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(54) **Titre : METHODES DE MODULATION DE L'ACTIVITE DE LA CYTOKINE ET AGENTS REACTIFS ASSOCIES**
(54) **Title: METHODS OF MODULATING CYTOKINE ACTIVITY; RELATED REAGENTS**

(57) **Abrégé/Abstract:**
Provided are methods of modulating cytokine activity, e.g., for the purpose of treating immune and inflammatory disorders, including tumors and cancer. Also provided are methods of administering agonists or antagonists of IL-33 and IL-33 receptor.

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(54) Title: USE FOR INTERLEUKIN-33 (IL33) AND THE IL-33 RECEPTOR COMPLEX

(57) Abstract: Provided are methods of modulating cytokine activity, e.g., for the purpose of treating immune and inflammatory disorders, including tumors and cancer. Also provided are methods of administering agonists or antagonists of IL-33 and IL-33 receptor.

METHODS OF MODULATING CYTOKINE ACTIVITY; RELATED REAGENTS

FIELD OF THE INVENTION

[0001] The present invention relates generally to uses of mammalian cytokines. More specifically, the invention discloses methods of using IL-33, and a receptor for IL-33.

BACKGROUND OF THE INVENTION

[0002] The immune system protects individuals from infective agents, e.g., bacteria, multi-cellular organisms, as well as cancers. This system includes several types of lymphoid and myeloid cells such as monocytes, macrophages, dendritic cells (DCs), eosinophils, T cells, B cells, and neutrophils. These lymphoid and myeloid cells often produce signaling proteins known as cytokines. Immune response includes inflammation, i.e., the accumulation of immune cells systemically or in a particular location of the body. In response to an infective agent or foreign substance, immune cells secrete cytokines which, in turn, modulate immune cell proliferation, development, differentiation, or migration. Immune response sometimes results in pathological consequences, that is, inflammatory disorders. These inflammatory disorders, which involve immune cells and cytokines, include, e.g., psoriasis, rheumatoid arthritis, Crohn's disease, multiple sclerosis, and atherosclerosis (see, e.g., Abbas, *et al.* (eds.) (2000) *Cellular and Molecular Immunology*, W.B. Saunders Co., Philadelphia, PA; Oppenheim and Feldmann (eds.) (2001) *Cytokine Reference*, Academic Press, San Diego, CA; Kaufmann, *et al.* (2001) *Immunobiol.* 204:603-613; Saurez and Schultz-Cheery (2000) *Dev. Comp. Immunol.* 24:269-283; van Reeth and Nauwynck (2000) *Vet. Res.* 31:187-213; Garcia-Sastre (2001) *Virology* 279:375-384; Katze, *et al.* (2002) *Nat. Rev. Immunol.* 2:675-687; van Reeth (2000) *Vet. Microbiol.* 74:109-116; Tripp (2003) *Curr. Pharm. Des.* 9:51-59).

[0003] The interleukin-1 (IL-1) family of cytokines contributes to the pathology of inflammatory disorders and proliferative conditions, e.g., arthritis and cancer. Cytokines of the IL-1 family include IL-1alpha, IL-1beta, IL-1delta, IL-1epsilon, basic fibroblast growth factor, IL-18, CREG and CREG2. IL-1alpha and IL-1beta are biosynthesized as 31 kDa polypeptides that are further processed to mature 17 kDa forms, while IL-1delta and IL-1epsilon appear not to possess a distinct pro-form (see, e.g., Debets, *et al.* (2001) *J. Immunol.* 167:1440-1446; McMahon, *et al.* (1997) *J. Biol. Chem.* 272:28202-28205; Irikura, *et al.* (2002) *New Engl. J. Med.* 169:393-398; Kim, *et al.* (2002) *J. Biol. Chem.* 277:10998-11003).

[0004] The IL-1 family also includes IL-1 receptors, i.e., IL-1RI, IL-1RII, and IL-1R accessory protein (a.k.a. IL-1R1, IL-1R2, and IL-1R3, respectively). IL-1alpha and IL-1beta trigger cell signaling by binding to IL-1R1, while IL-1RII can function as a molecule that absorbs circulating ligand. IL-1 receptor antagonist (IL-1Ra), another IL-1 family protein, binds to IL-1 receptor without transmitting a signal and serves as an inhibitor of IL-1. IL-1ra and IL-1delta play similar roles in antagonizing signaling through receptors, i.e., IL-1ra antagonizes IL-1alpha-mediated signaling via IL-1R1, while IL-1delta antagonizes IL-1epsilon-mediated signaling via IL-1R6 (see, e.g., You, *et al.* (2001) *New Engl. J. Med.* 193:101-109). Debets, *et al.* (2001) *J. Immunol.* 167:1440-1446; Apte and Voronov (2002) *Sem. Cancer Biol.* 12:277-290; Wong, *et al.* (1997) *Proc. Natl. Acad. Sci. USA* 94:227-232).

[0005] IL-1 family members play a role in inflammatory conditions, e.g., rheumatoid arthritis, psoriasis, asthma, chronic obstructive pulmonary disorder (COPD), sepsis, and inflammatory bowel disorder (IBD). Rheumatoid arthritis (RA) is a common chronic inflammatory disorder characterized by degradation of joints, e.g., the synovial membrane, cartilage, and bone. The disorder strikes about 1% of the population and cannot be cured. IL-1 stimulates a number of cells involved in arthritic inflammation, e.g., fibroblasts, osteoclasts, chondrocytes, and neutrophils, which may show abnormal proliferation and release enzymes causing joint destruction (see, e.g., (Debets, *et al.* (1997) *J. Immunol.* 158:2955-2963; Lacey, *et al.* (2003) *Arthritis Rheum.* 48: 103-109; Chung (2001) *Eur. Resp. J. Suppl.* 34: 50s-59s; Freeman and Buchman (2001) *Expert Opin. Biol. Ther.* 1:301-308; Dinarello (2000) *Chest* 118:503-508). Krause, *et al.* (2002) *J. Immunol.* 169:6610-6616; Choy and Panayi (2001) *New Engl. J. Med.* 344:907-916; Woolley (2003) *New Engl. J. Med.* 348:1709-1711; Williams, *et al.* (2000) *New Engl. J. Med.* 164: 7240-7245; Feldmann

and Maini (2001) *Annu. Rev. Immunol.* 19:163-196; Lacey, *et al.*, *supra*; Niki, *et al.* (2001) *J. Clin. Invest.* 107:1127-1135; Attur, *et al.* (2000) *J. Biol. Chem.* 51:40307-40315).

[0006] Proliferative disorders are the second most common cause of death in the United States (Anderson (2002) *National Vital Statistics Reports* 50:1-86; Toribara and Sleisenger (2003) *New Engl. J. Med.* 332:861-867; Janne and Mayer (2000) *New Engl. J. Med.* 342:1960-1968; Fuchs and Mayer (1995) *New Engl. J. Med.* 333:32-41). Cytokines of the IL-1 family have been implicated in the control and pathology of proliferative disorders, i.e., cancer. IL-1 modulates progression through the cell cycle, e.g., by changing expression of cyclin-dependent kinases and cyclin-dependent kinase inhibitors. High doses of IL-1beta promote tumor invasiveness, while low doses can promote immune eradication of tumors (see, e.g., Zeisler, *et al.* (1998) *Eur. J. Cancer* 34:931-933; Yoshida, *et al.* (2002) *Brit. J. Cancer* 86:1396-1400; Nesbit, *et al.* (1999) *Oncogene* 18:6469-6476; Dinarello, *et al.* (1998) *J. Leuko. Biol.* 63:658-664; Apte and Voronov, *supra*; Saijo, *et al.* (2002) *New Engl. J. Med.* 169: 469-475; Murai, *et al.* (2001) *J. Biol. Chem.* 276:6797-6806; Koudssi, *et al.* (1998) *J. Biol. Chem.* 273: 25796-25803; Zeki, *et al.* (1999) *J. Endocrinol.* 160:67-73; Osawa, *et al.* (2000) *J. Biochem.* 127:883-893).

[0007] There is an unmet need to treat inflammatory and immune disorders. The present invention fulfils this need by providing methods of using agonists and antagonists of IL-33 or IL-33 receptor.

SUMMARY OF THE INVENTION

[0008] The present invention is based, in part, upon the discovery that an agonist or antagonist of IL-33 or IL-33 receptor (previously known as IL-100 and IL-100 receptor) modulates response to a number of immune and inflammatory conditions.

[0009] The present invention provides a method of modulating an immune disorder or condition, comprising administering an effective amount of an agonist or antagonist of IL-33 or IL-33R complex. Also provided is the above method wherein the disorder or condition comprises: a) innate response; b) asthma or allergy; c) multiple sclerosis; d) an inflammatory bowel disorder; e) arthritis; f) infection; g) a cancer or tumor. Further provided is the above method wherein the infection comprises: a) an intracellular pathogen; b) a bacterium; c) a parasite; or d) a virus; and the above method wherein the intracellular pathogen is: a) *Leishmania sp.*; b) *Mycobacterium sp.*; c) *Listeria sp.*; d) *Toxoplasma sp.*; e) *Schistosoma*; or f) a respiratory virus. Moreover, the present invention provides the above method wherein the immune disorder or conditions comprises TH1-type response or TH2-type response; and the above method wherein the TH2-type response comprises an early event in TH2-type response; as well as the above method wherein the arthritis comprises rheumatoid arthritis; osteoarthritis; or psoriatic arthritis.

[0010] In another embodiment, the present invention provides the above method wherein the agonist comprises IL-33 or a nucleic acid; as well as the above method wherein the nucleic acid encodes IL-33; and the above method wherein the antagonist comprises a binding composition from an antibody that specifically binds IL-33 or a complex of IL-33, T1/ST2 and SIGIRR (IL-33R). In yet another embodiment, the present invention provides the above method wherein the binding composition from an antibody comprises a polyclonal antibody; a monoclonal antibody; a humanized antibody, or a fragment thereof; an Fab, Fv, or $F(ab')_2$ fragment; a peptide mimetic of an antibody; or a detectable label. Also provided is the above method, wherein wherein the antagonist comprises: a) a soluble IL-33R; b) a small molecule; or c) a nucleic acid; and the above method wherein the nucleic acid specifically hybridizes with a polynucleotide encoding IL-33; as well as the above method wherein the nucleic acid comprises anti-sense nucleic acid or small interference RNA (siRNA).

[0011] In another aspect, the present invention provides a method of modulating blood cell counts comprising administering an effective amount of an agonist or antagonist of IL-33; and the above method wherein the IL-33 agonist increases the counts of total white blood cells; neutrophils; lymphocytes; or eosinophils; as well as the above method wherein the IL-33 antagonist increases the count of platelets; and the above method wherein the IL-33 antagonist decreases the counts of total white blood cells; neutrophils; lymphocytes; or eosinophils.

[0012] Yet another aspect of the present invention provides a method of diagnosing the immune condition or disorder noted above, comprising contacting a binding composition to a biological sample, wherein the binding composition specifically binds to IL-33, and measuring or determining the specific binding of the binding composition to the biological sample. Also provided is a kit for the diagnosis of the immune condition or disorder of Claim 1, comprising a compartment and a binding composition that specifically binds to: IL-33; an IL-33R complex; a complex of IL-33 and IL-33R; or a nucleic acid encoding IL-33.

It is provided an antagonist of IL-33, wherein the antagonist comprises an antibody or an antigen-binding fragment thereof that specifically binds to IL-33.

It is also provided an antagonist of IL-33, wherein the antagonist comprises an antisense nucleic acid to IL-33; or a small interference RNA (siRNA) to IL-33.

It is further provided an agonist of IL-33, wherein the agonist comprises IL-33; or a nucleic acid encoding IL-33.

It is equally provided a kit comprising a compartment and an antibody that specifically binds to IL-33; or a nucleic acid probe for IL-33.

It is also provided the use of an antagonist of IL-33 in the preparation of a medicament for the treatment of a disorder or condition selected from the group consisting of asthma; allergy; multiple sclerosis; or arthritis, wherein the antagonist comprises an antibody or an antigen-binding fragment thereof that specifically binds to IL-33.

It is further provided the use of an antagonist of IL-33 in the preparation of a medicament for the treatment of a disorder or condition selected from the group consisting of: asthma; allergy; multiple sclerosis; or arthritis, wherein the antagonist comprises: an antisense nucleic acid to IL-33; or small interference RNA (siRNA) to IL-33.

It is equally provided the use of an agonist of IL-33 in the preparation of a medicament for the treatment of a cancer or tumor, wherein the agonist comprises: IL-33; or a nucleic acid encoding IL-33.

It is provided the use of an antagonist of IL-33 for treating a disorder or condition selected from the group consisting of: asthma; allergy; multiple sclerosis; or arthritis, wherein the antagonist comprises an antibody or an antigen-binding fragment thereof that specifically binds to IL-33.

It is further provided the use of an antagonist of IL-33 for treating a disorder or condition selected from the group consisting of: asthma; allergy; multiple sclerosis; or arthritis, wherein the antagonist comprises: an antisense nucleic acid to IL-33; or small interference RNA (siRNA) to IL-33.

It is also provided the use of an agonist of IL-33 for treating a cancer or tumor, wherein the agonist comprises: IL-33; or a nucleic acid encoding IL-33.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] Figure 1 shows IL-5 production in IL-33 + anti-IL-33 antibody treated mice versus IL-33 alone and isotype control antibody treated mice.

[0014] Figure 2 shows CIA disease scores for anti-IL-33 and isotype control treated mice.

[0015] Figure 3 shows the incidence of CIA in anti-IL-33 and isotype control treated mice.

[0016] Figure 4 shows the mean number of arthritic paws in mice treated with anti-IL-33 antibody or isotype control antibody.

[0017] Figure 5 shows the EAE disease scores of anti-IL-33 and isotype control treated mice.

[0018] Figure 6 shows the incidence of EAE in anti-IL-33 and isotype control treated mice.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0019] As used herein, including the appended claims, the singular forms of words such as "a," "an," and "the," include their corresponding plural references unless the context clearly dictates otherwise.

I. Definitions.

[0021] "Activation," "stimulation," and "treatment," as it applies to cells or to receptors, may have the same meaning, e.g., activation, stimulation, or treatment of a cell or receptor with a ligand, unless indicated otherwise by the context or explicitly. "Ligand" encompasses natural and synthetic ligands, e.g., cytokines, cytokine variants, analogues, muteins, and binding compositions derived from antibodies. "Ligand" also encompasses small molecules, e.g., peptide mimetics of cytokines and peptide mimetics of antibodies. "Activation" can refer to cell activation as regulated by internal mechanisms as well as by external or environmental factors. "Response," e.g., of a cell, tissue, organ, or organism, encompasses a change in biochemical or physiological behavior, e.g., concentration, density, adhesion, or migration within a biological compartment, rate of gene expression, or state of differentiation, where the change is correlated with activation, stimulation, or treatment, or with internal mechanisms such as genetic programming.

