the germline sequence are underlined. SEQ ID NO:30

MEAPAQLHFL LLLWLPDTTG EIVLTQSPAT LSLSPGERAT LSCRASQSVS SYLAWYQQK 60
GQAPRLLYID ASNRATGIPA RFSGSGSGTD FTLTISSLEP EDFAVYYCQO RSNWPITFG 120
PGTKVDIK 180

Amino acid sequence of cDNA of 136C8 heavy chain variable region (VH) [leader sequence (italics) and variable region]. The CDR sequences are in bold text and changes from the germline sequence are underlined. SEQ ID NO:31

MELGLCWFL VAILEGQCE VQLVESGGGL VQPGSGSRLS CAASGTFSS YSMNWVRQAP 60
GKGLEWSYI SYSTSIYYA DSVKGRFTIS RDNKNSLYL QMNRLEDST AVYYCARENY 120
YGSGSYEDFY DYWGQGTLVT VSS 180

Amino acid sequence of cDNA of 136C8 kappa light chain variable region (VL) [leader sequence (bold) and variable region]. The CDR sequences are in bold text and changes from the germline sequence are underlined. SEQ ID NO:32

MEAPAQLHFL LLLWLPDTTG EIVLTQSPAT LSLSPGERAT LSCRASQSVS SYLAWYQQK 60
GQAPRLLYID ASNRATGIPA RFSGSGSGTD FTLTISSLEP EDFAVYYCQO RSNWPITFG 120
PGTKVDIK 180

Amino acid sequence of cDNA of 136D29 heavy chain variable region (VH) [leader sequence (bold) and variable region]. The CDR sequences are in bold text and changes from the germline sequence are underlined. SEQ ID NO:33

MDCTWRILFL VAAATGTHAQ VQLVQSGAEV KKPGASVKVS CKVSGETLITE LSMHWVRQAP 60
GKGLEWMMGF DPDPGETIYA QKFQGRVSMET EDTSTDTAYM ELSSLRSEDAY VYTCGATGGY 120
YGPGVMDVWG QGTTVTVSS 180

Amino acid sequence of cDNA of 136D29 kappa light chain #2 variable region (VL2) [leader sequence (bold) and variable region]. The CDR sequences are in bold text and changes from the germline sequence are underlined. SEQ ID NO: 34

MDMRVLCLQL GLLLLGFPGA 17CDIQMTQP SLSASVYGDR VTITCRASQG ISIWLAWYQQ 60
KPEXAPKSLI YAASSLQSGV PSRSFSGSGG TDFTTLTSSL QPEDFATYYC QQNSYPLTF 120
136C5, 136C8 or 136D29 VH and VL were cloned into the IgG4PE expression vector. Briefly, oligonucleotide primers, containing 5'-SaII and 3'-Nhel restriction enzyme recognition sites were designed to amplify the variable region of the Heavy chain (VH) by PCR. PCR was performed using pTopoC5VH miniprep DNA as a template. 136C5H_F and 136C5H_R as primers (Table 2) with KOD Hot Start DNA polymerase. After digestion of the PCR product with Nhel and Sail, a 424 bp fragment was subcloned into the IgG4PE expression vector pN5KG4PE-Lark (IDEC Pharmaceuticals, U.S. Patent No. 6,001,358) that was pre-digested with Nhel and Sail (8.9 kilobases DNA fragment). The existence of variable region of the Heavy chain (VH) was analyzed by restriction digest and verified by DNA sequencing.

As the second step, VL was inserted into N5KG4PE Lark-VH vector as follows: the DNA vector was digested by two DNA restriction enzymes, BgIII and BsiWI. The 9.1 kb DNA fragment was isolated. Similarly to the Heavy chain construct, a primer set for PCR of VL was designed to contain the recognition sites for 5'BgII and 3'BsiWi. These primers (Table 2), 136C5K1_F and 12kl reverse BsiWI, were used to amplify VL from the pTopoC5VL miniprep plasmid DNA. The PCR product was digested with BgIII and BsiWI and isolated by agarose gel electrophoresis and gel purification. This fragment, containing C5VL, was ligated to the prepared 9.1 kb vector with T4 DNA ligase and used to transform TopIO cells (Invitrogen). Positive E. coli transformants were selected. This expression vector, pN5KG4PE136C5, was purified, and the presence of both C5VL and C5VH regions were confirmed by restriction analysis.

Generation of vectors to produce recombinant 136C5G4PE and 136D29G4PE antibodies was performed in the same manner. The resulting vectors, pN5KG4PE136C8 and pN5KG4PE136D29kl, and pN5KG4PE136D29kzl, were confirmed by restriction enzyme digest and sequencing.

These recombinant antibodies are variants of human IgG4 isotype in which serine 228 was replaced with a proline (S228P). This change in the Fc region of IgG4 reduces heterogeneity observed with hIgG4 and extends the serum half-life (Angal et al., Mol Immunol 30: 105 (1993)). A second mutation that replaces leucine 235 with a glutamic acid (L235E) eliminates the residual FR binding and complement binding activities (Alegre et al., J Immunol 148:3461 (1992)). The resulting antibody with both mutations is referred to as IgG4PE. The numbering of the hIgG4 amino acids was derived from Kabat at al., Sequences of Proteins of Immunological Interest, Fifth Edition (1991).

<table>
<thead>
<tr>
<th>SEQ ID NO</th>
<th>Name</th>
<th>Sequence 5' to 3'</th>
<th>Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>RACEUP5'</td>
<td>CTAATACGACTCACTATAGGGC</td>
<td>22-mer</td>
</tr>
<tr>
<td>36</td>
<td>IgGlP</td>
<td>TCTTGCCACCTTGCTGGGCTGGCTGGTGT</td>
<td>31-mer</td>
</tr>
<tr>
<td>37</td>
<td>HK5</td>
<td>AGGCACACAAAGAGGCCAGTCCACAGATTTTC</td>
<td>30-mer</td>
</tr>
<tr>
<td>38</td>
<td>M13F</td>
<td>GTAAAAGCAGGGCCAGTG</td>
<td>18-mer</td>
</tr>
<tr>
<td>39</td>
<td>M13R</td>
<td>CAGGAAAACAGCTATGAC</td>
<td>17-mer</td>
</tr>
<tr>
<td>40</td>
<td>136C5H_F</td>
<td>AGAGAGAGAGTCGACTCACCATGGACTGGGCTGTG</td>
<td>38-mer</td>
</tr>
</tbody>
</table>
Production of recombinant human anti-hIL-10Rα antibody from 293F cells: Suspension cultures of 293F cells were maintained in Freestyle 293 expression medium while shaking at ~120 rpm/min in an 8% CO₂ humidified incubator at 37°C. For transient expression of recombinant antibodies, 3 x 10⁷ 293F cells were transfected with 30 μg of each plasmid encoding the recombinant IgG4PE versions of either the 136C5, 136C8 or 136D29 anti-hIL-10Rα antibodies using 293-fectin (Invitrogen Corp.) following the manufacturer's instructions. Transfectants were allowed to grow in suspension in 30 mL of Freestyle 293 expression medium for 7 days under normal growth conditions. Growth medium was harvested and cells removed by centrifugation at a speed of 300 x g followed by filtration through a 0.22 μm filter. The antibody concentration present in this unpurified material determined by hlgG quantitation ELISA and used for in vitro assays to assess the functional properties of the subclass switched antibodies.

Example 3

This example includes a description of characterization of human monoclonal antibodies that bind to IL-10Rα.

