

(19) AUSTRALIAN PATENT OFFICE

(54) Title
Use for interleukin-33 (IL33) and the IL-33 receptor complex

(51)⁶ International Patent Classification(s)
C07K 14/54 (2006.01)20060101ALI2005100
C07K 16/24 (2006.01)⁸BMEP **C07K**
C07K 16/28 (2006.01)^{16/28}
A61K 38/00 (2006.01)20060101ALI2005100
C07K 14/54 ⁸BMEP **A61K**
20060101AFI2005100 38/00
⁸BMEP **C07K** 20060101ALN200510
16/24 08BMEP
PCT/US2005/004743

(21) Application No: 2005215528 (22) Application Date: 2005 .02 .15

(87) WIPO No: W005/079844

(30) Priority Data

(31) Number	(32) Date	(33) Country
60/545,730	2004 .02 .17	US

(43) Publication Date : 2005 .09 .01

(71) Applicant(s)
Schering Corporation

(72) Inventor(s)
Oft, Martin, Kastelein, Robert A., Schmitz, Jochen

(74) Agent/Attorney
Griffith Hack, Level 29, Northpoint 100 Miller Street, North Sydney, NSW, 2060

(56) Related Art
WO 2004/056868
Walzl et al. J. Exp. Med. 2001, 193(7), 785-792
Coyle et al. J. Exp. Med. 1999, 190(7), 895-902
Kumar et al. J. Biol. Chem. (1995), 270(46), 27905-27913
Sims et al. Current Opinion in Immunology (2002), 14(1), 117-122
WO 1999/032626
WO 1999/034217
US 5576191
O'Neill Nature Immunology (2003), 4(9), 823-824

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
1 September 2005 (01.09.2005)

PCT

(10) International Publication Number
WO 2005/079844 A3

(51) International Patent Classification⁷: **A61K 39/395**,
38/20, 38/17, A61P 11/06, 19/02, 25/02, 31/00, 35/00,
37/00, C07K 14/54, 16/24, 16/28, 14/715

(21) International Application Number:
PCT/US2005/004743

(22) International Filing Date: 15 February 2005 (15.02.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/545,730 17 February 2004 (17.02.2004) US

(71) Applicant (for all designated States except US): **SCHERING CORPORATION** [US/US]; 2000 Galloping Hill Road, Kenilworth, NJ 07033-0530 (US).

(72) Inventors; and
(75) Inventors/Applicants (for US only): **SCHMITZ, Jochen** [DE/US]; 4153-F El Camino Way, Palo Alto, CA 94306 (US). **OFT, Martin** [DE/US]; 645 Maroon Drive, Palo Alto, CA 94301 (US). **KASTELEIN, Robert, A.** [NL/US]; 463 Summit Drive, Redwood City, CA 94062 (US).

(74) Agent: **TRIOLO, Thomas, A.**; Schering-Plough Corporation, 2000 Galloping Hill Road, Patent Department K-6-1 1990, Kenilworth, NJ 07033-0530 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM,

AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HN, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MY, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LI, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:
— as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for all designations

Published:
— with international search report
— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

(88) Date of publication of the international search report:
24 November 2005

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2005/079844 A3

(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.* 343:7240-7245; 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

The present invention provides the following items (1) to (14):

- (1) A method of modulating an immune disorder or condition in a subject, comprising administering to said subject an effective amount of an IL-33 antagonist comprising:
 - a. a binding composition derived from the antigen binding site of an antibody that specifically binds to mature human IL-33;
 - b. anti-sense nucleic acid to IL-33; or
 - c. small interference RNA (siRNA) to IL-33,
wherein the mature human IL-33 comprises residues 112-270 of SEQ ID NO:2.

- (2) Use of an IL-33 antagonist comprising:
 - a. a binding composition derived from the antigen binding site of an antibody that specifically binds to mature human IL-33;
 - b. anti-sense nucleic acid to IL-33; or
 - c. small interference RNA (siRNA) to IL-33,
wherein the mature human IL-33 comprises residues 112-270 of SEQ ID NO:2,
in the manufacture of a medicament for modulating an immune disorder or condition in a subject.

- (3) The method of item (1), or the use of item (2), wherein the disorder or condition comprises:
 - a. asthma or allergy involving a pathogenic TH2-type response;
 - b. multiple sclerosis;
 - c. an inflammatory bowel disorder; or
 - d. arthritis.

- (4) The method of item (1), or the use of item (2), wherein the binding composition comprises:
 - a. a monoclonal antibody, or a fragment thereof;
 - b. a humanized antibody, or a fragment thereof; or
 - c. an Fab, Fv, or F(ab')₂ antibody fragment.