[0022] "Activity" of a molecule may describe or refer to the binding of the molecule to a ligand or to a receptor, to catalytic activity; to the ability to stimulate gene expression or cell signaling, differentiation, or maturation; to antigenic activity, to the modulation of activities of other molecules, and the like. "Activity" of a molecule may also refer to activity in modulating or maintaining cell-to-cell interactions, e.g., adhesion, or activity in maintaining a structure of a cell, e.g., cell membranes or cytoskeleton. "Activity" can also mean specific activity, e.g., [catalytic activity]/[mg protein], or [immunological activity]/[mg protein], concentration in a biological compartment, or the like. "Proliferative activity" encompasses an activity that promotes, that is necessary for, or that is specifically associated with, e.g., normal cell division, as well as cancer, tumors, dysplasia, cell transformation, metastasis, and angiogenesis.

[0023] "Administration" and "treatment," as it applies to the administration of an agonist or antagonist of IL-33, e.g., to an animal, human, experimental subject, cell, tissue, organ, or biological fluid, refers to contact of an exogenous pharmaceutical, therapeutic, diagnostic agent, compound, or composition to the animal, human, subject, cell, tissue, organ, or biological fluid. "Administration" and "treatment" can refer, e.g., to therapeutic, placebo, pharmacokinetic, diagnostic, research, and experimental methods. "Treatment of a cell" encompasses contact of a reagent to the cell, as well as contact of a reagent to a fluid, where the fluid is in contact with the cell. "Administration" and "treatment" also means *in*

vitro and *ex vivo* treatments, e.g., of a cell, by a reagent, diagnostic, binding composition, or by another cell. "Treatment," as it applies to a human, veterinary, or research subject, refers to therapeutic treatment, prophylactic or preventative measures, to research and diagnostic applications. "Treatment" as it applies to a human, veterinary, or research subject, or cell, tissue, or organ, encompasses contact of an IL-33 agonist or IL-33 antagonist to a human or animal subject, a cell, tissue, physiological compartment, or physiological fluid. "Treatment of a cell" also encompasses situations where the IL-33 agonist or IL-33 antagonist contacts IL-33 receptor (T1/ST2), e.g., in the fluid phase or colloidal phase, as well as situations where the agonist or antagonist contacts a fluid, e.g., where the fluid is in contact with a cell or receptor, but where it has not been demonstrated that the agonist or antagonist contacts the cell or receptor.

[0024] "Binding composition" refers to a molecule, small molecule, macromolecule, antibody, a fragment or analogue thereof, or soluble receptor, capable of binding to a target, where the target is, e.g., IL-33 or IL-33R. "Binding composition" also may refer to a complex of molecules, e.g., a non-covalent complex, to an ionized molecule, and to a covalently or non-covalently modified molecule, e.g., modified by phosphorylation, acylation, cross-linking, cyclization, or limited cleavage, which is capable of binding to a target. "Binding composition" may also refer to a molecule in combination with a stabilizer, excipient, salt, buffer, solvent, or additive, capable of binding to a target. "Binding" may be defined as an association of the binding composition with a target where the association results in reduction in the normal Brownian motion of the binding composition, in cases where the binding composition can be dissolved or suspended in solution.

[0025] "Conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences or, where the nucleic acid does not encode an amino acid sequence, to essentially identical nucleic acid sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids may encode any given protein.
As to amino acid sequences, one of skill will recognize that an individual substitution to a nucleic acid, peptide, polypeptide, or protein sequence which substitutes an amino acid or a small percentage of amino acids in the encoded sequence for a conserved amino acid is a "conservatively modified variant." Conservative substitution tables providing functionally

similar amino acids are well known in the art. An example of a conservative substitution is the exchange of an amino acid in one of the following groups for another amino acid of the same group (U.S. Pat. No. 5,767,063 issued to Lee, *et al.*; Kyte and Doolittle (1982) *J. Mol. Biol.* 157: 105-132):

(1) Hydrophobic: Norleucine, Ile, Val, Leu, Phe, Cys, or Met;

(2) Neutral hydrophilic: Cys, Ser, Thr;

(3) Acidic: Asp, Glu;

(4) Basic: Asn, Gln, His, Lys, Arg;

(5) Residues that influence chain orientation: Gly, Pro;

(6) Aromatic: Trp, Tyr, Phe;

(7) Small amino acids: Gly, Ala, Ser.

[0026] "Derived" can be used to describe, e.g., deriving the structure of a peptide, oligopeptide, or polypeptide from a parent peptide, oligopeptide, or polypeptide, such as an antibody. In this context, derived encompasses, e.g., peptide structures where the peptide has the same sequence as a sequence found within the parent, e.g., where the peptide is identical to the parent but with a truncation at the N-terminus, C-terminus, or both N- and C-termini of the parent, or with a truncation and a fusion, or with a fusion only. Derived also means that the peptide has the same sequence as found in the parent, but with conservative amino acid changes, or with deletions or insertions, where the deletions or insertions preserve a biological property in the peptide that is inherent in the parent. "Derived" encompasses situations where the peptide or polypeptide is synthesized using the parent as a starting compound, and where the peptide or polypeptide is synthesized *de novo*, using the structure of the parent as a guide.

[0027] "Effective amount" or "therapeutically effective amount," of the agonist or antagonist of the IL-33 of the present invention, means an amount sufficient to ameliorate a symptom or sign of a disorder or physiological condition or an amount sufficient to permit or facilitate a diagnosis of the disorder or physiological condition. An effective amount for a particular patient or veterinary subject may vary depending on factors such as the condition being treated, the overall health of the patient, the method route and dose of administration and the severity of side affects (see, e.g., U.S. Pat. No. 5,888,530 issued to Netti, *et al.*). An effective amount can be the maximal dose or dosing protocol that avoids significant side effects or toxic effects. The effect will result in an improvement of a

diagnostic measure, parameter, or detectable signal by at least 5%, usually by at least 10%, more usually at least 20%, most usually at least 30%, preferably at least 40%, more preferably at least 50%, most preferably at least 60%, ideally at least 70%, more ideally at least 80%, and most ideally at least 90%, where 100% is defined as the diagnostic parameter shown by a normal subject (see, e.g., Maynard, *et al.* (1996) *A Handbook of SOPs for Good Clinical Practice*, Interpharm Press, Boca Raton, FL; Dent (2001) *Good Laboratory and Good Clinical Practice*, Urch Publ., London, UK).

[0028] "Exogenous" refers to substances that are produced outside an organism, cell, or human body, depending on the context. "Endogenous" refers to substances that are produced within a cell, organism, or human body, depending on the context.

[0029] "Disorder" refers to a pathological state, or a condition that is correlated with or predisposes to a pathological state. "Infectious disorder" refers, e.g., to a disorder resulting from a microbe, bacterium, parasite, virus, and the like, as well as to an inappropriate, ineffective, or pathological immune response to the disorder. "Oncogenic disorder" encompasses a cancer, transformed cell, tumor, displasia, angiogenesis, metastasis, and the like, as well as to an inappropriate, ineffective, or pathological immune response to the disorder.

[0030] "Effective amount" means, e.g., an amount of an IL-33 agonist, IL-33 antagonist, binding compound or binding composition, sufficient to ameliorate a symptom or sign of a disorder, condition, or pathological state. "Effective amount" also relates to an amount of an IL-33 agonist, antagonist, or binding compound or composition, sufficient to allow or facilitate the diagnosis of a symptom or sign of a disorder, condition, or pathological state.

[0031] "Inhibitors" and "antagonists" or "activators" and "agonists" refer to inhibitory or activating molecules, respectively, e.g., for the activation of, e.g., a ligand, receptor, cofactor, a gene, cell, tissue, or organ. A modulator of, e.g., a gene, a receptor, a ligand, or a cell, is a molecule that alters an activity of the gene, receptor, ligand, or cell, where activity can be activated, inhibited, or altered in its regulatory properties. The modulator may act alone, or it may use a cofactor, e.g., a protein, metal ion, or small molecule. Inhibitors are compounds that decrease, block, prevent, delay activation, inactivate, desensitize, or down regulate, e.g., a gene, protein, ligand, receptor, or cell. Activators are compounds that increase, activate, facilitate, enhance activation, sensitize, or

up regulate, e.g., a gene, protein, ligand, receptor, or cell. An inhibitor may also be defined as a composition that reduces, blocks, or inactivates a constitutive activity. An "agonist" is a compound that interacts with a target to cause or promote an increase in the activation of the target. An "antagonist" is a compound that opposes the actions of an agonist. An antagonist prevents, reduces, inhibits, or neutralizes the activity of an agonist. An antagonist can also prevent, inhibit, or reduce constitutive activity of a target, e.g., a target receptor, even where there is no identified agonist.

[0032] To examine the extent of inhibition, for example, samples or assays comprising a given, e.g., protein, gene, cell, or organism, are treated with a potential activator or inhibitor and are compared to control samples without the inhibitor. Control samples, i.e., not treated with antagonist, are assigned a relative activity value of 100%. Inhibition is achieved when the activity value relative to the control is about 90% or less, typically 85% or less, more typically 80% or less, most typically 75% or less, generally 70% or less, more generally 65% or less, most generally 60% or less, typically 55% or less, usually 50% or less, more usually 45% or less, most usually 40% or less, preferably 35% or less, more preferably 30% or less, still more preferably 25% or less, and most preferably less than 25%. Activation is achieved when the activity value relative to the control is about 110%, generally at least 120%, more generally at least 140%, more generally at least 160%, often at least 180%, more often at least 2-fold, most often at least 2.5-fold, usually at least 5-fold, more usually at least 10-fold, preferably at least 20-fold, more preferably at least 40-fold, and most preferably over 40-fold higher.

[0033] Endpoints in activation or inhibition can be monitored as follows. Activation, inhibition, and response to treatment, e.g., of a cell, physiological fluid, tissue, organ, and animal or human subject, can be monitored by an endpoint. The endpoint may comprise a predetermined quantity or percentage of, e.g., an indicia of inflammation, oncogenicity, or cell degranulation or secretion, such as the release of a cytokine, toxic oxygen, or a protease. The endpoint may comprise, e.g., a predetermined quantity of ion flux or transport; cell migration; cell adhesion; cell proliferation; potential for metastasis; cell differentiation; and change in phenotype, e.g., change in expression of gene relating to inflammation, apoptosis, transformation, cell cycle, or metastasis (see, e.g., Knight (2000) *Ann. Clin. Lab. Sci.* 30:145-158; Hood and Cheresh (2002) *Nature Rev. Cancer* 2:91-100; Timme, *et al.* (2003) *Curr. Drug Targets* 4:251-261; Robbins and Itzkowitz (2002) *Med.*

Clin. North Am. 86:1467-1495; Grady and Markowitz (2002) *Annu. Rev. Genomics Hum. Genet.* 3:101-128; Bauer, *et al.* (2001) *Glia* 36:235-243; Stanimirovic and Satoh (2000) *Brain Pathol.* 10:113-126).

[0034] An endpoint of inhibition is generally 75% of the control or less, preferably 50% of the control or less, more preferably 25% of the control or less, and most preferably 10% of the control or less. Generally, an endpoint of activation is at least 150% the control, preferably at least two times the control, more preferably at least four times the control, and most preferably at least 10 times the control.

[0035] "Expression" refers to a measure of mRNA or polypeptide encoded by a specific gene. Units of expression may be a measure of, e.g., the number of molecules of mRNA or polypeptide/mg protein, the number of molecules of mRNA or polypeptide/cell, in measurements of expression by cell, tissue, cell extract, or tissue extract. The units of expression may be relative, e.g., a comparison of signal from control and experimental mammals or a comparison of signals with a reagent that is specific for the mRNA or polypeptide versus with a reagent that is non-specific.

[0036] "Hybridization" that is specific or selective typically occurs when there is at least about 55% homology over a stretch of at least about 30 nucleotides, preferably at least about 75% over a stretch of about 25 nucleotides, and most preferably at least about 90% over about 20 nucleotides (see, e.g., Kanehisa (1984) *Nucleic Acids Res.* 12:203-213). Hybridization under stringent conditions, e.g., of a first nucleic acid to a second nucleic acid, are those that: (1) Employ low ionic strength and high temperature for washing, for example, 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) Employ during hybridization a denaturing agent, such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serum albumin/0.1% Ficoll® (Sigma-Aldrich, St. Louis, MO)/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42°C.; (3) Employ 50% formamide, 5 X SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 X Denhardt's solution, sonicated salmon sperm DNA (50 ng/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 X SSC and 0.1% SDS; or (4) Employ a buffer of 10% dextran sulfate, 2 X SSC (sodium chloride/sodium citrate), and 50% formamide at 55°C, followed by a high-stringency wash consisting of 0.1 X SSC containing EDTA at 55°C (U.S. Pat. No. 6,387,657 issued to Botstein, *et al.*).

[0037] Stringent conditions for hybridization of nucleic acids are a function of salt, temperature, organic solvents, and chaotropic agents. Stringent temperature conditions will usually include temperatures in excess of about 30°C, more usually in excess of about 37°C, typically in excess of about 45°C, more typically in excess of about 50°C, preferably in excess of about 65°C, and more preferably in excess of about 70°C. Stringent salt conditions will ordinarily be less than about 1 M, more ordinarily less than about 500 mM, usually less than about 400 mM, more usually less than about 300 mM, typically less than about 200 mM, preferably less than about 100 mM, and more preferably less than about 80 mM, even down to less than about 20 mM. However, the combination of parameters is more important than the measure of any single parameter (Wetmur and Davidson (1968) *J. Mol. Biol.* 31:349-370).

[0038] "Immune condition" or "immune disorder" encompasses, e.g., pathological inflammation, an inflammatory disorder, and an autoimmune disorder or disease. "Immune condition" also refers to infections, persistent infections, and proliferative conditions, such as cancer, tumors, and angiogenesis, including infections, tumors, and cancers that resist irradication by the immune system. "Cancerous condition" includes, e.g., cancer, cancer cells, tumors, angiogenesis, and precancerous conditions such as dysplasia.

[0039] "Inflammatory disorder" means a disorder or pathological condition where the pathology results, in whole or in part, from, e.g., a change in number, change in rate of migration, or change in activation, of cells of the immune system. Cells of the immune system include, e.g., T cells, B cells, monocytes or macrophages, antigen presenting cells (APCs), dendritic cells, microglia, NK cells, NKT cells, neutrophils, eosinophils, mast cells, or any other cell specifically associated with the immunology, for example, cytokine-producing endothelial or epithelial cells.

[0040] "Inflammatory disorder" means a disorder or pathological condition where the pathology results, in whole or in part, from an increase in the number and/or increase in activation of cells of the immune system, e.g., of T cells, B cells, monocytes or macrophages, alveolar macrophages, dendritic cells, NK cells, NKT cells, neutrophils, eosinophils, or mast cells.

[0041] "IL-33 Receptor", "IL-33R", or "IL-33R complex" as used herein shall mean the association of two IL-1R family members, T1/ST2 and SIGIRR to form receptor complex responsive to stimulation with IL-33.