KM mice™ were immunized with soluble recombinant hIL-10Rα;hFc in RIBI. Several of the mice raised anti-human IL-10Rα specific antibodies, with a range in human IgG IL-10Rα specific titers. Splenocytes from the highest responders were fused with myeloma cells to generate human anti-human IL-10Rα producing hybridomas. The production of anti-IL-10Rα antibodies was determined by both ELISA and flow cytometry using recombinant soluble hIL-10Rα and CHO-hIL-10Rα transfectants, respectively. The positive hybridomas were cloned by limiting dilution to yield monoclonal hybridomas. Three human antibodies were further characterized for relative binding affinity for human IL-10Rα, the ability to be blocked by human IL-10 binding to the receptor in vitro, competition with each other, cross-reactivity with non-human primate IL-10Rα, and neutralization of IL-10 in vitro. These antibodies were also compared with commercially available anti-human IL-10Rα antibodies, 3F9 (Biolegend), SPM466 (Spring Biosystems) and 37607 (R&D Systems) (Table 3).

Table 3: Characteristics of Anti-Human IL-10Rα Monoclonal Antibodies

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Original Subclass</th>
<th>Binding Group</th>
<th>Binding to shIL-10Rα</th>
<th>Fold Reduction by IL-10</th>
<th>In vitro neutralizing activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>KD</td>
<td>BMAX</td>
<td></td>
</tr>
<tr>
<td>136C5h</td>
<td>hIgG1</td>
<td>A</td>
<td>0.038</td>
<td>1.79</td>
<td>1.88</td>
</tr>
<tr>
<td>Antibody</td>
<td>HLA Antibody</td>
<td>A</td>
<td>0.047</td>
<td>1.77</td>
<td>1.98</td>
</tr>
<tr>
<td>----------</td>
<td>--------------</td>
<td>---</td>
<td>--------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>136D29*</td>
<td>hlgG4</td>
<td>B</td>
<td>0.058</td>
<td>1.68</td>
<td>1.98</td>
</tr>
<tr>
<td>3F9*</td>
<td>Rat IgG2a</td>
<td>C</td>
<td>0.034</td>
<td>2.08</td>
<td>2.37</td>
</tr>
<tr>
<td>SPM466*</td>
<td>Rat IgG</td>
<td>C</td>
<td>0.043</td>
<td>1.71</td>
<td>NT</td>
</tr>
<tr>
<td>37607*</td>
<td>Mouse IgG2a</td>
<td>C</td>
<td>0.071</td>
<td>0.63</td>
<td>NT</td>
</tr>
</tbody>
</table>

*Human anti-human IL-10Ra antibodies

commercial rat or mouse anti-human IL-1 ORa antibodies

Binding group defined by all parameters measured including cross-blocking, cross-reactivity, and neutralizing activity

NT: not tested

ND: no neutralizing activity detected

**[0334]** Antibodies 136C5, 136C8, and 136D29 all bound specifically to human IL-10Ra expressed on monocytes and lymphocytes found in human peripheral blood. Binding could be inhibited by pre-incubation with soluble human IL-10Ra α:hFc (Figure 1). The binding of these human anti-human IL-10Ra antibodies was saturable and the KD and maximum binding (BMAX) of each antibody was determined by titrating the amount of antibody needed to bind to recombinant human IL-10Ra coated on an ELISA plate (Figure 2A and Table 3). These results were confirmed using a flow cytometry based assay and the human B cell line RPMI-8226. (Figure 2B). Binding of 37607 to this cell line is barely detectable above isotype control staining. The relative binding affinities of 136C5, 136C8, 136D29, and 3F9 were not significantly different from each other, all demonstrated approximately three-fold higher relative binding affinities than 37607 in the ELISA. Binding of a third commercial antibody, SPM466, was very similar to 3F9 in both assays. The KD value is equal to the effective concentration of half maximal binding (EC50) and was determined by non-linear regression analysis of the sigmoidal dose response in the ELISA and flow cytometric assays using the following equation. Y = bottom + (Top - Bottom)/(1+10^-((LogEC50-X))). X is the logarithm of concentration. Y is the response. Y starts at Bottom and goes to Top with a sigmoid shape (Graphpad Prism 4 Software, San Diego, CA). The BMAX is the highest OD or geometric mean fluorescence intensity (geo mean) observed for an individual antibody. The binding max can be variable depending on the source and lot of the secondary antibody used to detect the anti-human IL-10Ra antibodies.

**[0335]** Recombinant IL-10 binding to the IL-10R expressed by RPMI-8226 cells reduced binding of the anti-human IL-10Ra antibodies (Figure 3A). The reduction in antibody binding was similar for all antibodies tested. Figure 3B illustrates the binding of IL-10 to the RPMI-8226 cells. The fold reduction in binding by DL-10 (Table 3) was determined by dividing the geo mean of the antibody binding in the absence of IL-10 by the geo mean in the presence of IL-10. These data show that 136C5, 136C8, 136D29, and 3F9 recognize sequences within the IL-10 binding site. Blockade of antibody binding by IL-10 was not complete because saturating amounts of IL-10 were not used.

**[0336]** Several single nucleotide polymorphisms (SNP) have been identified in the extracellular domain of human IL-10Ra (Gasche, et al., J Immunol 170:5578 (2003)). The SNP that result in changes to the amino acid sequence of human BL-10Ra have the potential to affect the binding of anti-human IL-
lORα antibodies. This possibility was assessed by generating recombinant-Fc fusion proteins of each of the extracellular human IL-I ORα SNP variants that result in amino acid changes (SEQ ID NO. 63, 64, 65, 67, 69, 72) and testing their binding to IL-IO and to anti-IL-10Rα antibodies by ELISA (Figure 12). IL-IO binding to variant S159G (SEQ ID NO. 67) was reduced as was previously reported (Gasche, et al., J Immunol 170:5578 (2003)). Binding of 136C5, 136C8, 136D29, 3F9, and SPM466 to all variants is superior than 37607, similar to binding to the consensus human protein. 136C8 shows significantly better binding to IL-I ORα variant R212E (SEQ ID NO. 69) than 136D29, 3F9, SPM466 and 37607 binding to IL-I ORα variant R212E (SEQ ID NO. 69). These results demonstrate unique binding of antibody 136C8 compared to other described antibodies, and that therapeutic use of an antibody that binds to R212E, such as 136C8, would cover a broader population. Individual antibody binding to L61I (SEQ ID NO. 63) and V233M (SEQ ID NO. 71) variants is similar to binding to the consensus human IL-I ORα for all antibodies tested. 3F9 binding to V1131 (SEQ ID NO. 65) and I224V (chimp) (SEQ ID NO. 6) is reduced compared with 136C8 and 136D29. These changes are due to the nature of different secondary anti-rat antibodies used to detect 3F9 in different assays as these data differ from Figure 7A, where a different lot of anti-rat IgG was used to detect binding of 3F9.

**Example 4**

This example includes a description of cross block studies.