[0042] "Ligand" refers, e.g., to a small molecule, peptide, polypeptide, and membrane associated or membrane-bound molecule, or complex thereof, that can act as an agonist or antagonist of a receptor. "Ligand" also encompasses an agent that is not an agonist or antagonist, but that can bind to the receptor without significantly influencing its biological properties, e.g., signaling or adhesion. Moreover, "ligand" includes a membrane-bound ligand that has been changed, e.g., by chemical or recombinant methods, to a soluble version of the membrane-bound ligand. By convention, where a ligand is membrane-bound on a first cell, the receptor usually occurs on a second cell. The second cell may have the same or a different identity as the first cell. A ligand or receptor may be entirely intracellular, that is, it may reside in the cytosol, nucleus, or some other intracellular compartment. The ligand or receptor may change its location, e.g., from an intracellular compartment to the outer face of the plasma membrane. The complex of a ligand and receptor is termed a "ligand receptor complex." Where a ligand and receptor are involved in a signaling pathway, the ligand occurs at an upstream position and the receptor occurs at a downstream position of the signaling pathway.

[0043] A "first polypeptide chain" and a "second polypeptide chain" refers to two polypeptide chains not linked together by way of a classical peptide bond. Typically, the first polypeptide chain comprises an N-terminus and C-terminus, and the second polypeptide chain comprises another N-terminus and another C-terminus, that is, altogether there are two N-termini and two C-termini. The first polypeptide chain can be encoded by a first vector, while the second polypeptide chain can be encoded by a second vector. The first polypeptide chain and second polypeptide chain can be encoded by one vector, where a first promoter can be operably linked with the first polypeptide chain and a second promoter can be operably linked with the second polypeptide chain or, in another embodiment, expression of both the first and second polypeptide chains can be operably linked to the same promoter.

[0044] "Sensitivity," e.g., sensitivity of receptor to a ligand, means that binding of a ligand to the receptor results in a detectable change in the receptor, or in events or molecules specifically associated with the receptor, e.g., conformational change, phosphorylation, nature or quantity of proteins associated with the receptor, or change in genetic expression mediated by or associated with the receptor.

[0045] "Small molecules" are provided for the treatment of physiology and disorders of tumors and cancers. "Small molecule" is defined as a molecule with a molecular weight that is less than 10 kD, typically less than 2 kD, and preferably less than 1 kD. Small molecules include, but are not limited to, inorganic molecules, organic molecules, organic molecules containing an inorganic component, molecules comprising a radioactive atom, synthetic molecules, peptide mimetics, and antibody mimetics. As a therapeutic, a small molecule may be more permeable to cells, less susceptible to degradation, and less apt to elicit an immune response than large molecules. Small molecules, such as peptide mimetics of antibodies and cytokines, as well as small molecule toxins are described (see, e.g., Casset, *et al.* (2003) *Biochem. Biophys. Res. Commun.* 307:198-205; Muyldermans (2001) *J. Biotechnol.* 74:277-302; Li (2000) *Nat. Biotechnol.* 18:1251-1256; Apostolopoulos, *et al.* (2002) *Curr. Med. Chem.* 9:411-420; Monfardini, *et al.* (2002) *Curr. Pharm. Des.* 8:2185-2199; Domingues, *et al.* (1999) *Nat. Struct. Biol.* 6:652-656; Sato and Sone (2003) *Biochem. J.* 371:603-608; U.S. Patent No. 6,326,482 issued to Stewart, *et al*).

[0046] "Soluble receptor" refers to receptors that are water-soluble and occur, e.g., in extracellular fluids, intracellular fluids, or weakly associated with a membrane. Soluble receptor further refers to receptors that are engineered to be water soluble. For T1/ST2, the soluble or extracellular domain is defined as residues 1-337 of SEQ ID NO: 6 (human) and residues 1-342 of SEQ ID NO: 8 (mouse). For SIGIRR, the soluble or extracellular domain is defined as residues 1-118 of SEQ ID NO: 10 (human) and residues 1-117 of SEQ ID NO: 12 (mouse).

[0047] "Specificity of binding," "selectivity of binding," and the like, refer to a binding interaction between a predetermined ligand and a predetermined receptor that enables one to distinguish between the predetermined ligand and other ligands, or between the predetermined receptor and other receptors. "Specifically" or "selectively" binds, when referring to a ligand/receptor, antibody/antigen, or other binding pair, indicates a binding reaction that is determinative of the presence of the protein in a heterogeneous population of proteins and other biologics. Thus, under designated conditions, a specified ligand binds to a particular receptor and does not bind in a significant amount to other proteins present in the sample. The antibody, or binding composition derived from the antigen-binding site of an antibody, binds to its antigen with an affinity that is at least two fold greater, preferably at least ten times greater, more preferably at least 20-times greater, and most preferably at least

100-times greater than the affinity to any other antigen. In a preferred embodiment the antibody will have an affinity that is greater than about 10^9 liters/mol (see, e.g., Munsen, *et al.* (1980) *Analyt. Biochem.* 107:220-239).

II. General.

[0048] The present invention provides methods for the modulation or treatment of a number of immune conditions and disorders. In particular, the present invention provides agonists and antagonists of IL-33 for the treatment and diagnosis of, e.g., asthma, allergies, arthritis, and response to intracellular pathogens, such as parasites, and response to disorders involving granulomas, e.g., tuberculosis, sarcoidosis, and Crohn's disease.

[0049] Naïve T cells appear not to express T1/ST2 on their surface, whereas expression is induced after contact with antigens on differentiated TH2 effector cells. T1/ST2 has been used as a marker for TH2-type T cells. T1/ST2 is also expressed on mast cells and fibroblasts

[0050] Studies with T1/ST2 knockout mice seems to suggest that T1/ST2 does not play a part in the differentiation of naïve $CD4^+$ T cells to TH2-type T cells, though these results appear to be a function of the nature of the assays used, e.g., which pathogenic organism is used in challenge studies, or which phase of TH2-response is studied. Evidence also suggests a role for T1/ST2 in early events in TH2-response (see, e.g., Kropf, et al. (2002) Infect. Immunity 70:5512-5520; Hoshino, et al. (1999) J. Exp. Med. 190:1541-1547; Senn, et al. (2000) Eur. J. Immunol. 30:1929-1938; Townsend, et al. (2000) J. Exp. Med. 191:1069-1075).

[0051] Anti-T1/ST2 antibodies have been used in a number of studies addressing the role of T1/ST2 in immune function, while other studies have examined T1/ST2 expression animal models for immune response. Treatment with anti-T1/ST2 antibodies resulted in decreased TH2-type immune responses. The antibody inhibited eosinophil infiltration, IL-5 production, and IgE-production. Infections by *Schistosoma* provoked an up-regulation of T1/ST2, e.g., as determined by assessing expression in lung and liver granulomas. Animal models for asthma, e.g., treatment with house dust mite extract or with ovalbumin, resulted in increased expression of T1/ST2 on $CD4^+$ T cells, indicating a role for T1/ST2 in allergic or asthmatic responses. Studies with BALB/c mice revealed that treating with anti-T1/ST2 antibody induced higher TH1-type response, enhancing the ability of $CD4^+$ T cells to

respond to IL-12. Anti-T1/ST2 antibodies also reduce lesions due to *Leishmania major* infections, and reduced expression of TH2-type cytokines. An animal model of arthritis (collagen induced arthritis; CIA) was exacerbated by anti-T1/ST2 antibodies. In particular, T1/ST2 functions in early events in the generation of TH2-type responses. Chronic exposure to various allergens resulted in increased expression of T1/ST2 on $CD4^+$ T cells. T1/ST2 plays a role in mediating innate response, as anti-T1/ST2 antibodies exacerbate the toxic effects of lipopolysaccharide (LPS). Antibodies to T1/ST2 also modulated immune response to viruses, e.g., respiratory syncytial virus (see, e.g., Xu, et al. (1998) J. Exp. Med. 187:787-794; Lohning, et al. (1998) Proc. Natl. Acad. Sci. USA 95:6930-6935; Coyle, et al. (1999) J. Exp. Med. 190:895-902; Lohning, et al. (1999) J. Immunol. 162:3882-3889; Johnson, et al. (2003) Am. J. Respir. Crit. Care Med. 169:378-385; Kropf, et al. (2003) Infect. Immunity 71:1961-1971; Xu, et al. (1998) J. Exp. Med. 187:787-794; Kropf, et al. (2002) Eur. J. Immunol. 32:2450-2459; Swirski, et al. (2002) J. Immunol. 169:3499-3506; Sweet, et al. (2001) J. Immunol. 166:6633-6639; Walzl, et al. (2001) J. Exp. Med. 193:785-792.

[0052] IL-1 family members typically bind to a heterodimeric members of the IL-1 receptor family. It was shown that another known IL-1R family member, SIGIRR (single Ig IL-1 receptor related protein), complexes with T1/ST2 to form the functional receptor complex for IL-33. SIGIRR was originally found as an orphan IL-1R member (see, e.g., Garlanda, et al. (2004) Proc. Natl. Acad. Sci. 101:3522-3526; Clark, et al. (2003) Genome Res. 13:2265-2270; Thomassen et al. (1999) Cytokine 11:389-399; GenBank Accession No. NP_068577; GenBank Accession No. NM_021805; GenBank Accession No. NP_075546; and GenBank Accession No. NM_0230459). SIGIRR is a widely expressed IL-1R member.

[0053] In precipitation experiments using biotinylated mature human IL-33 (residues 112-270 of SEQ ID NO: 2), T1/ST2-Fc fusion, and SIGIRR-Fc fusion, it was shown that IL-33 could bind both receptor fusion proteins, however, the binding of IL-33 to SIGIRR was weaker as compared to IL-33 and T1/ST2 binding. To test the signaling capabilities of either or both receptors, an NF-κB-dependent assay was run. Co-expression of both T1/ST2 and SIGIRR was both necessary and sufficient to activate NF-κB signaling and MAP kinase upon stimulation with IL-33. Activation of JNK kinases was also observed.

III. Agonists, Antagonists, and Binding Compositions.

[0054] The present invention provides agonists and antagonists of IL-33, including binding compositions that specifically bind to IL-33 or to IL-33 receptor complex (T1/ST2 and SIGIRR). Binding compositions include antibodies, antibody fragments, and soluble receptors. The present invention contemplates blocking antibodies that bind to IL-33 or to IL-33R, or agonistic antibodies that stimulate signaling via the IL-33R complex. The binding compositions of the present invention also include nucleic acids that specifically hybridize to nucleic acids encoding IL-33 or IL-33R, e.g., anti-sense nucleic acids and small interference RNA (siRNA) Anti-idiotypic antibodies may also be used. Human IL-33 is disclosed by GenBank NM_033439. Regions of increased antigenicity, suitable for preparing anti-IL-33 antibodies, occur at, e.g., amino acids 1-23; 30-38; 61-78; 84-93; 99-106; 127-133; 139-144; 148-158; 166-180; 196-204; 231-237; and 252-257, of GenBank NM_033439, according to a Parker plot using Vector NTI® Suite (Informax, Inc, Bethesda, MD).

[0055] Receptors based on these extracellular regions are not limited by these exact N-terminal and C-terminal amino acids, but may be longer or shorter, e.g., by one, two, three, or more amino acids, as long as the ligand binding properties are substantially maintained. Fusion proteins based on the soluble receptors are also contemplated, e.g., for facilitating purification or stability or for providing a functional domain, e.g., a toxic polypeptide.

[0056] Monoclonal, polyclonal, and humanized antibodies can be prepared (see, e.g., Sheperd and Dean (eds.) (2000) *Monoclonal Antibodies*, Oxford Univ. Press, New York, NY; Kontermann and Dubel (eds.) (2001) *Antibody Engineering*, Springer-Verlag, New York; Harlow and Lane (1988) *Antibodies A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 139-243; Carpenter, *et al.* (2000) *J. Immunol.* 165:6205; He, *et al.* (1998) *J. Immunol.* 160:1029; Tang, *et al.* (1999) *J. Biol. Chem.* 274:27371-27378; Baca, *et al.* (1997) *J. Biol. Chem.* 272:10678-10684; Chothia, *et al.* (1989) *Nature* 342:877-883; Foote and Winter (1992) *J. Mol. Biol.* 224:487-499; U.S. Pat. No. 6,329,511 issued to Vasquez, *et al.*). Muteins and variants of antibodies and soluble receptors are contemplated, e.g., pegylation or mutagenesis to remove or replace deamidating Asn residues.

[0057] Purification of antigen is not necessary for the generation of antibodies. Immunization can be performed by DNA vector immunization, see, e.g., Wang, *et al.* (1997) *Virology* 228:278-284. Alternatively, animals can be immunized with cells bearing the antigen of interest. Splenocytes can then be isolated from the immunized animals, and the splenocytes can fused with a myeloma cell line to produce a hybridoma (Meyaard, *et al.* (1997) *Immunity* 7:283-290; Wright, *et al.* (2000) *Immunity* 13:233-242; Preston, *et al.* (1997) *Eur. J. Immunol.* 27:1911-1918). Resultant hybridomas can be screened for production of the desired antibody by functional assays or biological assays, that is, assays not dependent on possession of the purified antigen. Immunization with cells may prove superior for antibody generation than immunization with purified antigen (Kaithamana, *et al.* (1999) *J. Immunol.* 163:5157-5164).

[0058] Antibodies will usually bind with at least a K_D of about 10^{-3} M, more usually at least 10^{-6} M, typically at least 10^{-7} M, more typically at least 10^{-8} M, preferably at least about 10^{-9} M, and more preferably at least 10^{-10} M, and most preferably at least 10^{-11} M (see, e.g., Presta, *et al.* (2001) *Thromb. Haemost.* 85:379-389; Yang, *et al.* (2001) *Crit. Rev. Oncol. Hematol.* 38:17-23; Carnahan, *et al.* (2003) *Clin. Cancer Res.* (Suppl.) 9:3982s-3990s).

[0059] Soluble receptors comprising the extracellular domains of the IL-33 receptor complex (T1/ST2 and SIGIRR) can be prepared, as the cytoplasmic, transmembrane, and extracellular regions of each of the subunits have been identified (see, e.g., Lecart, et al. (2002) Eur. J. Immunol. 32:2979-2987; Mitcham, et al. (1996) *J. Biol. Chem.* 271:5777-5783; and the Sequence Listing below).

[0060] Soluble receptors can be prepared and used according to standard methods (see, e.g., Jones, *et al.* (2002) *Biochim. Biophys. Acta* 1592:251-263; Prudhomme, *et al.* (2001) *Expert Opinion Biol. Ther.* 1:359-373; Fernandez-Botran (1999) *Crit. Rev. Clin. Lab Sci.* 36:165-224). Also provided are compositions for siRNA interference (see, e.g., Arenz and Schepers (2003) *Naturwissenschaften* 90:345-359; Sazani and Kole (2003) *J. Clin. Invest.* 112:481-486; Pirollo, *et al.* (2003) *Pharmacol. Therapeutics* 99:55-77; Wang, *et al.* (2003) *Antisense Nucl. Acid Drug Devel.* 13:169-189).

IV. Therapeutic Compositions, Methods.

[0061] The present invention provides methods for treating and diagnosing innate response, asthma, allergies, and arthritis.

[0062] To prepare pharmaceutical or sterile compositions including an agonist or antagonist of IL-33, the reagent is mixed with a pharmaceutically acceptable carrier or excipient. Formulations of therapeutic and diagnostic agents can be prepared by mixing with physiologically acceptable carriers, excipients, or stabilizers in the form of, e.g., lyophilized powders, slurries, aqueous solutions, lotions, or suspensions (see, e.g., Hardman, *et al.* (2001) *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, McGraw-Hill, New York, NY; Gennaro (2000) *Remington: The Science and Practice of Pharmacy*, Lippincott, Williams, and Wilkins, New York, NY; Avis, *et al.* (eds.) (1993) *Pharmaceutical Dosage Forms: Parenteral Medications*, Marcel Dekker, NY; Lieberman, *et al.* (eds.) (1990) *Pharmaceutical Dosage Forms: Tablets*, Marcel Dekker, NY; Lieberman, *et al.* (eds.) (1990) *Pharmaceutical Dosage Forms: Disperse Systems*, Marcel Dekker, NY; Weiner and Kotkoskie (2000) *Excipient Toxicity and Safety*, Marcel Dekker, Inc., New York, NY).

[0063] Selecting an administration regimen for a therapeutic depends on several factors, including the serum or tissue turnover rate of the entity, the level of symptoms, the immunogenicity of the entity, and the accessibility of the target cells in the biological matrix. Preferably, an administration regimen maximizes the amount of therapeutic delivered to the patient consistent with an acceptable level of side effects. Accordingly, the amount of biologic delivered depends in part on the particular entity and the severity of the condition being treated. Guidance in selecting appropriate doses of antibodies, cytokines, and small molecules are available (see, e.g., Wawrzynczak (1996) *Antibody Therapy*, Bios Scientific Pub. Ltd, Oxfordshire, UK; Kresina (ed.) (1991) *Monoclonal Antibodies, Cytokines and Arthritis*, Marcel Dekker, New York, NY; Bach (ed.) (1993) *Monoclonal Antibodies and Peptide Therapy in Autoimmune Diseases*, Marcel Dekker, New York, NY; Baert, *et al.* (2003) *New Engl. J. Med.* 348:601-608; Milgrom, *et al.* (1999) *New Engl. J. Med.* 341:1966-1973; Slamon, *et al.* (2001) *New Engl. J. Med.* 344:783-792; Beniaminovitz, *et al.* (2000) *New Engl. J. Med.* 342:613-619; Ghosh, *et al.* (2003) *New Engl. J. Med.* 348:24-32; Lipsky, *et al.* (2000) *New Engl. J. Med.* 343:1594-1602).

[0064] Antibodies, antibody fragments, and cytokines can be provided by continuous infusion, or by doses at intervals of, e.g., one day, one week, or 1-7 times per week. Doses may be provided intravenously, subcutaneously, topically, orally, nasally, rectally, intramuscular, intracerebrally, or by inhalation. A preferred dose protocol is one involving the maximal dose or dose frequency that avoids significant undesirable side effects. A total weekly dose is generally at least 0.05 μg/kg body weight, more generally at least 0.2 μg/kg, most generally at least 0.5 μg/kg, typically at least 1 μg/kg, more typically at least 10 μg/kg, most typically at least 100 μg/kg, preferably at least 0.2 mg/kg, more preferably at least 1.0 mg/kg, most preferably at least 2.0 mg/kg, optimally at least 10 mg/kg, more optimally at least 25 mg/kg, and most optimally at least 50 mg/kg (see, e.g., Yang, *et al.* (2003) *New Engl. J. Med.* 349:427-434; Herold, *et al.* (2002) *New Engl. J. Med.* 346:1692-1698; Liu, *et al.* (1999) *J. Neurol. Neurosurg. Psych.* 67:451-456; Portielji, *et al.* (20003) *Cancer Immunol. Immunother.* 52:133-144). The desired dose of a small molecule therapeutic, e.g., a peptide mimetic, natural product, or organic chemical, is about the same as for an antibody or polypeptide, on a moles/kg body weight basis. The desired plasma concentration of a small molecule therapeutic is about the same as for an antibody, on a moles/kg body weight basis.

[0065] An effective amount for a particular patient may vary depending on factors such as the condition being treated, the overall health of the patient, the method route and dose of administration and the severity of side affects (see, e.g., Maynard, *et al.* (1996) *A Handbook of SOPs for Good Clinical Practice*, Interpharm Press, Boca Raton, FL; Dent (2001) *Good Laboratory and Good Clinical Practice*, Urch Publ., London, UK).

[0066] Typical veterinary, experimental, or research subjects include monkeys, dogs, cats, rats, mice, rabbits, guinea pigs, horses, and humans.

[0067] Determination of the appropriate dose is made by the clinician, e.g., using parameters or factors known or suspected in the art to affect treatment or predicted to affect treatment. Generally, the dose begins with an amount somewhat less than the optimum dose and it is increased by small increments thereafter until the desired or optimum effect is achieved relative to any negative side effects. Important diagnostic measures include those of symptoms of, e.g., the inflammation or level of inflammatory cytokines produced. Preferably, a biologic that will be used is derived from the same species as the animal targeted for treatment, thereby minimizing a humoral response to the reagent.

[0068] Methods for co-administration or treatment with a second therapeutic agent, e.g., a cytokine, steroid, chemotherapeutic agent, antibiotic, or radiation, are well known in the art (see, e.g., Hardman, *et al.* (eds.) (2001) *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 10th ed., McGraw-Hill, New York, NY; Poole and Peterson (eds.) (2001) *Pharmacotherapeutics for Advanced Practice:A Practical Approach*, Lippincott, Williams & Wilkins, Phila., PA; Chabner and Longo (eds.) (2001) *Cancer Chemotherapy and Biotherapy*, Lippincott, Williams & Wilkins, Phila., PA). An effective amount of therapeutic will decrease the symptoms typically by at least 10%; usually by at least 20%; preferably at least about 30%; more preferably at least 40%, and most preferably by at least 50%.

[0069] The route of administration is by, e.g., topical or cutaneous application, injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial, intracerebrospinal, intralesional, or pulmonary routes, or by sustained release systems or an implant (see, e.g., Sidman *et al.* (1983) *Biopolymers* 22:547-556; Langer, *et al.* (1981) *J. Biomed. Mater. Res.* 15:167-277; Langer (1982) *Chem. Tech.* 12:98-105; Epstein, *et al.* (1985) *Proc. Natl. Acad. Sci. USA* 82:3688-3692; Hwang, *et al.* (1980) *Proc. Natl. Acad. Sci. USA* 77:4030-4034; U.S. Pat. Nos. 6,350466 and 6,316,024).

V. Kits and Diagnostic Reagents.

[0070] Diagnostic methods for inflammatory disorders, e.g., psoriasis, Crohn's disease, rheumatoid arthritis, asthma or allergy, atherosclerosis, and cancers, based on antibodies, nucleic acid hybridization, and the PCR method, are available.

[0071] This invention provides polypeptides of IL-33, fragments thereof, nucleic acids of IL-33, and fragments thereof, in a diagnostic kit, e.g., for the diagnosis of viral disorders, including of influenza A, and viral disorders of the respiratory tract and of mucosal tissues. Also provided are binding compositions, including antibodies or antibody fragments, for the detection of IL-33, and metabolites and breakdown products thereof. Typically, the kit will have a compartment containing either a IL-33 polypeptide, or an antigenic fragment thereof, a binding composition thereto, or a nucleic acid, such as a nucleic acid probe, primer, or molecular beacon (see, e.g., Rajendran, *et al.* (2003) *Nucleic Acids Res.* 31:5700-5713; Cockerill (2003) *Arch. Pathol. Lab. Med.* 127:1112-1120;

Zammatteo, *et al.* (2002) *Biotech. Annu. Rev.* 8:85-101; Klein (2002) *Trends Mol. Med.* 8:257-260).

[0072] A method of diagnosis can comprise contacting a sample from a subject, e.g., a test subject, with a binding composition that specifically binds to a polypeptide or nucleic acid of IL-33 or IL-33 receptor. The method can further comprise contacting a sample from a control subject, normal subject, or normal tissue or fluid from the test subject, with the binding composition. Moreover, the method can additionally comprise comparing the specific binding of the composition to the test subject with the specific binding of the composition to the normal subject, control subject, or normal tissue or fluid from the test subject. Expression or activity of a test sample or test subject can be compared with that from a control sample or control subject. A control sample can comprise, e.g., a sample of non-affected or non-inflamed tissue in a patient suffering from an immune disorder. Expression or activity from a control subject or control sample can be provided as a predetermined value, e.g., acquired from a statistically appropriate group of control subjects.

[0073] The kit may comprise, e.g., a reagent and a compartment, a reagent and instructions for use, or a reagent with a compartment and instructions for use. The reagent may comprise an agonist or antagonist of IL-33, or an antigenic fragment thereof, a binding composition, or a nucleic acid in a sense and/or anti-sense orientation. A kit for determining the binding of a test compound, e.g., acquired from a biological sample or from a chemical library, can comprise a control compound, a labeled compound, and a method for separating free labeled compound from bound labeled compound. The control compound can comprise a segment of the polypeptide of IL-33 or IL-33 receptor or a nucleic acid encoding IL-33 or IL-33 receptor. The segment can comprise zero, one, two, or more antigenic fragments.

[0074] A composition that is "labeled" is detectable, either directly or indirectly, by spectroscopic, photochemical, biochemical, immunochemical, isotopic, or chemical methods. For example, useful labels include ^{32}P, ^{33}P, ^{35}S, ^{14}C, ^{3}H, ^{125}I, stable isotopes, fluorescent dyes, electron-dense reagents, substrates, epitope tags, or enzymes, e.g., as used in enzyme-linked immunoassays, or fluorettes (Rozinov and Nolan (1998) *Chem. Biol.* 5:713-728).

[0075] Diagnostic assays can be used with biological matrices such as live cells, cell extracts, cell lysates, fixed cells, cell cultures, bodily fluids, or forensic samples.

Conjugated antibodies useful for diagnostic or kit purposes, include antibodies coupled to dyes, isotopes, enzymes, and metals, see, e.g., Le Doussal, *et al.* (1991) *New Engl. J. Med.* 146:169-175; Gibellini, *et al.* (1998) *J. Immunol.* 160:3891-3898; Hsing and Bishop (1999) *New Engl. J. Med.* 162:2804-2811; Everts, *et al.* (2002) *New Engl. J. Med.* 168:883-889. Various assay formats exist, such as radioimmunoassays (RIA), ELISA, and lab on a chip (U.S. Pat. Nos. 6,176,962 and 6,517,234).

[0076] Gene expression data is useful tool in the diagnosis and treatment of diseases and pathological conditions (see, e.g., Li and Wong (2001) *Genome Informatics* 12:3-13; Lockhart, *et al.* (1996) *Nature Biotechnol.* 14:1675-1680; Homey, *et al.* (2000) *J. Immunol.* 164:3465-3470; Debets, *et al.* (2000) *J. Immunol.* 165:4950-4956).

VI. Uses.

[0077] The present invention provides methods for the treatment and diagnosis of inflammatory and immune disorders, including inappropriate or ineffective response to infection. Provided are methods relating to, e.g., asthma, allergies, arthritis, disorders involving eosinophilic inflammation, and disorders involving pathogenic or ineffective TH2-type response.

[0078] The present invention provides methods for stimulating immune defense against bacteria, parasites, and viruses, intracellular pathogens, and cancers and tumors. Provided are methods for the treatment of intracellular bacteria. Intracellular bacterial species include *Salmonella sp.*, *Shigella sp.*, *Listeria sp.*, *Francisella sp.*, *Mycobacteria sp.*(tuberculosis; leprosy), *Legionella sp.*, *Rickettsia sp.*, *Orienta sp.*, *Ehrlichia sp.*, *Anaplasma sp.*, *Neorickettsia sp.*, *Chlamydia sp.*, and *Coxiella sp.* Additionally, IFNgamma mediates response to parasites, e.g., *Plasmodia sp.* (malaria), *Toxoplasma sp.*, *Leishmania sp.*, *Trypanosoma sp.*, and *Cryptosporidium sp.* Provided are methods for treating viruses, e.g., HIV, orthopoxviruses, such as variola virus and vaccinia virus (smallpox), and herpesviruses, including alphaherpesviruses, e.g., *Herpes Simplex* virus, and betaherpesviruses, e.g., *Cytomegalovirus*. Also provided are methods for the treatment of chronic inflammatory disorders (see, e.g., Kent, *et al.* (2000) *Vaccine* 18:2250-2256; Ismail, *et al.* (2002) *FEMS Microbiol. Lett.* 207:111-120; Kaufmann (2001) *Nature Revs. Immunol.* 1:20-30; Goebel and Gross (2001) *TRENDS Microbiol.* 9:267-273; Heussler, *et al.* (2001) *Int. J. Parasitol.* 31:1166-1176; Luder, *et al.* (2001) Carsten, *et al.* (2001) *TRENDS*

Parasitol. 17:480-486; Rook, *et al.* (2001) *Eur. Resp. J.* 17:537-557; Stenger and Rollinghoff (2001) *Ann. Rheum. Dis.* 60:iii43-iii46; Haas, *et al.* (2002) *Am. J. Dermatopathol.* 24:319-323; Dorman and Holland (2000) *Cytokine Growth Factor Revs.* 11:321-333; Smith, *et al.* (2002) *J. Gen. Virol.* 83 (Pt. 12) 2915-2931; Cohrs and Gilden (2001) *Brain Pathol.* 11:465-474; Tannenbaum and Hamilton (2002) *Sem. Cancer Biol.* 10:113-123; Ikeda, *et al.* (2002) *Cytokine Growth Factor Revs.* 13:95-109; Klimp, *et al.* (2002) *Crit. Rev. Oncol. Hematol.* 44:143-161; Frucht, *et al.* (2001) *TRENDS Immunol.* 22:556-560).

[0079] The present invention provides methods of treating or diagnosing a proliferative condition or disorder, e.g., cancer of the uterus, cervix, breast, prostate, testes, penis, gastrointestinal tract, e.g., esophagus, oropharynx, stomach, small or large intestines, colon, or rectum, kidney, renal cell, bladder, bone, bone marrow, skin, head or neck, skin, liver, gall bladder, heart, lung, pancreas, salivary gland, adrenal gland, thyroid, brain, ganglia, central nervous system (CNS) and peripheral nervous system (PNS), and immune system, e.g., spleen or thymus. The present invention provides methods of treating, e.g., immunogenic tumors, non-immunogenetic tumors, dormant tumors, virus-induced cancers, e.g., epithelial cell cancers, endothelial cell cancers, squamous cell carcinomas, papillomavirus, adenocarcinomas, lymphomas, carcinomas, melanomas, leukemias, myelomas, sarcomas, teratocarcinomas, chemically-induced cancers, metastasis, and angiogenesis. The invention also contemplates reducing tolerance to a tumor cell or cancer cell antigen, e.g., by modulating activity of a regulatory T cell (Treg) (see, e.g., Ramirez-Montagut, *et al.* (2003) *Oncogene* 22:3180-3187; Sawaya, *et al.* (2003) *New Engl. J. Med.* 349:1501-1509; Farrar, *et al.* (1999) *J. Immunol.* 162:2842-2849; Le, *et al.* (2001) *J. Immunol.* 167:6765-6772; Cannistra and Niloff (1996) *New Engl. J. Med.* 334:1030-1038; Osborne (1998) *New Engl. J. Med.* 339:1609-1618; Lynch and Chapelle (2003) *New Engl. J. Med.* 348:919-932; Enzinger and Mayer (2003) *New Engl. J. Med.* 349:2241-2252; Forastiere, *et al.* (2001) *New Engl. J. Med.* 345:1890-1900; Izbicki, *et al.* (1997) *New Engl. J. Med.* 337:1188-1194; Holland, *et al.* (eds.) (1996) *Cancer Medicine Encyclopedia of Cancer, 4th ed.*, Academic Press, San Diego, CA).