The antibodies were evaluated by ELISA to determine if they compete with each other for binding to soluble human EL-I ORα as a means of determining how many antibody reactivities or epitopes are recognized by this panel of antibodies. Three epitopes groups (Groups I, II and III), were identified by the ability of the antibodies to completely (>70%, Group I), partially (>50% - <70%, Group II), or marginally (<50%, Group III) inhibit binding of each other (Figure 4, Table 4). Using this criteria 136C5 and 136C8 are in the same group (I). 136D29 is closely related to 136C5 and 136C8, but distinct (group II) in that binding of 136D29 is only marginally affected by the other antibodies. The commercial antibody 3F9 binds to a third epitope (group III). Assignment of SPM466 to an epitope group by this method is difficult. Its ability to block the other antibodies is similar to 3F9, which it partially blocks, but SPM466 binding is inhibited by all other antibodies. The blocking by 136C5, 136C8, and 136D29 may be due to steric hindrance. Assignment of antibody 37607 to an epitope group is difficult due to its apparent low affinity. Antibody 37607 binding to EL-I ORα is completely blocked by all the antibodies tested here, but 37607 only partially or marginally affected binding of 136C5, 136C8, 136D29 and 3F9 antibodies. Complete blockade of SPM466 suggests a shared or overlapping epitope.

The epitope is not the only parameter that can affect antibody binding in this experimental approach, the affinities and avidities of the antibodies for hEL-10Rα will affect how they compete with one another for binding. An example of this is that 37607 has a relatively low binding affinity for human IL-10R (Figure 2 and Table 3), in this assay 37607 is significantly inhibited by the human anti-human EL-10R antibodies, yet it only partially or marginally affects the binding of the human anti-EL-10Rα antibodies. In addition, steric hindrance due to binding of the antibodies to close or overlapping
sequences will also lead to competition, this can also be affected by the subclass of the antibody as some isotypes, such as IgG4, are more flexible than others. The IL-10 binding site of the IL-1 ORα protein has been mapped (Reineke, et al Protein Sci 7:951 (1998)). In their paper, Reineke, et al. describe that the IL-10 binding site on IL-1 ORα is composed of 5 non-contiguous peptides that form the three-dimensional IL-10 binding site and that the 37607 antibody binds to 2 of 5 peptides. If the human anti-human EL-10Ra antibodies described here bind to just one of the two peptides recognized by 37607 as described by Reineke, et al., they would reduce binding of 37607 in our assay. This model suggests that antibodies could compete with each other but not share the exact same binding site. We used ELISA and dot blot methods to test the binding of 37607 and 136C8 to peptides that overlap the DL-10 binding sites of EL-10Ra described by Reineke, et al. By the methods we employed we were unable confirm Reineke’s data for binding of the commercial antibody 37607 to any of the peptides that comprise the reported IL-10 binding site of IL-1 ORα (SEQ ID NO 81 - 87). We also did not detect binding of 136C5, 136C8, 136D29, or 3F9 to these peptides. Furthermore, our data demonstrate that these antibodies only recognize native protein and not reduced or denatured DL-10Ra as demonstrated by dot blot and western blot analyses. These results demonstrate that the exemplary antibodies disclosed herein, 136C5, 136C8, and 136D29, recognize conformational epitopes, and not linear epitopes.

Table 4: Percent IL-10Ra binding inhibition by anti-IL-10Ra antibodies.

<table>
<thead>
<tr>
<th>Coated Ab</th>
<th>136C5</th>
<th>136C8</th>
<th>136D29</th>
<th>3F9</th>
<th>SPM466</th>
<th>37607</th>
</tr>
</thead>
<tbody>
<tr>
<td>136C5*</td>
<td>93.93</td>
<td>77.91</td>
<td>48.00</td>
<td>40.36</td>
<td>83.22</td>
<td>78.80</td>
</tr>
<tr>
<td>136C8*</td>
<td>94.21</td>
<td>79.52</td>
<td>43.81</td>
<td>34.42</td>
<td>83.32</td>
<td>79.08</td>
</tr>
<tr>
<td>136D29*</td>
<td>83.43</td>
<td>82.54</td>
<td>80.00</td>
<td>46.50</td>
<td>84.56</td>
<td>74.21</td>
</tr>
<tr>
<td>3F9*</td>
<td>67.20</td>
<td>77.60</td>
<td>58.00</td>
<td>69.40</td>
<td>90.25</td>
<td>70.78</td>
</tr>
<tr>
<td>SPM466*</td>
<td>72.10</td>
<td>64.55</td>
<td>55.04</td>
<td>52.16</td>
<td>90.59</td>
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<td>37607*</td>
<td>59.30</td>
<td>65.20</td>
<td>38.70</td>
<td>1</td>
<td>84.38</td>
<td>74.20</td>
</tr>
</tbody>
</table>

Blocking Ab

Grey highlight = antibody’s blockade of site

*Human anti-human IL-1 ORα antibodies

*commercial rat or mouse anti-human IL-1 ORα antibodies

Example 5

[0340] This example includes a description of in vitro functional analysis of the human anti-human IL-1 ORα antibodies.

[0341] To study the in vitro neutralization activity of the anti-human IL-10Ra antibodies, human peripheral blood mononuclear cells (PBMC) were treated with lipopolysaccharide (LPS) to induce secretion of TNF-α. Addition of recombinant human IL-10 blocks TNF-α secretion. If addition of an anti-human EL-10Ra antibody to the culture restores or enhances TNF-α secretion, then the antibody is considered to have IL-10 neutralizing activity. This was observed with the human and commercial anti-human IL-1 ORα antibodies (Figure 5). At the 20 (Figure 5) and 48 hour timepoints, 136C5, 136C8,
136D29, 3F9 and SPM466 (not shown) all enhanced TNF-α secretion from PBMC treated with LPS and IL-10 with similar dose responses. The mouse anti-human DL-1ORa antibody 37607 was less effective, but still neutralized (i.e., inhibited, reduced, antagonized, prevented or blocked) IL-10. PBMC treated with LPS also produce IL-10 and at 48 hours this amount of endogenous IL-10 reduces TNF-α secretion. The anti-human IL-I ORa antibodies effectively enhanced TNF-α secretion in the presence of endogenous IL-10 as well as in the presence of exogenous IL-10. LPS treatment of PBMC also induces production of IFN-gamma, IL-6 and IL-1β, which are suppressed by addition of IL-10. Neutralization (i.e., inhibition, reduction, antagonism, prevention, or blockade) of IL-10 with 136C5, 136C8, 136D29, 3F9, SPM466, and 37607 restored production of IFN-gamma, DL-6, and IL-1β from the LPS treated PBMC with similar efficacy as observed for restoration of TNF-α. These results demonstrate the ability of invention antibodies to enhance production of TNF-α, IL-6, IL-1β, and IFN-γ secretion in the presence of exogenous IL-10, which results increased immune responses.

[0342] IL-10 has been described as an immunosuppressive agent that reduces the cytokine production by activated T cells (de Waal Malefyt, et al., J Exp Med 174:1209 (1991); Fiorentino, et al., J Immunol 146:3444 (1991); Matsuda, et al., J Exp Med 180:2371 (1994)). Natural Killer T (NKT) cells are a subset of T cells that express NK cell markers such as CD56 and CD161. Approximately 10 - 25% of human T cells in the peripheral blood express NK cell markers (Lanier, et al., J Immunol 153:2417 (1994); Kronenberg, Annu Rev Immunol 23:877 (2005)). Within the NKT cell population there exists a very small subset of cells, 0.01 - 0.5%, that expresses the Vα24 chain of the T cell receptor. These invariant NKT cells are activated by and expand in the presence of a synthetic glycol lipid, α-galactosylceramide, (also known as KRN7000, which was originally discovered by Kirin Brewery Co. Ltd. (Kawano, et al., Science, 278:1626 (1997); Kobayashi, et al., Oncol Res 7:259 (1995))). Stimulation of human NKT cell lines with KRN7000 bound to CD1d expressed on antigen presenting cells results in secretion of large amounts of cytokines such as IFN-γ, TNF-α, GM-CSF, BL-4 and IL-5 and induces cytotoxic activity against target cells that present KRN7000. IL-10 inhibits cytokine secretion from these invariant NKT cells.

[0343] The ability of the human IL-I ORa antibodies to neutralize this function of IL-10 was studied using NKT cell lines previously described by Rogers, et al. (Rogers, et al., J Immunol Meth 285:197 (2004)). NKT cell lines were stimulated with the antigen KRN7000 and allogeneic PBMC, in the presence or absence of IL-10. Antigen stimulation resulted in secretion of IFN-γ and TNF-α at 24 and 48 hours and was inhibited by IL-10. 136C5, 136C8, 136D9, and 3F9 all restored secretion of these cytokines in a dose dependent manner (Figure 10). GM-CSF and IL-5 secretion were also restored by neutralization of IL-10 by the human anti-human IL-I ORa antibodies. These data show that human antibody 136C8 has superior neutralizing (i.e., inhibiting, reducing, antagonizing, preventing or blocking) activity compared to the other antibodies. Restoration or enhancement of cytokine secretion, whether induced by Toll Like Receptor (TLR) ligands such as LPS or by antigen stimulation, will lead to
increased immune responses that are beneficial in appropriate physiological settings, such as a chronic viral infection.

[0344] In addition to suppression of cytokine secretion, IL-10 induces the down-regulation of MHC class II and co-stimulatory molecules (de Waal Malefyt, et al., J Exp Med 174:1209 (1991); de Waal Malefyt, et al., 7 Exp Med 174:9 15 (1991)). Treatment of human PBMC with the anti-human IL-10Ra antibodies in the presence of IL-10 partially restored expression of the HLA-DR MHC class II molecule (Figure 1IA). Thus, these antibodies may restore or enhance antigen presentation of IL-10 suppressed antigen presenting cells. Treatment of the PBMC with the antibodies alone had no effect on the level of HLA-DR expression indicating that the antibodies were not agonistic in nature.

[0345] Signaling through the IL-10R results in activation of STAT3, which can be measured by detecting phosphorylation of Tyrosine 705 (phosphoSTAT3) (OFarrell, et al., Embo J 17:1006 (1998); OTwrell, et al., J Immunol 164:4607 (2000); Rahimi, et al, J Immunol 174:7823 (2005)). Western blot analysis of lysates from human PBMC stimulated with IL-10 confirmed activation of STAT3. Treatment with the anti-human IL-10Ra antibodies blocked IL-10 induced phosphorylation of STAT3 in a dose dependent manner (Figure 1IB, left panel) and IL-10 inhibition by 136C8 was greater than inhibition by 37607 or SPM466 at the highest dose tested, 3 µg/ml (right panel). These data show that human antibody 136C8 has superior neutralizing (i.e., inhibiting, reducing, antagonizing, preventing or blocking) activity compared to the other antibodies. The antibodies alone did not induce STAT3 activation even when crosslinked with an anti-human IgG antibody (Figure 1IC). These data demonstrate that the anti-human IL-10Ra antibodies can neutralize (i.e., inhibit, reduce, antagonize, prevent or block) many of the pleiotropic effects of IL-10, including TNF-α and IFN-γ induction, HLA-DR expression, and STAT3 activation. In the absence of exogenously added IL-10 the antibodies retain their neutralizing activity and despite binding to IL-10R do not induce the signaling pathways resulting from IL-10 binding to IL-10R.

Example 6

[0346] This example includes a description of cross-reactivity with non-human IL-10Ra

[0347] Additional information about the binding specificity of the human anti-human IL-10Ra monoclonal antibodies was obtained by evaluating the ability of the antibodies to bind to rodent, chimpanzee, and cynomolgus macaque IL-10Ra on primary peripheral blood mononuclear cells (PBMC). Neither the human or commercial anti-human IL-10Ra monoclonal antibodies bound to IL-10Ra on mouse or rat splenocytes. 136C5, 136C8, 136D29 and 3F9 bound to IL-10Ra on the surface of chimpanzee and cynomolgus lymphocytes (Figure 6) and monocytes. Binding could be inhibited by pre-incubation with recombinant soluble human IL-10Ra:hFc, demonstrating the specific cross-reactivity of the antibodies. Binding of 37607 to human, chimpanzee, and cynomolgus macaque IL-10Ra was barely detectable above the isotype control staining. Binding of 136C5, 136C8, 136D29, 3F9, and 37607 to soluble chimp IL-10R α:hFc was tested by ELISA and was found to be similar for all antibodies to binding to human IL-10Ra:hFc (Figure 7A and Table 5). Binding to cynomolgus macaque recombinant
IL-10R α:hFc confirmed the reduced binding of 136D29 and 37607 compared with binding of this antibody to human and chimp IL-1 ORa (Figure 7B and Table 5). In contrast to the flow cytometry data, SPM466 and 3F9 bound well to the soluble recombinant form of cynomolgus IL-10R α:hFc, indicating differences between the conformation of soluble and surface expressed IL-1 ORa and further confirming the uniqueness of the human anti-human IL-1 ORa antibodies disclosed herein compared with previously described antibodies.

**Table 5: Binding (BMAX) of anti-human IL-10Rα monoclonal antibodies to human, chimp, and cynomolgus IL-10Rα**

<table>
<thead>
<tr>
<th></th>
<th>Human</th>
<th>Chimp</th>
<th>Cynomolgus</th>
</tr>
</thead>
<tbody>
<tr>
<td>136C5</td>
<td>1.789</td>
<td>1.743</td>
<td>1.643</td>
</tr>
<tr>
<td>136C8</td>
<td>1.764</td>
<td>1.654</td>
<td>1.656</td>
</tr>
<tr>
<td>136D29</td>
<td>1.646</td>
<td>1.449</td>
<td>1.315</td>
</tr>
<tr>
<td>3F9</td>
<td>2.094</td>
<td>2.10</td>
<td>1.493</td>
</tr>
<tr>
<td>SPM466</td>
<td>1.71</td>
<td>NT</td>
<td>1.536</td>
</tr>
<tr>
<td>37607</td>
<td>0.61</td>
<td>0.46</td>
<td>1.126</td>
</tr>
</tbody>
</table>

*Human anti-human IL-10Rα antibodies
*commercial rat or mouse anti-human IL-10Rα antibodies