[0080] The present invention provides methods for treating a proliferative condition, cancer, tumor, or precancerous condition such as a dysplasia, with an agonist or antagonist of IL-33, with at least one additional therapeutic or diagnostic agent. The at least one

additional therapeutic or diagnostic agent can be, e.g., a cytokine or cytokine antagonist, such as interferon-alpha, or anti-epidermal growth factor receptor, doxorubicin, epirubicin, an anti-folate, e.g., methotrexate or fluoruracil, irinotecan, cyclophosphamide, radiotherapy, hormone or anti-hormone therapy, e.g., androgen, estrogen, anti-estrogen, flutamide, or diethylstilbestrol, surgery, tamoxifen, ifosfamide, mitolactol, an alkylating agent, e.g., melphalan or cis-platin, etoposide, vinorelbine, vinblastine, vindesine, a glucocorticoid, a histamine receptor antagonist, an angiogenesis inhibitor, radiation, a radiation sensitizer, anthracycline, vinca alkaloid, taxane, e.g., paclitaxel and docetaxel, a cell cycle inhibitor, e.g., a cyclin-dependent kinase inhibitor, a monoclonal antibody, a complex of monoclonal antibody and toxin, a T cell adjuvant, bone marrow transplant, or antigen presenting cells, e.g., dendritic cell therapy. Vaccines can be provided, e.g., as a soluble protein or as a nucleic acid encoding the protein (see, e.g., Le, *et al.*, supra; Greco and Zellefsky (eds.) (2000) *Radiotherapy of Prostate Cancer*, Harwood Academic, Amsterdam; Shapiro and Recht (2001) New Engl. J. Med. 344:1997-2008; Hortobagyi (1998) *New Engl. J. Med.* 339:974-984; Catalona (1994) *New Engl. J. Med.* 331:996-1004; Naylor and Hadden (2003) *Int. Immunopharmacol.* 3:1205-1215; The Int. Adjuvant Lung Cancer Trial Collaborative Group (2004) *New Engl. J. Med.* 350:351-360; Slamon, *et al.* (2001) *New Engl. J. Med.* 344:783-792; Kudelka, *et al.* (1998) *New Engl. J. Med.* 338:991-992; van Netten, *et al.* (1996) *New Engl. J. Med.* 334:920-921).

[0081] A number of biomarkers and methods for scoring inflammatory disorders, e.g., psoriasis, Crohn's disease, and rheumatoid arthritis are available (see, e.g., Bresnihan (2003) *Arthritis Res. Ther.* 5:271-278; Barnero and Delmas (2003) *Curr. Opin. Rheumatol.* 15:641-646; Gionchetti, *et al.* (2003) *Dig. Dis.* 21:157-167; Wiik (2002) *Autoimmune Rev.* 1:67-72; Sostegni, *et al.* (2003) *Aliment Pharmacol. Ther.* 17 (Suppl.2):11-17).

[0082] Biomarkers and methods for scoring cancer are also described (see, e.g., Alison (ed.) (2001) *The Cancer Handbook*, Grove's Dictionaries, Inc., St. Louis, MO; Oldham (ed.) (1998) *Principles of Cancer Biotherapy, 3rd*. ed., Kluwer Academic Publ., Hingham, MA; Thompson, *et al.* (eds.) (2001) *Textbook of Melanoma*, Martin Dunitz, Ltd., London, UK; Devita, *et al.* (eds.) (2001) *Cancer:* Principles *and Practice of Oncology, 6th ed.*, Lippincott, Phila, PA; Holland, *et al.* (eds.) (2000) *Holland-Frei Cancer Medicine*, BC Decker, Phila., PA; Garrett and Sell (eds.) (1995) *Cellular Cancer Markers*, Humana Press, Totowa, NJ; MacKie (1996) *Skin Cancer, 2nd ed.*, Mosby, St. Louis; Moertel (1994) *New*

Engl. J. Med. 330:1136-1142; Engleman (2003) *Semin. Oncol.* 30(3 Suppl. 8):23-29; Mohr, *et al.* (2003) *Onkologie* 26:227-233).

[0083] The broad scope of this invention is best understood with reference to the following examples, which are not intended to limit the inventions to the specific embodiments.

EXAMPLES

I. General Methods.

[0084] Standard methods in biochemistry and molecular biology are described (see, e.g., Maniatis, *et al.* (1982) *Molecular* Cloning, *A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Sambrook and Russell (2001) *Molecular Cloning, 3rd ed.,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Wu (1993) *Recombinant DNA*, Vol. 217, Academic Press, San Diego, CA). Standard methods also appear in Ausbel, *et al.* (2001) *Current Protocols in Molecular Biology, Vols.1-4*, John Wiley and Sons, Inc. New York, NY, which describes cloning in bacterial cells and DNA mutagenesis (Vol. 1), cloning in mammalian cells and yeast (Vol. 2), glycoconjugates and protein expression (Vol. 3), and bioinformatics (Vol. 4).

[0085] Methods for protein purification including immunoprecipitation, chromatography, electrophoresis, centrifugation, and crystallization are described (Coligan, *et al.* (2000) *Current Protocols in Protein* Science, *Vol. 1*, John Wiley and Sons, Inc., New York). Chemical analysis, chemical modification, post-translational modification, production of fusion proteins, glycosylation of proteins are described (see, e.g., Coligan, *et al.* (2000) *Current Protocols in Protein Science, Vol. 2*, John Wiley and Sons, Inc., New York; Ausubel, *et al.* (2001) *Current Protocols in Molecular Biology, Vol. 3*, John Wiley and Sons, Inc., NY, NY, pp. 16.0.5-16.22.17; Sigma-Aldrich, Co. (2001) *Products for Life Science Research*, St. Louis, MO; pp. 45-89; Amersham Pharmacia Biotech (2001) *BioDirectory*, Piscataway, N.J., pp. 384-391). Methods for the production, purification, and fragmentation of polyclonal and monoclonal antibodies are described (Coligan, *et al.* (2001) *Current Protcols in Immunology, Vol. 1*, John Wiley and Sons, Inc., New York; Harlow and Lane (1999) *Using Antibodies*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Harlow and Lane, *supra*). Standard techniques for characterizing ligand/receptor

interactions are available (see, e.g., Coligan, *et al.* (2001) *Current Protcols in Immunology, Vol. 4*, John Wiley, Inc., New York).

[0086] Methods for flow cytometry, including fluorescence activated cell sorting (FACS), are available (see, e.g., Owens, *et al.* (1994) *Flow Cytometry Principles for Clinical Laboratory Practice*, John Wiley and Sons, Hoboken, NJ; Givan (2001) *Flow Cytometry, 2nd ed.*; Wiley-Liss, Hoboken, NJ; Shapiro (2003) *Practical Flow Cytometry*, John Wiley and Sons, Hoboken, NJ). Fluorescent reagents suitable for modifying nucleic acids, including nucleic acid primers and probes, polypeptides, and antibodies, for use, e.g., as diagnostic reagents, are available (see, e.g., Molecular Probes (2003) *Catalogue*, Molecular Probes, Inc., Eugene, OR; Sigma-Aldrich (2003) *Catalogue*, St. Louis, MO).

[0087] Standard methods of histology of the immune system are described (see, e.g., Muller-Harmelink (ed.) (1986) *Human Thymus: Histopathology and Pathology*, Springer Verlag, New York, NY; Hiatt, *et al.* (2000) *Color Atlas of Histology*, Lippincott, Williams, and Wilkins, Phila, PA; Louis, *et al.* (2002) *Basic Histology:Text and Atlas*, McGraw-Hill, New York, NY).

[0088] Methods for using animal models, e.g., knockout mice, and cell-based assays for the testing, evaluation, and screening of diagnostic, therapeutic, and pharmaceutical agents are available (see, e.g., Car and Eng (2001) *Vet. Pathol.* 38:20-30; Kenyon, *et al.* (2003) *Toxicol. Appl. Pharmacol.* 186:90-100; Deurloo, *et al.* (2001) *Am. J. Respir. Cell Mol. Biol.* 25:751-760; Zuberi, *et al.* (2000) *J.* Immunol. 164:2667-2673; Temelkovski, *et al.* (1998) *Thorax* 53:849-856; Horrocks, *et al.* (2003) *Curr. Opin. Drug Discov. Devel.* 6:570-575; Johnston, *et al.* (2002) *Drug Discov. Today* 7:353-363).

[0089] Software packages and databases for determining, e.g., antigenic fragments, leader sequences, protein folding, functional domains, glycosylation sites, and sequence alignments, are available (see, e.g., GenBank, Vector NTI® Suite (Informax, Inc, Bethesda, MD); GCG Wisconsin Package (Accelrys, Inc., San Diego, CA); DeCypher® (TimeLogic Corp., Crystal Bay, Nevada); Menne, *et al.* (2000) *Bioinformatics* 16: 741-742; Menne, *et al.* (2000) *Bioinformatics Applications Note* 16:741-742; Wren, *et al.* (2002) *Comput. Methods Programs Biomed.* 68:177-181; von Heijne (1983) *Eur. J. Biochem.* 133:17-21; von Heijne (1986) *Nucleic Acids Res.* 14:4683-4690).

II. Interleukin-100.

[0090] Similarity of the amino acid sequence of human IL-33 to other members of the IL-1 family is as follows. Similarity with IL-1Ra is 34% similarity; IL-1delta is 36%; IL-1F10 is 36%; IL-1zeta is 9%; IL-1F8 is 32%; IL-1epsilon is 40%; and IL-1F9 is 31%.

[0091] Interleukin-100 was discovered by using computational sequence analyses and identified as a new IL-1 family member by secondary structure comparison with members of the IL-1 family, respectively IL-1beta and IL-18. IL-1 family members are highly inflammatory regulators of the immune system, released in response to pathogenic challenges. Human and mouse homologs of IL-33 were identified as well as a rat and dog IL-33. Gene expression analysis showed that human IL-33 was expressed in epithelial cells, smooth muscle cells, and mesangial cells. Upon stimulation with IL-1β and TNF-α, human IL-33 mRNA levels are highly induced in primary normal human dermal and lung fibroblasts as well as in bronchial smooth muscle cells. Expression of IL-33 mRNA in psoriatic skin samples as well as in lung pulmonary alveolar proteinosis was significantly elevated.

[0092] Similar to IL-1 and IL-18, IL-33 has no signal peptide. In stead, IL-33 is made and secreted as a large preproprotein that requires extensive processing to release the mature, biologically active form. It is likely that prepro IL-33 is processed by a caspase. We identified a caspase cleavage site in the protein sequence and showed that in vitro translated human IL-33 is cleaved by recombinant constitutively active caspase-1. To investigate the putative biological role of IL-33, recombinant proteins were expressed and purified in E.coli. Therefore, the IL-33 gene was cloned into a pET3a bacterial expression vector. The N-terminus of the recombinant protein was selected by comparing the mature sequence of IL-33 to IL-1β and IL-18 so that the mature, biologically active protein would lack the pro-domain. Amino acid 112 of the full-length protein was selected as the N-terminal amino acid. Recombinant protein was expressed and purified from E.coli. In vivo studies were carried out. The intraperitoneal (IP) injection of recombinant human IL-33 into mice (C57BL/6J), with a dosage of either 5 ug/day or 50 ug/day, led to severe eosinophilia and splenomegaly after 7 days. Serum levels of several cytokines were tested on day 3 and day 7. An induction of IL-5 up to 10000 pg/ml in the 50 ug rhIL-33/day group and up to 1000 pg/ml in the 5 ug rhIL-33/day group was observed on day 3. The serum levels of IL-5 decreased to 1100 pg/ml in the 50 ug rhIL-33/day group and in the 5 ug rhIL-33/day group to 500 pg/ml. IL-13 serum levels were also detectable at day 7 after treatment with either

5ug or 50 ug IL-33/day at 30 pg/ml and 100 pg/ml, respectively. No IL-5 and IL-13 could be detected in the PBS-treated control group. Furthermore, no elevated levels for IFN-γ, TNF-α, IL-12, IL-10, IL-6, IL-4, IL-2 or MCP-1 could be detected in IL-33 treated mice or in the control group.

[0093] After IP injection of 50 ug rhIL-33/day for 2 days liver lymphocytes were harvested. Cells were plated into culture dishes with 2x10e6/ml of culture medium and were stimulated with 50 ng/ml PMA and 1uM ionomycin for 4hr. During the last 2hr, Brefelding A , a secretion inhibitor, was added. Cells were surface stained for CD3 and NK1.1 and intracellularly stained either for IL-5 or IL-4 and analyzed by FACS. CD3/NK1.1 positive liver lymphocytes cells derived from mice treated for 2 days with IL-33 showed an accumulation of IL-5 and IL-4. These results suggest that IL-33 activates NKT cells to secrete IL-4 and IL-5.

[0094] To test if IL-33 binds NKT cells, biotinylated IL-33 was used for a binding experiment. Human NKT cells, derived from PBMCs, were incubated either with Strepdavidin-PE or biotinylated rhIL-33 and Strepdavidin-PE. The stained cells were analyzed by FACS. Binding of rhIL-33 to human NKT cells was observed. This binding could be competed with unbiotinylated rhIL-33.

[0095] IL-1 family members exert their biological response by interacting with cell surface receptors. We have identified the orphan IL-1 receptor ST2/T1 as a cellular receptor for IL-33. FACS staining of a mouse mast cell line with a ST2/T1-specific monoclonal antibody showed that this mast cell line expresses the ST2/T1 receptor. This staining could be specifically and dose-dependently competed by incubating the mast cell line with IL-33 protein.

[0096] NKT cells are essential for airway inflammation and the production of IL-4 and IL-13 in allergen-induced airway-hyper-reactivity in mouse models of asthma. The induction of IL-5 and IL-13 by IL-33 in NKT cells suggests that IL-33 can play a role in the induction of these diseases. The generation of therapeutic antibodies neutralizing IL-33 may be beneficial in the treatment of these diseases.

[0097] NKT cells have been implicated in many diseases. Identification of IL-33 as a modulator of NKT cells suggest that IL-33 can affect other diseases, such as lupus, multiple sclerosis, malignancies, airway inflammation and infectious diseases. The

induction of Th2 cytokines by IL-33, such as IL-5 and IL-13, may help in the protection against microbial infection or in the protection against tumors.

[0098] IL-1 family members typically bind to two different members of the IL-1 receptor family to form a complete receptor complex. The identification of IL-33 and ST2/T1 as one of the subunits that make up the receptor for IL-33, makes it possible to identify the second IL-33 receptor subunit. Identification of the complete IL-33 receptor will allow detailed identification of the biological responses induced by IL-33.

[0099] Real time PCR analysis using Taqman* revealed that IL-33 was expressed by a number of cells and tissues (Table 1). The present invention provides agonists and antagonists of IL-33 for the modulation of inflammatory and autoimmune disorders and conditions, e.g., psoriasis, asthma, allergies, and inflammatory bowel disease, e.g., gastric inflammation, ulcerative colitis, Crohn's disease, celiac disease, and irritable bowel syndrome.

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Table 1. Real time PCR analysis of IL-33 expression, relative to ubiquitin (1.0).			
Human skin psoriasis vulgaris		Human skin, normal adjacent	
Skin sample no.	Expression	Skin sample no.	Expression
PS-034	757	PS-034	261
PS-037	731	PS-037	267
PS-028	443	PS-028	285
PS-025	446	PS-025	261
PS-023	602	PS-023	235
Colon control	0.5		
Colon Crohn's no.4003197A	87		
Colon Crohn's no.9609C144	125		
Colon Crohn's no.403242A	114		

Expression of cytokines with *Nippostrongylus brasiliensis* infection, as determined by real time PCR Taqman analysis.					
sample	Cytokine tested				
	IL-25	IL-13	IL-4	IL-5	IL-33
Untreated stomach	0.86	0.03	0.51	0.01	9.43
stomach 2 day	1.46	0.69	0.09	0.02	56.4
stomach 4 day	0.73	0.67	0.39	0.09	80.32
stomach 8 day	1.04	3.30	0.33	0.44	84.9
stomach 11 days	0.27	1.22	1.77	0.01	8.86
stomach 16 days	0.35	0.38	1.31	0.12	3.43
Untreated control lung	0.29	0.01	0.57	0.66	56.8
Lung nippo 2 days	0.91	0.37	0.61	0.65	90.37
Lung nippo 4 days	0.59	13.34	4.65	3.78	356.49
Lung nippo 8 days	0.36	55.88	8.78	1.14	48.76
Lung nippo 11 days	0.35	33.93	16.05	2.11	116.79
Lung nippo 16 days	0.04	23.77	25.48	0.84	30.65

[00100] IL-33 induction by IL-1β plus TNF-α (8 hours) was compared with IL-33 induction with medium alone. Induction was studied in the indicated cell type (Table 2).

Table 2. IL-33 induction. ND means not detectable. Numbers are relative to ubiquitin (1.0).

Cell	IL-1β plus TNF-α	Medium only
NHDF	3250	50
NHEK	ND	ND
NHBE	25	25
PAEC	100	200
NHLF	25	ND
BSMC	2025	350

[00101] In vitro translated human IL-33 was found to be cleaved by caspase-1. Without caspase treatment, analysis by SDS PAGE revealed a band at about 32 kDa, corresponding to pro-human IL-33. Treatment with caspase-1 for 1 hour at 37° resulted in two bands of about equal intensity, one corresponding to pro-IL-33, and the other migrating at about 20-22 kDa (mature human IL-33). Treatment for 2 hours at 37° resulted in the same two bands, but with about two thirds of the protein migrating at about 22 kDa. Similar studies demonstrated that in vitro translated human IL-33 could also be cleaved by elastase or by cathepsin G, to species migrating at about 20-22 kDa, whereas MMP-3 treatment did not result in cleavage under the conditions used. Amino acid 112 of IL-33 is believed to be the position of cleavage, producing the mature IL-33, due to homology with other IL-1 family members.

[00102] T1/ST2 was identified as at least one subunit of the receptor for human IL-33. Expression of T1/ST2 was as follows (Table 3).

Table 3. Expression of T1/ST2 by human or mouse cells.		
Cell type	Human cells	Mouse cells
TH1-type T cells	-/+	-/+
TH2-type T cells	-/+	+++
Mast cells	+++	++
monocytes/PBMC treated with LPS	++	not determined
Dendritic cells ex BM	(not determined)	+

[00103] Human IL-33 was cloned in a pET3a vector, and expressed in E. coli. The cloned protein began with amino acid 112, and was 158 amino acids long (18 kDa). IPTG was used to induced expression, and the protein was found to be water-soluble. The expressed protein was purified using a A-column and Sephadex* gel filtration. The purified preparation was tested for endotoxin, where the results demonstrated about 0.023 EU per microgram protein. Analysis by SDS PAGE using a non-reducing conditions revealed that at least 95% of the protein migrated at a single molecular weight of 18 kDa.

[00104] IL-33 was injected intraperitoneally (i.p.) into B6/Balb/c mice. Three groups of mice were used: (1) Injection with phosphate buffered saline (PBS); (2) hIL-33 (5 micrograms/day); and (3) hIL-33 (50 micrograms/day). The protocol also involved injections (i.p.) for 3 days, with sacrifice after three days of treatment, or injections (i.p.) for 7 days, with sacrifice after seven days of treatment. Blood, serum, blood smears, white blood cell differentials, histology, was performed. Thymus/spleen cells suspensions were analyzed by FACS analysis.

[00105] IL-33 treatment induces IL-5 and IL-13, as determined by measuring serum levels of IL-5 and IL-13 (Table 4). The cytokines IL-4, IL-5, and IL-13 were also measured in various organs with IL-33 administration. The organs tested were thymus, lung, spleen, and liver. These three cytokines were all found to be induced, as determined after 7 days treatment with IL-33. Increases were found at both levels of IL-33 (5 and 50 microgram IL-33). For example, in lung, IL-4, IL-5, and IL-13 expression with saline treatment was about 1.0, or less. But with IL-33 (50 micrograms), expression of IL-4 was 8.0; of IL-5 was 11.0; and of IL-13 was 41.0. IL-33 treatment also provoked increases in serum IgE and IgA. With 7 days treatment, IgE levels were 30,000 ng/ml (PBS) and 17,000 ng/ml (50

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micrograms IL-33). With 7 days treatment, IgA levels were 90 ng/ml (PBS) and 420 ng/ml (50 micrograms IL-33). IL-33 treatment also resulted in splenomegaly, where spleen mass in the PBS (control) treated mouse was about 80 mg, 5 micrograms with IL-33 for 7 days (150 mg spleen), and 50 micrograms with IL-33 for 7 days (190 mg spleen). IL-33 treatment also produced extramedullary hematopoiesis in the spleen, and thymus hypoplasia (decrease in thymus size), and hypoplasia of the cortex of the thymus.

Table 4. IL-33 treatment and white blood cell counts, platelet counts, and cytokine levels, at days 3 and 7.			
White blood cell counts (white blood cells/microliter blood)			
	PBS	5 micrograms IL-33/.day	50 micrograms IL-33/day
Day 3	12,000	12,000	14,000
Day 7	12,000	20,000	25,000
Platelets (platelets/microliter blood)			
Day 3			
Day 7	1,300,000	1,000,000	600,000
Neutrophils			
Day 3	500	350	520
Day 7	750	700	1200
Lymphocytes			
Day 3	10,000	10,000	7,500
Day 7	11,000	15,500	18,000
Monocytes			
Day 3	230	130	200
Day 7	400	250	300
Eosinophils			
Day 3	420	220	130
Day 7	500	2000	1750
Serum levels of IL-5 (pg/ml)			
	PBS	5 micrograms IL-33/.day	50 micrograms IL-33/day
Day 3	ND	500	7500
Day 7	ND	500	1100
Serum levels of IL-7 (pg/ml)			
Day 7	ND	40	78

[00106] Transformed and non-transformed cells were injected into mice, followed by an incubation period of the cells within the mouse, and retrieval and purification of the injected cells, with assessment of T1/ST2 expression (Table 5). Non-transformed mammary gland cells and ras-transformed mammary gland cells were used. With injection of the ras-transformed cells into a host immune-deficient nude mouse, the ras-transformed cells expressed T1/ST2 at increased levels, that is, expression was 103 (Table 5). With injection of the ras-transformed cells into a host mouse having an intact immune system, retrieval of the transformed cell, and Taqman® analysis, revealed greater increases in T1/ST2 expression. It is believed that these increases in T1/ST2 expression reflect increases in soluble T1/ST2, where the soluble T1/ST2 acts as a decoy. When soluble T1/ST2 acts as a decoy, it binds to IL-33, and inhibits the host from mounting a TH2-type immune response against the tumor cells. The host mice with intact immune systems that were used, were Xtb mice and XBalb mice (Table 6). The soluble version of T1/ST2 (also known as Fit 1) is described (see, e.g., Bergers, et al. (1994) *EMBO J.* 13:1176-1188; Reikerstorfer, et al. (1995) *J. Biol. Chem.* 270:17645-17648).

[00107] IL-33 was administered to mice harboring 4T1 breast cancer. Administered IL-33 was effective in reducing tumor size (Table 6). The present invention provides agonists of IL-33, e.g., IL-33 or a nucleic acid encoding IL-33, for the treatment of proliferative conditions, including cancers and tumors.

Table 5. Injection of ras-transformed cells into nude mice and immunocompetant mice, followed by Taqman analysis of T1/ST2 expression by recovered ras-transformed cells.

	Expression of T1/ST2
Nude mouse host	103
Xtb mouse host	421
XBalb mouse host	669

Table 6. Administered IL-33 reduces tumor size in mice.

Treatment	Tumor size (mm^3)										
	23	24	25	26	27	28	29	30	31	32	33
IL-33	175	125	125	200	180	170	205	200	200	240	240
no IL-33	175	200	225	240	280	300	370	405	430	470	500

[00108] Taqman analysis of human tissue samples revealed decreased expression of IL-33 in various cancers, e.g., breast cancer and ovarian cancer (Table 7). The results indicate that the tumor cells refrain from producing IL-33, in order to avoid activating the immune system to mount an anti-tumor response (Table 7).

Table 7. Real time PCR analysis of IL-33 expression by human cancer tissues.

	Cancerous tissue	Normal adjacent tissue
Human breast 6221 (infiltrating duct)	30.4	435.0
Human breast 6612 (infiltrating duct)	59.7	54.7
Human breast 6652 (infiltrating duct)	2.4	292.0
Human breast 7748/02 lobular	2.6	411.0
Human breast 7156 lobular	77.4	189.1
Human ovary papillary serous cystadenocarcinoma 1590	1872.7	609.4
Human ovary papillary serous cystadenocarcinoma 3572/02	206.6	793.9
Human ovary papillary serous cystadenocarcinoma 246869	235.1	1052.7

[00109] IL-33 treatment prolonged experimental autoimmune encephalitis, an animal model for multiple sclerosis. EAE was induced by proteolipid protein (PLP) (Kjellen, et al. (2001) *J. Neuroimmunol.* 120:25-33; Laman, et al. (2001) *J. Neuroimmunol.* 119:124-130; Fife, et al. (2001) *J. Immunol.* 166:7617-7624). Three groups of mice were used for injections of PBS, 0.5 micrograms IL-33, or 2.0 micrograms of IL-33, with daily i.p. injections from day 0 to day 12. Disease scores were assessed on days 7 to 23 (Table 8). The results demonstrated that in PBS-treated mice, the disease spontaneously resolved. However, with treatment with either dose of IL-33, the disease was prolonged, higher than that found with PBS treatment, and maintained itself at a disease score of between 2.5-3.0 (Table 8). The present invention provides an antagonist of IL-33 for the treatment of

autoimmune disorders, including autoimmune disorders of the central nervous system, e.g., multiple sclerosis.

Table 8. EAE disease score in mice at selected days after treatment with PBS or with IL-33.

	Day 11	Day 13	Day 15	Day 17	Day 19	Day 21
PBS	0	2.05	1.75	0.45	0.20	0.0
0.5 micrograms IL-33	0	0.75	1.15	2.4	2.9	2.9
2.0 micrograms IL-33	0	1.95	1.95	3.1	2.3	2.55

[00110] IL-33 treatment led to IL-5 induction in NKT cells. NKT cells were identified by the presence of both the NK1.1 marker and the CD3 marker. Black-6 mice were treated for two days with PBS or 50 micrograms/day of IL-33. Liver lymphocytes were isolated, and restimulated with PMA ionomycin for 3 hours and brefeldin for 1 hour. The results demonstrated that IL-33 induced IL-5 in NKT cells.

[00111] Anti-T1/ST2 antibody was tested for its ability to bind wild type mast cells (WTMC), and the influence of added IL-33 on binding of this antibody to the mast cells. Adding IL-33 abolished the ability of anti-T1/ST2 antibody to bind the mast cells, demonstrating that the receptor of IL-33 is T1/ST2.

III. In vivo effects of IL-33 antibody treatment

[00112] A mouse monoclonal antibody against human IL-33 was raised using methods well known in the art (see above). To test the ability of this antibody to antagonize IL-33 activity, Balb/c mice were injected subcutaneously with 0.2 mg of anti-IL-33 antibody on day 0. On day 1, the mice were injected intraperitonally with 100 ng of mIL-33. Serum was collected on day 2, and IL-5 levels measured. Treatment with the anti-IL-33 antibody resulted in little to no production of IL-5 when compared to mice treated with IL-33 alone and mice treated with isotype control antibody and IL-33 (See Figure 1).

IV. Treatment of Collagen Induced Arthritis (CIA)

[00113] B10.RIII mice, known to be susceptible to developing CIA, were injected with bovine collagen type II (bovine CII; Sigma) in complete Freund's adjuvant (Difco). Mice were injected above the tail base with 100ul of a 1mg/ml emulsion of bovine CII. A second boost dose was administered at day 21. Mice were assessed by the following clinical scale: 0 = normal; 1 = redness and/or swelling at one joint/site; 2 = redness and/or swelling at more than on joint/site; and 3 = redness and or swelling in the entire paw. CIA induced mice have a percent disease onset of 70-90%.

[00114] Mice were treated at day 23 with 1 mg of anti-IL-33 antibody or isotype control antibody. Antibodies were administered every 7 days for two more treatments. Anti-IL-33 treated mice showed decreased disease scores as well as a lower percent incidence of disease onset (see Figures 2 and 3). The anti-IL-33 treated mice also had a lower mean number of arthritic paws (see Figure 4).

V. Treatment of Experimental Autoimmune Encephilitis (EAE)

[00115] C57BL/6 mice were immunized with 50 ug of myelin oligodendrocyte glycoprotein (MOG) peptide to induce EAE. The clinical assessment of the disease is scored as follows: 1 = limp tail; 2 = hind limb weakness; 3 = inability to right + single hind limb weakness; 4 = inability to right + single hind limb paralysis; 5 = bilateral hind limb paralysis; 6 = bilateral hind limb paralysis + abdomen collapse; and 7 = 6 + moribund. EAE mice were treated subcutaneously with either 100 mg of anti-IL-33 antibody or isotype control antibody. Anti-IL-33 treated mice showed lower disease scores and lower disease incidence than the control group (see Figures 5 and 6).