Antibody binding does not insure functional cross-reactivity. The antibodies were tested in the TNF-α enhancement assay using chimpanzee (Figure 8) or cynomolgus (Figure 9) PBMC and recombinant human IL-10. Human EL-IO inhibited LPS induced TNF-α secretion from both chimpanzee and cynomolgus PBMC. 136C5, 136C8, 136D29, and 3F9 neutralized (i.e., inhibited, reduced, antagonized, prevented or blocked) the effects of human IL-10 on chimpanzee TNF-α secretion. These data demonstrate the functional cross-reactivity of the anti-human DL-10Rα monoclonal antibodies with chimpanzee EL-10Rα. The 37607 and SPM466 antibodies were not evaluated in this assay. Differences were observed in the ability of the antibodies to functionally cross-react with cynomolgus EL-10Rα (Figure 9A-B). Only the human anti-human EL-10Rα monoclonal antibodies 136C5 and 136C8 neutralized the effect of human EL-10 on cynomolgus PBMC. In contrast, 136D29, 3F9, SPM466 and 37607 antibodies did not significantly enhance TNF-α secretion. These results demonstrate that 136C5 and 136C8 functionally neutralize (i.e., inhibit, reduce, antagonize, prevent or block one or more functions) cynomolgus macaque EL-10Rα, while 136D29, 3F9, and 37607 do not neutralize cynomolgus macaque EL-10Rα responses. This is in agreement with the epitope mapping performed by competition ELISA (Table 4), in which 136C5 and 136C8 are in a different group (I) than the other antibodies and clearly demonstrates unique characteristics of these antibodies, placing them in a unique binding group designated group (A). This difference in binding will enable in vivo pre-clinical safety and efficacy studies to be performed in cynomolgus macaques, which will aid in clinical development of anti-EL-10Rα antibody.
Blockade of viral IL-10 suppression. Human and murine cytomegaloviruses (CMV) encode homologues of IL-10, which are capable of binding IL-10Ra and suppressing immune responses (Redpath, et al., *J Immunol* 162:6701 (1999); Jones, et al., *Proc Natl Acad Sci USA* 99:9404 (2002); Spencer, et al., *J Virol* 76:1285 (2002); Chang, et al., *J Virol* 78:8720 (2004)). IL-10Ra antibodies were tested for their ability to neutralize recombinant CMV IL-10 suppression of LPS-induced TNF-α secretion from PBMC isolated from two donors (Figure 13A-B). All of the antibodies studied neutralized suppressive activity of CMV IL-10 in vitro. The ability of the antibodies of the invention to neutralize CMV IL-10 indicates that the antibodies can be used as a therapeutic for treatment of latent or acute CMV infection.
What is Claimed:

1. A human or humanized antibody or subsequence thereof that specifically binds to IL-10 Receptor alpha protein and that reduces, inhibits or competes for binding of an antibody designated 136C5, 136C8, or 136D29, or an antibody comprising a heavy chain variable region sequence of any of SEQ ID NOs:29, 31 or 33, and a light chain variable region sequence of any of SEQ ID NOs:30, 32, or 34, to the IL-10 Receptor alpha protein.

2. An isolated or purified antibody or subsequence thereof that specifically binds to IL-10 Receptor alpha protein and that reduces, inhibits or competes for binding of an antibody designated 136C5, 136C8, or 136D29, or an antibody comprising a heavy chain variable region sequence of any of SEQ ID NOs:29, 31 or 33, and a light chain variable region sequence of any of SEQ ID NOs:30, 32, or 34, to the IL-10 Receptor alpha protein.

3. An isolated or purified antibody or subsequence thereof that specifically binds to IL-10 Receptor alpha protein and that does not detectably reduce, inhibit or compete for binding of antibody designated 3F9, SPM466 or 37607 to the IL-10 Receptor alpha protein.

4. An antibody or subsequence thereof that specifically binds to IL-10 Receptor alpha protein and that binds to an epitope distinct from the epitope that antibody designated 3F9, SPM466 or 37607 binds.

5. An isolated or purified antibody or subsequence thereof that specifically binds to a human IL-10 Receptor alpha protein and specifically binds to a chimpanzee IL-10 Receptor alpha protein, and modulates an IL-lOR/IL-10 signaling activity.

6. An isolated or purified antibody or subsequence thereof that specifically binds to a human IL-10 Receptor alpha protein, specifically binds to a chimpanzee IL-10 Receptor alpha protein, and specifically binds to a macaque IL-10 Receptor alpha protein, and modulates an IL-lOR/IL-10 signaling activity.

7. The antibody of claims 5 or 6, wherein the antibody or subsequence thereof increases TNF-alpha, IL-6, IL-1β or IFN-gamma expression or secretion by human, chimpanzee or macaque PBMCs treated with LPS in vitro in the presence of IL-10.

8. The antibody of claims 1 to 6, wherein the antibody or subsequence thereof reduces or inhibits less than about 50% of the binding of an antibody designated 136C5, 136C8, or 136D29, or an antibody comprising a heavy chain variable region sequence of any of SEQ ID NOs:29, 31 or 33 and a light chain variable region sequence of any of SEQ ID NOs:30, 32, or 34, to IL-10 Receptor alpha protein.

9. The antibody of claims 1 to 6, wherein the antibody or subsequence thereof reduces or inhibits about 50% or more of the binding of an antibody designated 136C5, 136C8, or 136D29, or an antibody comprising a heavy chain variable region sequence of any of SEQ ID NOs:29, 31 or 33 and a light chain variable region sequence of any of SEQ ID NOs:30, 32, or 34 to IL-10 Receptor alpha protein.
10. The antibody of claims 1 to 6, wherein the antibody or subsequence thereof binds to or recognizes a conformational epitope, and not a linear epitope.

11. The antibody of claims 1 to 6, wherein the antibody or subsequence thereof binds with greater affinity to IL-10Rα variant R212E (SEQ ID NO. 69) than binding of 136D29, 3F9, SPM466 or 37607 antibody to IL-10Ra variant R212E (SEQ ID NO. 69).

12. The antibody of claims 1 to 6, wherein the binding affinity, KD, of the antibody or subsequence thereof to IL-10 Receptor alpha protein is within about 1-1000-fold of the binding affinity, KD, of an antibody designated 136C5, 136C8, or 136D29, or an antibody comprising a heavy chain variable region sequence of any of SEQ ID NOs:29, 31 or 33 and a light chain variable region sequence of any of SEQ ID NOs:30, 32, or 34, to IL-10 Receptor alpha protein.

13. The antibody of claims 1 to 6, wherein the binding affinity, KD, of the antibody or subsequence thereof to IL-10 Receptor alpha protein is greater than or less than the binding affinity, KD, of an antibody designated 136C5, 136C8, or 136D29, or an antibody comprising a heavy chain variable region sequence of any of SEQ ID NOs:29, 31 or 33 and a light chain variable region sequence of any of SEQ ID NOs:30, 32, or 34, to IL-10 Receptor alpha protein.

14. An isolated or purified antibody or subsequence thereof that specifically binds to IL-10 Receptor alpha protein, wherein said antibody or subsequence comprises a sequence at least 80% identical to any heavy chain variable region sequence set forth as SEQ ID NOs:29, 31 or 33, and a sequence at least 80% identical to any light chain variable region sequence set forth as SEQ ID NOs:30, 32, or 34.

15. The isolated or purified antibody or subsequence thereof of claim 14, wherein said antibody or subsequence comprises a sequence at least 85% identical to any heavy chain variable region sequence set forth as SEQ ID NOs:29, 31 or 33, and a sequence at least 85% identical to any light chain variable region sequence set forth as SEQ ID NOs:30, 32, or 34.

16. The isolated or purified antibody or subsequence thereof of claim 14, wherein said antibody or subsequence comprises a sequence at least 90% identical to any heavy chain variable region sequence set forth as SEQ ID NOs:29, 31 or 33, and a sequence at least 90% identical to any light chain variable region sequence set forth as SEQ ID NOs:30, 32, or 34.

17. A single chain antibody that specifically binds to IL-10 Receptor alpha protein, comprising a heavy chain variable region sequence at least 80% identical to any of SEQ ID NOs:29, 31 or 33 and a light chain variable region sequence at least 80% identical to any of SEQ ID NOs:30, 32, or 34.

18. An isolated or purified antibody or subsequence thereof that specifically binds to IL-10 Receptor alpha protein, wherein said antibody or subsequence comprises any heavy chain variable region sequence set forth as SEQ ID NOs:29, 31 or 33, and any light chain variable region sequence set forth as SEQ ID NOs:30, 32, or 34, wherein said antibody or subsequence thereof has one or more amino acid additions, deletions or substitutions of SEQ ID NOs:29, 31 or 33, or SEQ ID NOs:30, 32, or 34.
19. The isolated or purified antibody or subsequence thereof of claim 18, wherein said antibody or subsequence comprises a sequence at least 80% identical to any heavy chain variable region sequence set forth as SEQ ID NOs:29, 31 or 33, and a sequence at least 80% identical to any light chain variable region sequence set forth as SEQ ID NOs:30, 32, or 34.