VI. Pull-down Assay to Identify IL-33R Complex

[00116] IL-33 was biotinylated with EX-LINK Supho-NHS-Biotin (Pierce). Pull-down of 2 µg biotinylated IL-33 was performed in 500 µl RIPA-Lysis buffer (upstate cell singaling solution) with 50 µl of a 50% Slurry of Agarose bound Avidin D (Vector Laborities). 5 µg of either recombinant extra-cellular ST2 -Fc (R&D Systems) or SIGIRR-Fc (R&D Systems) was used. After incubation overnight at 4°C precipitates were washed 3x with 500 µl RIPA-Lysis buffer. The precipitated proteins were separated by SDS-Page, electroblotted, and visualized by Western blot/ECL reaction with antibodies specific against

ST2 (R&D Systems) or SIGIRR (R&D Systems). Pull-down of biotinylated IL-33 with ST2-Fc or SIGIRR-Fc was performed in the same manner as above only Protein G-Sepharose* (Amersham Bioscience) was used instead of Agarose bound Avidin D. IL-33 presents was visualized *via* a Streptavidin-HRP conjugate (Pierce) and ECL reaction.

VII. Phosphorylation of NF-κB and MAP Kinases

[00117] The mastcell line WTMC was described previously (see, e.g., Wright, et al. (2003). J. Immunol. 171:3034-3046.). Cells were lysed in RIPA lysis Buffer (Upstate) containing Complete Mini protease inhibitor cocktail (Roche) and 10 mM Na_3VO_4. Proteins were separated by SDS-Page, transferred to Immobilon-P* membranes (Millipore) and immunoblotted using antibodies to phosphorylated p65 NF-κB, p65 NF-κB, phosphorylated p44/42 MAP kinases, p44/42 MAP kinases, phosphorylated p38 MAP kinase and p38 MAP kinase (all Antibodies form Cell Signaling Technology).

VIII. Transient Transfection and Reporter Gene Assays

[00118] HEK293FT cells were seeded before transfection with an NF-κB–driven GFP reporter gene construct (pNF-κB-hrGFP; Stratagene) and with a combination of plasmids encoding for ST2, or SIGIRR or both, as indicated with Fugene-6* (Roche) according to manufacturer's recommendations. Cells were split 24 hours after transfection. After 24 hours cells were either left untreated or stimulated with mouse IL-33 at the concentration of 50 ng/ml. Sixteen hours after stimulation, cells were analyzed for GFP-expression by FACS.

***Trade-mark**

Table 9: Sequence Identifiers	
SEQ ID NO: 1	Human IL-33 nucleic acid sequence
SEQ ID NO: 2	Human IL-33 polypeptide sequence
SEQ ID NO: 3	Mouse IL-33 nucleic acid sequence
SEQ ID NO: 4	Mouse IL-33 nucleic acid sequence
SEQ ID NO: 5	Human T1/ST2 nucleic acid sequence
SEQ ID NO: 6	Human T1/ST2 polypeptide sequence
SEQ ID NO: 7	Mouse T1/ST2 nucleic acid sequence
SEQ ID NO: 8	Mouse T1/ST2 polypeptide sequence
SEQ ID NO: 9	Human SIGIRR nucleic acid sequence
SEQ ID NO: 10	Human SIGIRR polypeptide sequence
SEQ ID NO: 11	Mouse SIGIRR nucleic acid sequence
SEQ ID NO: 12	Mouse SIGIRR polypeptide sequence

[00120] Many modifications and variations of this invention, as will be apparent to one of ordinary skill in the art can be made to adapt to a particular situation, material, composition of matter, process, process step or steps, to preserve the objective and scope of the invention. All such modifications are intended to be within the scope of the claims appended hereto without departing from the scope of the invention. The specific embodiments described herein are offered by way of example only, and the invention is to be limited by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled; and the invention is not to be limited by the specific embodiments that have been presented herein by way of example.

1. An antagonist of IL-33, wherein the antagonist comprises an antibody or an antigen-binding fragment thereof that specifically binds to IL-33.

2. The antagonist of claim 1, wherein the antibody or antigen-binding fragment thereof comprises:

a) a monoclonal antibody; or

b) an Fab, Fv, or $F(ab')_2$ fragment.

3. An antagonist of IL-33, wherein the antagonist comprises:

a) an antisense nucleic acid to IL-33; or

b) a small interference RNA (siRNA) to IL-33.

4. The antagonist of any one of claims 1 to 3 for use in the treatment of a disorder or condition selected from the group consisting of:

a) asthma;

b) allergy;

c) multiple sclerosis; and

d) arthritis.

5. The antagonist of claim 4, wherein the arthritis comprises rheumatoid arthritis.

6. An agonist of IL-33 for use in the treatment of breast cancer, wherein the agonist comprises:

a) IL-33; or

b) a nucleic acid encoding IL-33.

7. The agonist of claim 6, wherein the IL-33 agonist increases the counts of:

a) total white blood cells;

b) neutrophils;

c) lymphocytes; or

d) eosinophils.

8. The IL-33 agonist of claim 6, wherein the IL-33 agonist decreases the count of platelets.

9. Use of an antagonist of IL-33 in the preparation of a medicament for the treatment of a disorder or condition selected from the group consisting of:

a) asthma;

b) allergy;

c) multiple sclerosis; and

d) arthritis,

wherein the antagonist comprises an antibody or an antigen-binding fragment thereof that specifically binds to IL-33.

10. The use of claim 9, wherein the antibody or antigen-binding fragment thereof comprises:

a) a monoclonal antibody; or

b) an Fab, Fv, or $F(ab')_2$ fragment.

11. Use of an antagonist of IL-33 in the preparation of a medicament for the treatment of a disorder or condition selected from the group consisting of:

a) asthma;

b) allergy;

c) multiple sclerosis; and

d) arthritis,

wherein the antagonist comprises:

i) an antisense nucleic acid to IL-33; or

ii) small interference RNA (siRNA) to IL-33.

12. The use of any one of claims 9 to 11, wherein the arthritis comprises rheumatoid arthritis.

13. Use of an agonist of IL-33 in the preparation of a medicament for the treatment of breast cancer, wherein the agonist comprises:

a) IL-33; or

b) a nucleic acid encoding IL-33.

14. The use of claim 13, wherein the medicament increases the counts of:

a) total white blood cells;

b) neutrophils;

c) lymphocytes; or

d) eosinophils.

15. The use of claim 13, wherein the medicament decreases the count of platelets.

16. Use of an antagonist of IL-33 for treating a disorder or condition selected from the group consisting of:

a) asthma;

b) allergy;

c) multiple sclerosis; and

d) arthritis,

wherein the antagonist comprises an antibody or an antigen-binding fragment thereof that specifically binds to IL-33.

17. The use of claim 16, wherein the antibody or antigen-binding fragment thereof comprises:

a) a monoclonal antibody; or

b) an Fab, Fv, or $F(ab')_2$ fragment.

18. Use of an antagonist of IL-33 for treating a disorder or condition selected from the group consisting of:

a) asthma;

b) allergy;

c) multiple sclerosis; and

d) arthritis,

wherein the antagonist comprises:

i) an antisense nucleic acid to IL-33; or

ii) small interference RNA (siRNA) to IL-33.

19. The use of any one of claims 16 to 18, wherein the arthritis comprises rheumatoid arthritis.

20. Use of an agonist of IL-33 for treating breast cancer, wherein the agonist comprises:

a) IL-33; or

b) a nucleic acid encoding IL-33.

21. The use of claim 20, wherein the agonist of IL-33 increases the counts of:

a) total white blood cells;

b) neutrophils;

c) lymphocytes; or

d) eosinophils.

22. The use of claim 21, wherein the agonist of IL-33 decreases the count of platelets.

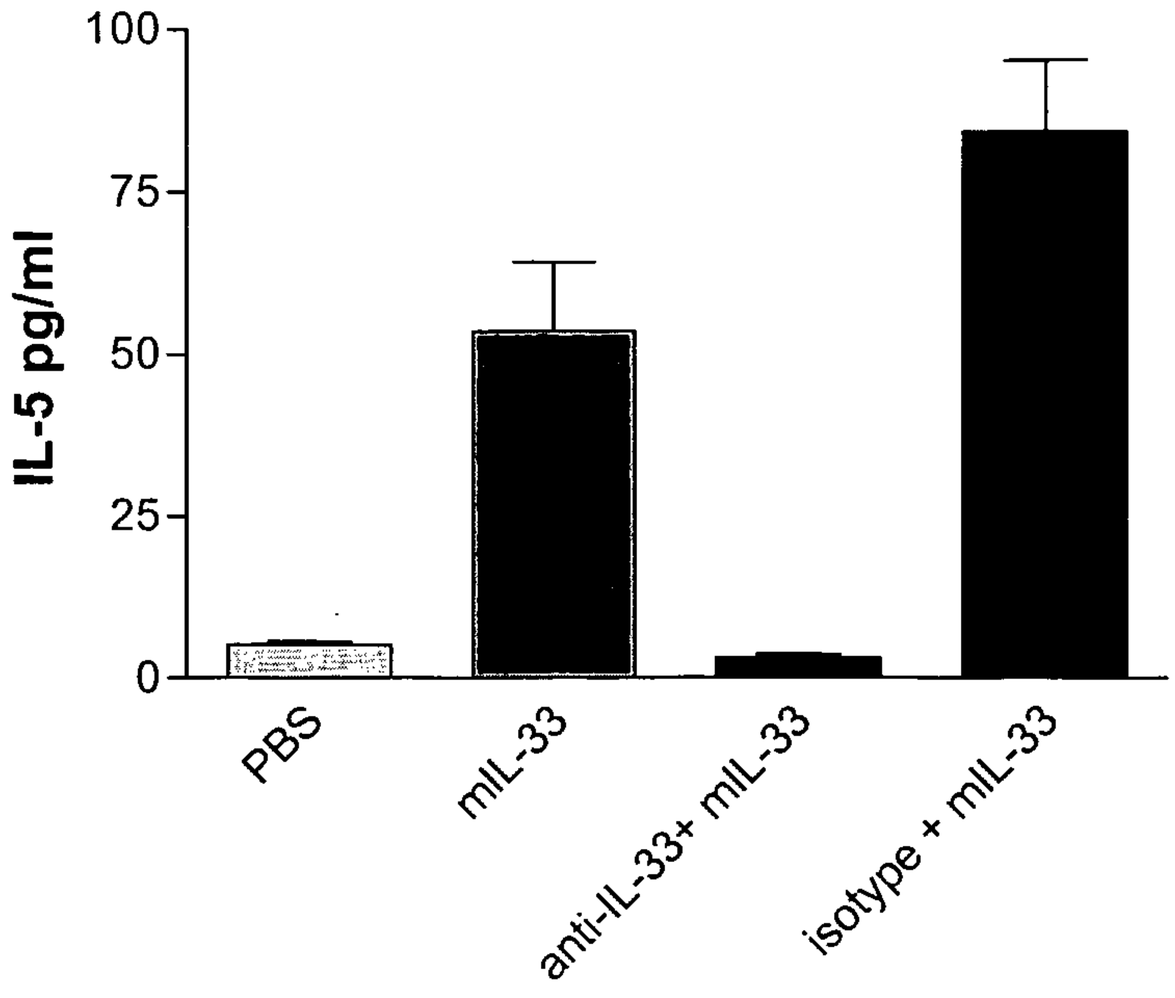
IL-5 pg/ml
100
75
50
25
0
PBS
mIL-33
anti-IL-33+ mIL-33
isotype + mIL-33

FIG. 1

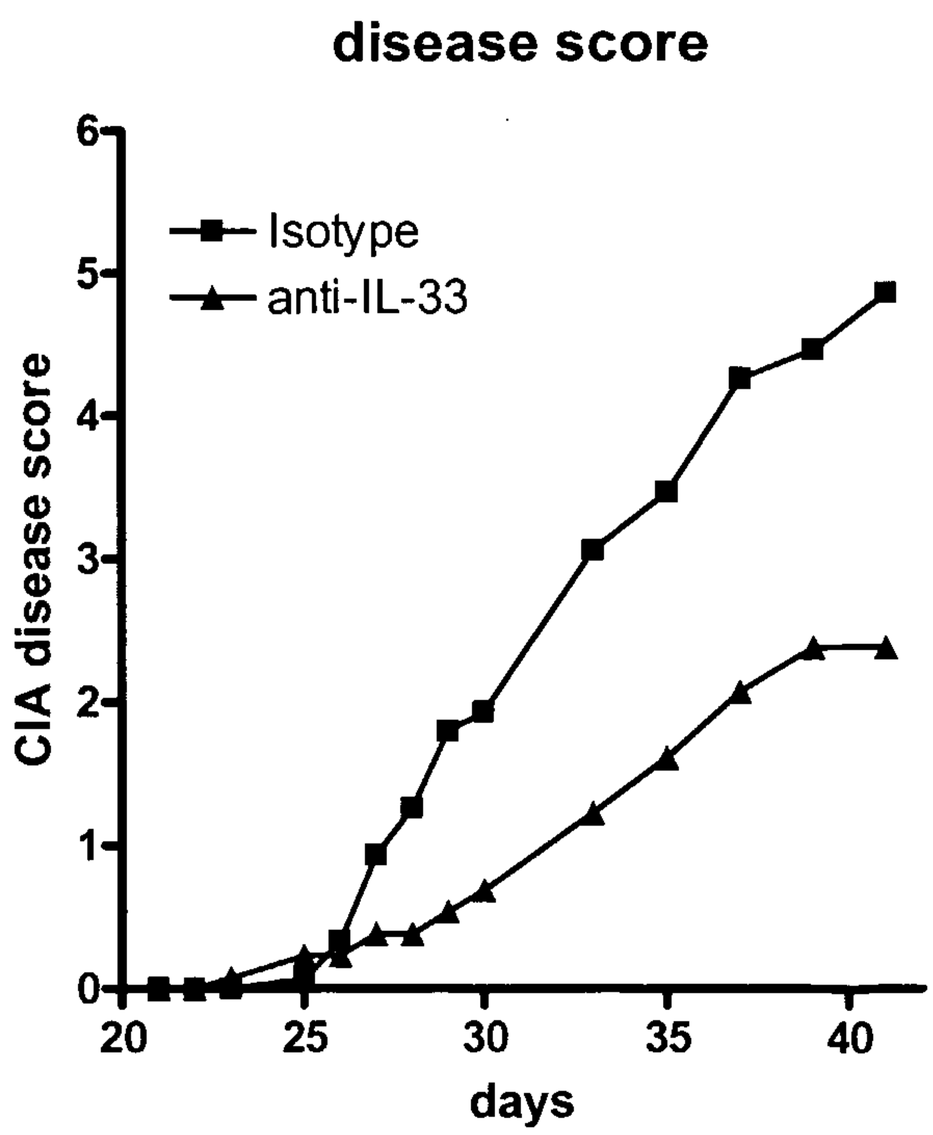
disease score
Isotype
anti-IL-33
CIA disease score
6
5
4
3
2
1
0
20
25
30
35
40
days

FIG. 2

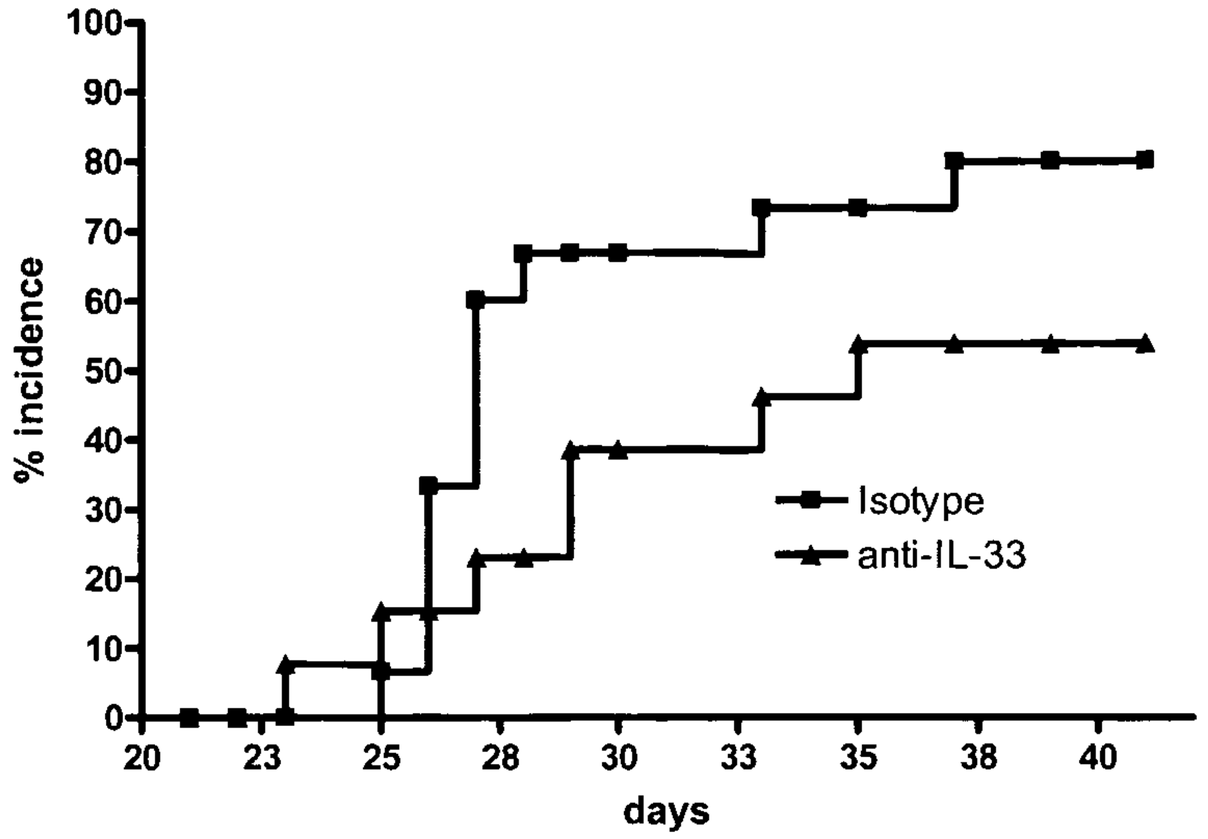
incidence of disease
% incidence
100
90
80
70
60
50
40
30
20
10
0
20
23
25
28
30
33
35
38
40
days
Isotype
anti-IL-33

FIG. 3

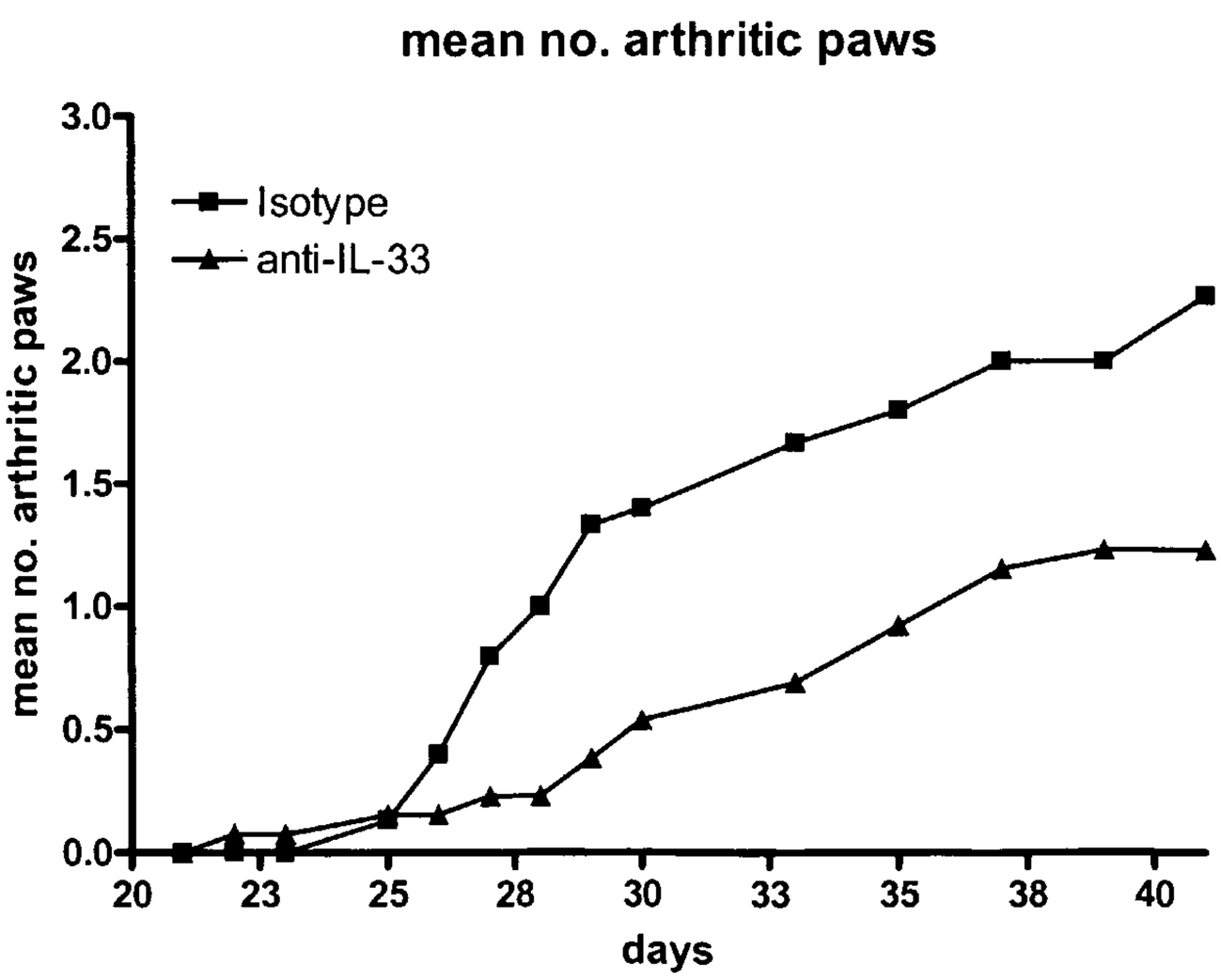
mean no. arthritic paws
mean no. arthritic paws
3.0
2.5
2.0
1.5
1.0
0.5
0.0
20
23
25
28
30
33
35
38
40
days
Isotype
anti-IL-33

FIG. 4

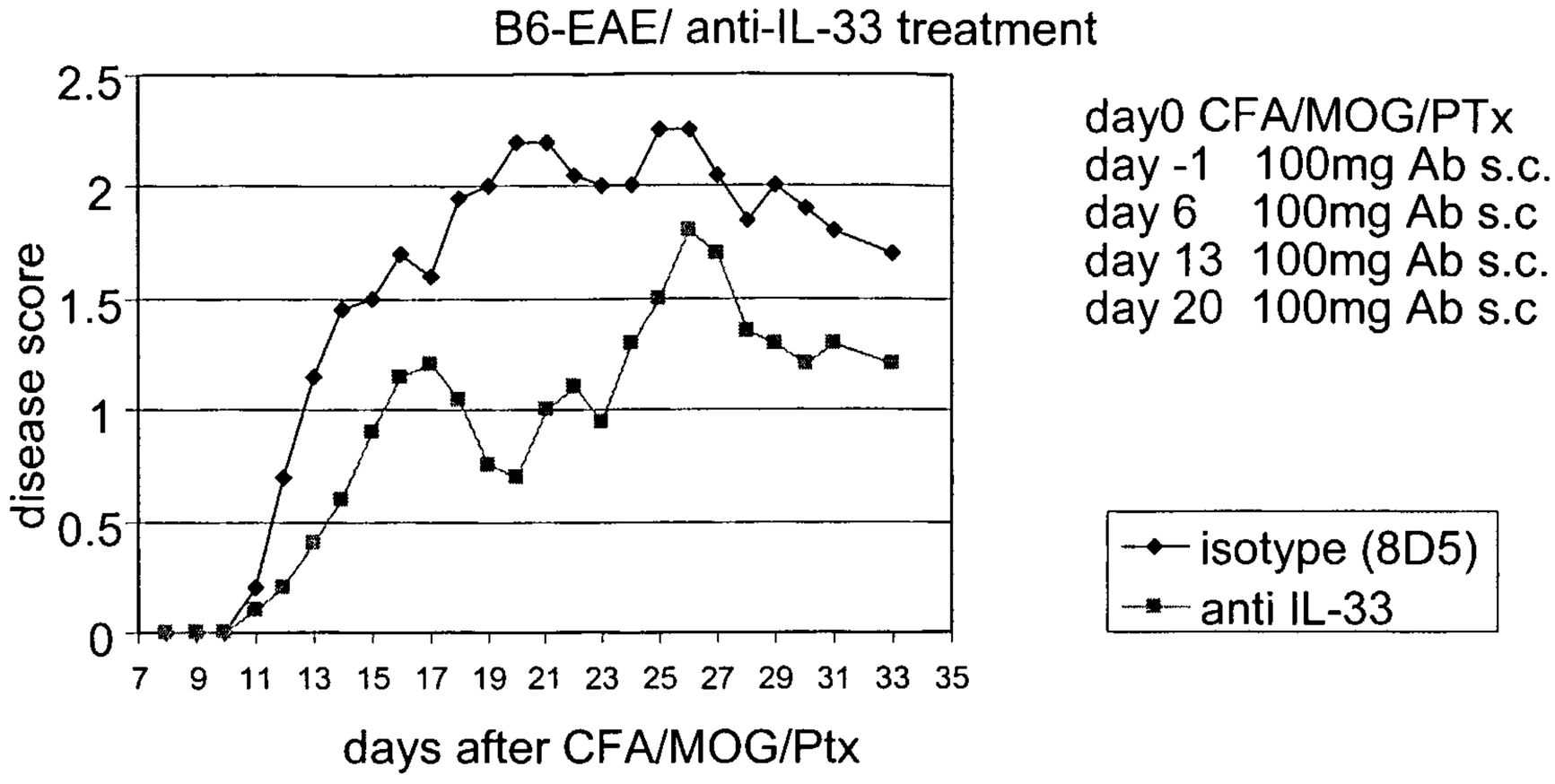
B6-EAE/ anti-IL-33 treatment
disease score
2.5
2
1.5
1
0.5
0
7 9 11 13 15 17 19 21 23 25 27 29 31 33 35
days after CFA/MOG/Ptx
day0 CFA/MOG/PTx
day -1 100mg Ab s.c.
day 6 100mg Ab s.c
day 13 100mg Ab s.c.
day 20 100mg Ab s.c
isotype (8D5)
anti IL-33

FIG. 5

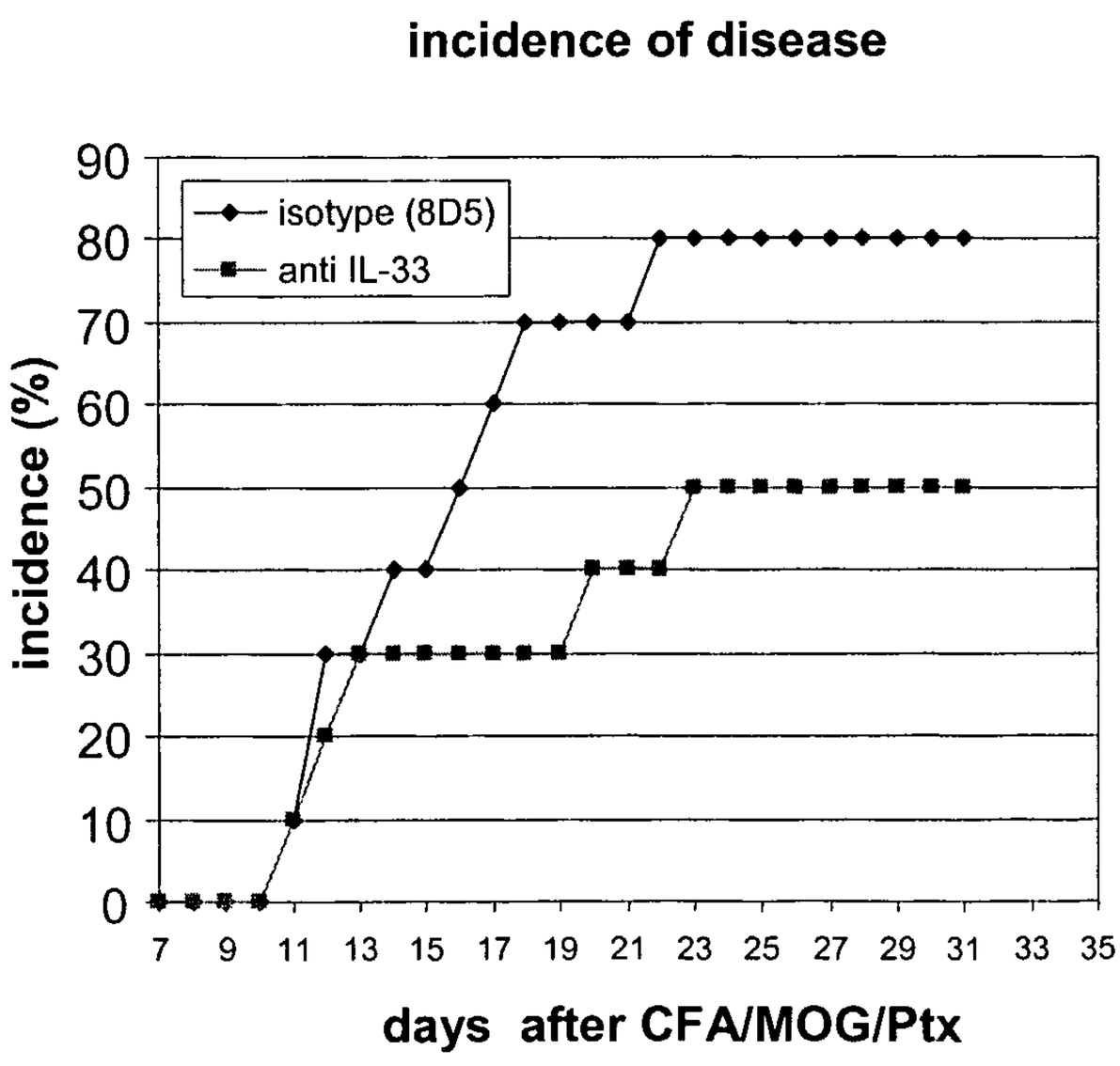
incidence of disease
incidence (%)
90
80
70
60
50
40
30
20
10
0
7 9 11 13 15 17 19 21 23 25 27 29 31 33 35
days after CFA/MOG/Ptx
isotype (8D5)
anti IL-33

FIG. 6