20. The antibody or the subsequence thereof of claim 18, wherein said substitution is a conservative or a non-conservative amino acid substitution.

21. The antibody or the subsequence thereof of claim 18, wherein said substitution is located within a complementarity determining region (CDR) or within a framework region (FR).

22. The antibody or the subsequence thereof of claim 18, wherein said substitution is located outside of a CDR or a FR.

23. The antibody or subsequence thereof of any of claims 1 to 6, 13, 17 and 18, wherein binding of the antibody or subsequence thereof to IL-10 Receptor alpha protein is reduced or inhibited by IL-10 binding to IL-10 Receptor alpha protein expressed on a cell in vivo or in vitro, or in solution.

24. The antibody or subsequence thereof of any of claims 1 to 6, 13, 17 and 18, wherein the antibody or subsequence thereof reduces or inhibits IL-10 signaling activity.

25. The antibody or subsequence thereof of claim 24, wherein the reduction or inhibition of IL-10 signaling activity is greater than the reduction or inhibition of IL-10 signaling activity by any of 136D29, 3F9, SPM466 or 37607 antibodies.

26. The antibody or subsequence thereof of any of claims 1 to 6, 13, 17 and 18, wherein the antibody or subsequence thereof increases or induces TNF-alpha or EFN-gamma expression by PBMC or NKT cells in the presence of IL-10, at least partially restores expression of the HLA-DR MHC class II molecule in the presence of EL-10, or inhibits or reduces EL-10 induced phosphorylation of STAT3.

27. The antibody or subsequence thereof of any of claims 1 to 6, 13, 17 and 18, wherein the antibody or subsequence thereof reverses or inhibits the EL-10 inhibition of TNF alpha expression or secretion by human PBMC treated with lipopolysaccharide (LPS).

28. The antibody or subsequence thereof of any of claims 1 to 6, 13, 17 and 18, wherein the antibody or subsequence thereof induces, promotes, stimulates or increases a proinflammatory or adaptive immune response or production of a cytokine or chemokine.

29. The antibody or subsequence thereof of claim 28, wherein the proinflammatory immune response comprises one or more of: CD4+ or CD8+ T cell or NKT cell proliferation, CD4+ or CD8+ T cell or NKT cell production of IL-2, EFN-gamma, EL-4, EL-5 or TNF-alpha, macrophage or dendritic cell activation or CD4+ or CD8+ T cell cytotoxic activity.

30. The antibody or subsequence thereof of claim 28, wherein the cytokine comprises one or more of: EL-1 alpha, EL-1 beta, TNF-alpha, EL-6, EL-9, EL-12, EL-18, or GM-CSF.

31. The antibody or subsequence thereof of claim 28, wherein the chemokine comprises one or more of: MCP1, MCP5, RANTES, EL-8, EP-IO, or MEP-2.
32. The antibody or subsequence thereof of any of claims 1 to 6, 13, 17 and 18, wherein the IL-10 Receptor alpha protein is a mammalian IL-10 Receptor alpha protein.

33. The antibody or subsequence thereof of claim 32, wherein the IL-10 Receptor alpha protein is a primate IL-10 Receptor alpha protein.

34. The antibody or subsequence thereof of claim 32, wherein the IL-10 Receptor alpha protein is a human, a chimp or a macaque IL-10 Receptor alpha protein.

35. The antibody or subsequence thereof of any of claims 1 to 6, 13, 17 and 18, wherein the antibody or subsequence binds to a chimp and a macaque IL-10 Receptor alpha protein.

36. The antibody or subsequence thereof of any of claims 1 to 6, 13, 17 and 18, wherein the antibody or subsequence thereof binds to an IL-10 Receptor alpha protein set forth as SEQ ID NO:2 or SEQ ID NO:8, or an EL-10 Receptor alpha protein extracellular domain of SEQ ID NO:4, or SEQ ID NO:6 or SEQ NO: 10.

37. The antibody or subsequence thereof of any of claims 1 to 6, 13, 17 and 18, wherein the antibody isotype comprises an IgM, IgG, IgA, IgD or IgE isotype.

38. The antibody or subsequence thereof of any claim 37, wherein the IgG or IgA isotype is selected from IgGl, IgG2, IgG3, and IgG4; and IgAl and IgA2.

39. The antibody subsequence of any of claims 1 to 6, 13, 17 and 18, wherein the antibody subsequence is selected from Fab, Fab’, F(ab’) 2, Fv, Fd, single-chain Fv (scFv), disulfide-linked Fvs (sdFv), light chain variable region V_L, heavy chain variable region V_H, trispecific (Fab_3), bispecific (Fab_2), diabody ((V_L-V_H)_2 or (V_H-V_L)_2), triabody (trivalent), tetrabody (tetravalent), minibody ((scF_v-C_H^3)_2), bispecific single-chain Fv (Bis-scFv), IgGdeltaCH2, scFv-Fc, (ScFv)_2^-Fc and EgG4PE.

40. The antibody or the subsequence thereof of any of claims 1 to 6, 13, 17 and 18, wherein the antibody comprises a heavy chain variable region sequence with at least 80 amino acids identical to any of SEQ NO:29, 31 or 33, and a light chain variable region sequence with at least 80 amino acids identical to any of SEQ NO:30, 32, or 34.

41. The antibody or the subsequence thereof of any of claims 1 to 6, 13, 17 and 18, wherein the antibody comprises a heavy chain variable region sequence with at least 90 amino acids identical to any of SEQ NO:29, 31 or 33, and a light chain variable region sequence with at least 90 amino acids identical to any of SEQ NO:30, 32, or 34.

42. The antibody or the subsequence thereof of any of claims 1 to 6, 13, 17 and 18, wherein the antibody is primatized, humanized or human.

43. An antibody or subsequence thereof that specifically binds to EL-10 Receptor alpha protein, comprising a heavy chain CDR within any of SEQ NO:29, 31 or 33, or a light chain CDR within any of SEQ NO:30, 32, or 34.

44. An antibody or subsequence thereof that specifically binds to EL-10 Receptor alpha protein, comprising a heavy chain CDR within any of SEQ NO:29, 31 or 33, and a light chain CDR within any of SEQ NO:30, 32, or 34.
45. An antibody or subsequence thereof that specifically binds to IL-10 Receptor alpha protein, comprising all heavy chain CDRs within any one of SEQ ID NOs: 29, 31 or 33, and all light chain CDRs within any one of SEQ ID NOs: 30, 32, or 34.

46. The antibody or subsequence of any of claims 44 to 46, wherein the heavy chain CDR is selected from: SYSMN; YISTGSSTIYYADSVKG; ENYYGGSGYEDYFDY; YISTRSSIYYADSVKG; ELSMH; GFPDDGETIY AQKFQG; and GGGYGPVGMDV.

47. The antibody or subsequence of any of claims 44 to 46, wherein the light chain CDR is selected from: RASQSVSSYLA; DASNRAT; QQRSNWPIFT; RASQGISIWLA; AASSLQS; and QQYNYSYPLT.

48. An antibody or subsequence thereof that specifically binds to IL-10 Receptor alpha protein, wherein the antibody or subsequence thereof comprises any of a heavy chain variable region sequence with CDR1 (SYSMN), CDR2 (YISTGSSTIYYADSVKG), CDR3 (ENYYGGSGYEDYFDY) and a light chain variable region sequence with CDR1 (RASQSVSSYLA), CDR2 (DASNRAT), CDR3 (QQRSNWPIFT); a heavy chain variable region sequence with CDR1 (SYSMN), CDR2 (YISTRSSIYYADSVKG), CDR3 (ENYYGGSGYEDYFDY) and a light chain variable region sequence with CDR1 (RASQSVSSYLA), CDR2 (DASNRAT), CDR3 (QQRSNWPIFT); and heavy chain variable region sequence with CDR1 (ELSMH), CDR2 (GFPDDGETIY AQKFQG), CDR3 (GGGYGPVGMDV) and a light chain variable region sequence with CDR1 (RASQGISPWLA), CDR2 (AASSLQS), CDR3 (QQYNYSYPLT).

49. The antibody or the subsequence thereof of any of claims 1 to 6, 13, 17, 18, 44 to 46 or 48, wherein the antibody is produced by a hybridoma cell, a CHO cell line or a HEK293F cell.

50. The antibody or the subsequence thereof of any of claims 1 to 6, 13, 17, 18, 44 to 46 or 48, wherein the antibody comprises an antibody multimer.

51. The antibody or the subsequence thereof of any of claims 1 to 6, 13, 17, 18, 44 to 46 or 48, further comprising one or more heterologous domains.

52. The antibody or the subsequence of claim 51, wherein the heterologous domain comprises a label or tag.

53. The antibody or the subsequence of claim 51, wherein the heterologous domain comprises an amino acid sequence.

54. A heavy chain variable region sequence set forth as SEQ ID NOs: 29, 31 or 33.

55. A light chain variable region sequence set forth as SEQ ID NOs: 30, 32, or 34.

56. A nucleic acid that encodes a heavy or light chain variable region sequence of the antibody or the subsequence thereof of any of claims 1 to 6, 13, 17, 18, 44 to 46 or 48.

57. The nucleic acid of claim 56, further comprising nucleic acid that encodes a constant region sequence of an antibody.

58. The nucleic acid of claim 56, further comprising an expression control element.
59. A vector comprising a nucleic acid that encodes the antibody or the subsequence of any of claims 1 to 6, 13, 17, 18, 44 to 46 or 48.

60. A host cell that expresses the antibody or the subsequence of any of claims 1 to 6, 13, 17, 18, 44 to 46 or 48.

61. A host cell transformed with a nucleic acid that encodes the antibody or the subsequence of any of claims 1 to 6, 13, 17, 18, 44 to 46 or 48.

62. A non-human animal that expresses or produces a heavy or light chain variable region sequence of the antibody or the subsequence of any of claims 1 to 6, 13, 17, 18, 44 to 46 or 48.

63. A non-human animal that expresses or produces the antibody or the subsequence of any of claims 1 to 6, 13, 17, 18, 44 to 46 or 48.

64. The non-human animal of claim 63, wherein the antibody is expressed from a gene locus encoding human immunoglobulin lambda or kappa light chain.

65. The non-human animal of claim 63, wherein the non-human animal comprises an animal that contains a human immunoglobulin nucleic acid sequence.

66. The non-human animal of claim 63, wherein the non-human animal comprises a transchromosomic animal.

67. The non-human animal of claim 63, wherein the non-human animal comprises a mammal.

68. The non-human animal of claim 63, wherein the non-human animal comprises a mouse, rat, guinea pig, rabbit, sheep, goat, cow, pig, or horse.

69. A plant that expresses the antibody or subsequence thereof of any of claims 1 to 6, 13, 17, 18, 44 to 46 or 48.

70. A pharmaceutical composition, comprising the antibody or subsequence thereof of any of claims 1 to 6, 13, 17, 18, 44 to 46 or 48 and a pharmaceutically acceptable excipient or carrier.

71. A composition, comprising at least two antibodies or subsequences thereof of any of claims 1 to 6, 13, 17, 18, 44 to 46 or 48.

72. A composition, comprising the antibody or subsequence thereof of any of claims 1 to 6, 13, 17, 18, 44 to 46 or 48 and a pathogen antigen or an epitope thereof, live or attenuated pathogen, or a nucleic acid encoding a pathogen antigen or an epitope thereof, or an immune stimulating agent or compound.

73. An antibody designated as 136C5 (produced by hybridoma deposited on April 8, 2008, with deposit designation of PTA-9131), 136C8 (produced by hybridoma deposited on April 8, 2008, with deposit designation of PTA-9132), or 136D29 (produced by hybridoma deposited on April 8, 2008, with deposit designation of PTA-9133), or a functional subsequence thereof.

74. A kit, comprising the antibody or subsequence thereof of any of claims 1 to 6, 13, 17, 18, 44 to 46, 48 or 73.

75. A method of treating a subject for a pathogen infection, comprising administering to a subject in need thereof an amount of the antibody or subsequence thereof of any of claims 1 to 6, 13, 17, 18, 44 to 46, 48 or 73 sufficient to treat the subject.
76. A method of providing a subject with protection against a pathogen infection, or reactivation from latency, comprising administering to a subject in need thereof a pathogen antigen or an epitope thereof, live or attenuated pathogen, or a nucleic acid encoding a pathogen antigen or an epitope thereof, and an amount of the antibody or subsequence thereof of any of claims 1 to 6, 13, 17, 18, 44 to 46, 48 or 73 sufficient to provide the subject with protection against the pathogen infection, or reactivation from latency.

77. The method of claim 75, wherein the pathogen infection is chronic or acute.

78. The method of claim 75, wherein the pathogen infection is a latent infection.

79. The method of claims 75 or 76, wherein the subject is a mammal.

80. The method of claims 75 or 76, wherein the subject is a human.

81. The method of claims 75 or 76, wherein the pathogen comprises a virus, bacterium, parasite, or fungus.

82. The method of claim 81, wherein the virus comprises a poxvirus, herpesvirus, hepatitis virus, immunodeficiency virus, flavivirus, papilloma virus (PV), polyoma virus, rhabdovirus, a myxovirus, an arenavirus, a coronavirus, adenovirus, reovirus, picornavirus, togavirus, bunyavirus, parvovirus or retrovirus.

83. The method of claim 82, wherein the poxvirus comprises a vaccinia virus, Molluscum contagiosum, variola major or variola minor smallpox virus, cow pox, camel pox, sheep pox, or monkey pox.

84. The method of claim 82, wherein the herpesvirus comprises an alpha-herpesvirus, beta-herpesvirus, gamma-herpesvirus, Epstein Bar Virus (EBV), Cytomegalovirus (CMV), varicella zoster virus (VZV/HHV-3), or human herpes virus 1, 2, 4, 5, 6, 7, or 8 (HHV-8, Kaposi's sarcoma-associated virus).

85. The method of claim 82, wherein the hepatitis virus comprises hepatitis A, B, C, D, E or G.

86. The method of claim 82, wherein the immunodeficiency virus comprises human immunodeficiency virus (HIV).

87. The method of claim 86, wherein the HIV comprises HIV-I, HIV-2 or HIV-3.

88. The method of claim 82, wherein the flavivirus comprises Hepatitis C virus, Yellow Fever virus, Dengue virus, Japanese Encephalitis or West Nile viruses.

89. The method of claim 82, wherein the papilloma virus comprises a human papilloma virus (HPV).

90. The method of claim 89, wherein the human papilloma virus comprises HPV strain 1, 6, 11, 16, 18, 30, 31, 42, 43, 44, 45, 51, 52, or 54.

91. The method of claim 82, wherein the polyoma virus comprises BK virus (BKV) or JC virus (JCV).

92. The method of claim 82, wherein the rhabdovirus comprises rabies virus or vesiculovirus.

93. The method of claim 82, wherein the myxovirus comprises a paramyxovirus or orthomyxovirus.

94. The method of claim 93, wherein the paramyxovirus comprises measles, mumps, pneumovirus or respiratory syncytial virus (RSV).
95. The method of claim 93, wherein the orthomyxovirus comprises an influenza virus.
96. The method of claim 95, wherein the influenza virus comprises influenza A, influenza B or influenza C.
97. The method of claim 82, wherein the arenavirus comprises lymphocytic choriomeningitis virus (LCMV), Junín virus, Lassa virus, Guanarito virus, Sabia virus or Machupo virus.
98. The method of claim 82, wherein the coronavirus comprises a virus that causes a common cold or severe acute respiratory syndrome (SARS).
99. The method of claim 82, wherein the adenovirus comprises a viral infection of the bronchii, lung, stomach, intestine (gastroenteritis), eye (conjunctivitis), bladder (cystitis) or skin.
100. The method of claim 82, wherein the reovirus comprises a rotavirus, cypovirus or orbivirus.
101. The method of claim 82, wherein the picornavirus comprises a rhinovirus, apthovirus, hepatovirus, enterovirus, coxsackie B virus, or cardiovirus.
102. The method of claim 101, wherein the rhinovirus causes a common cold.
103. The method of claim 82, wherein the togavirus comprises an alphavirus, sindbis virus, or rubellavirus.
104. The method of claim 82, wherein the bunyavirus comprises a hantavirus, phlebovirus or nairovirus.
105. The method of claim 82, wherein the retrovirus comprises an alpha, beta, delta, gamma, epsilon, lentivirus, spumavirus or human T-cell leukemia virus.
106. The method of claim 105, wherein the lentivirus comprises an immunodeficiency virus.
107. The method of claim 106, wherein the immunodeficiency virus comprises a bovine, porcine, equine, canine, feline or primate virus.
108. The method of claim 105, wherein the human T-cell leukemia viruses comprises human T-cell leukemia virus 1 or 2 (HTLV-I and HTLV-2).
109. The method of claims 75 or 76, wherein the bacterium comprises a Mycobacterium, listeria monocytogenes, Helicobacter, bordetella, streptococcus, salmonella or chlamydia.
110. The method of claims 75 or 76, wherein the parasite comprises a protozoa or nematode.
111. The method of claim 110, wherein the protozoa comprises a Toxoplasma gondii, Leishmania, Plasmodium, or Trypanosoma cruzi.
112. The method of claim 110, wherein the nematode comprises a Schistosoma mansoni, or a helminth.
113. The method of claims 75 or 76, wherein the fungus comprises Candida albicans.
114. The method of claims 75 or 76, wherein the treatment is sufficient to protect against pathogen infection or pathology, decrease, reduce, or limit susceptibility to pathogen infection or pathology, or decrease, reduce, inhibit, suppress, limit or control pathogen numbers or titer, decrease, reduce, inhibit, suppress, limit or control pathogen proliferation or replication, decrease, reduce, inhibit, suppress, limit or control the amount of a pathogen protein, or decrease, reduce, inhibit, suppress, limit or control the amount of a pathogen nucleic acid.
115. The method of claims 75 or 76, wherein the treatment is sufficient to increase, promote, enhance, induce, augment, or stimulate an immune response against a pathogen.

116. The method of claims 75 or 76, wherein the treatment is sufficient to increase, promote, enhance, induce, augment or stimulate pathogen clearance or removal, decrease, reduce, inhibit, suppress, limit or control reactivation from latency, or decrease, reduce, inhibit, suppress, limit or control pathogen transmission to another subject.

117. The method of claims 75 or 76, wherein the treatment is sufficient to decrease, reduce, inhibit, suppress, limit, control or improve one or more adverse symptoms, disorders, illnesses, pathologies or diseases, or complications caused by or associated with pathogen infection or pathogen reactivation from latency.

118. The method of claims 75 or 76, wherein the antibody or subsequence thereof is administered prior to, substantially contemporaneously with or following exposure to or infection of the subject with a pathogen.

119. The method of claims 75 or 76, wherein the antibody or subsequence thereof is administered prior to, substantially contemporaneously with or following pathogen infection, a pathology or adverse symptom, disorder, illness, disease, or complication caused by or associated with pathogen infection, or reactivation from latency.

120. A method of increasing numbers or activation of an immune cell in a subject with or at risk of a pathogen infection, comprising administering to a subject an amount of the antibody or subsequence thereof of any of claims 1 to 6, 13, 17, 18, 44 to 46, 48 or 73 sufficient to increase numbers or activation of the immune cell in the subject.

121. The method of claim 120, wherein the immune cell comprises a T cell, natural killer T cell (NKT) dendritic cell (DC), macrophage, neutrophil, eosinophil, or mast cell.

122. The method of claim 121, wherein IL-12 is produced by the dendritic cell (DC) or macrophages.

123. The method of claim 120 wherein the immune cell comprises one or more of: CD4+, CD8+, CD 14+, CD 1 lb+ or CD 1 lc+ cells.

124. A method of increasing or inducing an anti-pathogen CD8+ or CD4+ T cell response in a subject with or at risk of a pathogen infection, comprising administering to a subject an amount of the antibody or subsequence thereof of any of claims 1 to 6, 13, 17, 18, 44 to 46, 48 or 73 sufficient to increase or induce an anti-pathogen CD8+ or CD4+ T cell response in the subject.

125. A method of increasing production of a Th1 or Th2 cytokine in a subject in need of increased production of a Th1 or Th2 cytokine, comprising administering to a subject an amount of the antibody or subsequence thereof of any of claims 1 to 6, 13, 17, 18, 44 to 46, 48 or 73 sufficient to increase Th1 cytokine production in the subject.

126. The method of claim 125, wherein the subject has or is at risk of having a pathogen infection.

127. The method of claim 125, wherein the cytokine comprises interferon (IFN) gamma, TNF-alpha, IL-1 alpha, EL-1 beta, IL-4, IL-5, IL-2, IL-6, EL-8, IL-12, IL-18 or GM-CSF.
128. The method of claim 127, wherein IFN gamma is produced by CD8+ T cells specific for a pathogen comprising the pathogen infection.
Figures 1A-1B

A

B

136C5

136C8

136D29

3F9

37607
Figures 3A-3B

A.  

136C5  136C8  136D29  3F9

B.  

IL10
Figure 4

![Bar graph showing blocking antibody levels for 136C5, 136C8, and 136D29. The graph compares the levels of these antibodies on a scale from 0 to 100.]
Figures 5A-5B

A.

- 3F9
- 136C5
- 136C8
- 136D29
- LPS + IL10
- LPS only

B.

- 3F9
- 37607
- 136C8
- hIgG
- LPS
- LPS+IL10
- Untreated
Figures 6A-6C
Figures 7A-7B
Figure 8
Figures 9A-9B

A.  
![Graph A](image_a)

- X Untreated
- O LPS
- LPS+IL10
- 136C5
- 136C8
- 136D29
- 3F9
- 37607
- hlgG

B.  
![Graph B](image_b)

- 136C8
- D29
- 3F9
- 37607
- SPM466
- O LPS
- LPS+IL10

9/13