2005215528 30 Mar 2009

4A

- (5) A method of modulating an immune disorder or condition in a subject, comprising administering to said subject an effective amount of an IL-33 agonist comprising:
- a. mature human IL-33 consisting of residues 112-270 of SEQ ID NO:2; or
 - b. a nucleic acid encoding mature human IL-33.
- (6) Use of an IL-33 agonist comprising:
- a. mature human IL-33 consisting of residues 112-270 of SEQ ID NO:2; or
 - b. a nucleic acid encoding mature human IL-33,
- in the manufacture of a medicament for modulating an immune disorder or condition in a subject.
- (7) The method of item (5), or the use of item (6), wherein the immune disorder or condition comprises a TH1-type response.
- (8) The method of item (5), or the use of item (6), wherein the immune disorder or condition comprises:
- a. innate response;
 - b. infection; or
 - c. a cancer or tumor.
- (9) A method of modulating an immune disorder or condition in a subject comprising administering to said subject an effective amount of a composition that blocks the binding of human IL-33 (residues 112-270 of SEQ ID NO: 2) to a receptor comprising human T1/ST2, wherein the composition comprises:
- a. a binding composition derived from the antigen-binding site of an antibody that specifically binds to human IL-33;
 - b. anti-sense nucleic acid to IL-33; or
 - c. small interference RNA (siRNA) to IL-33.
- (10) The method of item (9), wherein the disorder or condition comprises:
- a. asthma or allergy involving a pathogenic TH2-type response;

- b. multiple sclerosis;
 - c. an inflammatory bowel disorder; or
 - d. arthritis.
- (11) The method of item (9), wherein the binding composition comprises:
- a. a monoclonal antibody, or a fragment thereof;
 - b. a humanized antibody, or a fragment thereof; or
 - c. an Fab, Fv, or F(ab')₂ antibody fragment.
- (12) A complex comprising:
- a. mature human IL-33 (residues 112-270 of SEQ ID NO: 2); and
 - b. human T1/ST2.
- (13) A method of inhibiting the activity of T1/ST2 in a subject comprising administering to said subject a therapeutically effective amount of an antagonist of IL-33 selected from the group consisting of:
- a. a binding composition derived from the antigen-binding site of an antibody that specifically binds to human IL-33;
 - b. anti-sense nucleic acid to IL-33; and
 - c. small interference RNA (siRNA) to IL-33.
- (14) Use of an antagonist of IL-33 selected from the group consisting of:
- a. a binding composition derived from the antigen-binding site of an antibody that specifically binds to human IL-33;
 - b. anti-sense nucleic acid to IL-33; and
 - c. small interference RNA (siRNA) to IL-33,
- in the manufacture of a medicament for inhibiting the activity of T1/T2 in a subject.

[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] Described herein is 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. Also described is 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.

Also described is 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). Also described is 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 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).

2005215528 30 Mar 2009

[0010] Also described herein is 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.

[0011] Also described herein is 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 described herein, 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.

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.
- [0020] All references cited herein are incorporated herein by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

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 effects (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 Cheresch (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 irradiation 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- κ B-dependent assay was run. Co-expression of both T1/ST2 and SIGIRR was both necessary and sufficient to activate NF- κ B signaling and MAP kinase upon stimulation with IL-33. Activation of JNK kinases was also observed.

III. Agonists, Antagonists, and Binding Compositions.

[0054] Described herein are 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 disclosure 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 described herein 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 be fused with a myeloma cell line to produce a hybridoma (Meygaard, *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] Described herein are 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 $\mu\text{g}/\text{kg}$ body weight, more generally at least 0.2 $\mu\text{g}/\text{kg}$, most generally at least 0.5 $\mu\text{g}/\text{kg}$, typically at least 1 $\mu\text{g}/\text{kg}$, more typically at least 10 $\mu\text{g}/\text{kg}$, most typically at least 100 $\mu\text{g}/\text{kg}$, 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 effects (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] Described herein are 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 , ^3H , ^{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] Described herein are 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] Described herein are 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.*, *Oriente sp.*, *Ehrlichia sp.*, *Anaplasma sp.*, *Neorickettsia sp.*, *Chlamydia sp.*, and *Coxiella sp.* Additionally, IFN γ 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] Described herein are 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. Described are 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. Also contemplated are methods of 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] Also described herein are 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; Ausbel, 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 Protocols 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 Protocols 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-1 β 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-1 β 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 2×10^6 /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 Strepavidin-PE or biotinylated rhIL-33 and Strepavidin-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.

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 <i>Nippostrongylus brasiliensis</i> 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-1 β 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-1 β 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).

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

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 Fil 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).

	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.

	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 intraperitoneally with 100 ng of mL-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 one 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 Encephalitis (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 signaling solution) with 50 µl of a 50% Slurry of Agarose bound Avidin D (Vector Laboratories). 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- κ B 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 Na₃VO₄. Proteins were separated by SDS-Page, transferred to Immobilon-P membranes (Millipore) and immunoblotted using antibodies to phosphorylated p65 NF- κ B, p65 NF- κ B, 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- κ B-driven GFP reporter gene construct (pNF- κ B-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.

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

[00119] All citations herein are incorporated herein by reference to the same extent as if each individual publication, patent application, or patent was specifically and individually indicated to be incorporated by reference including all figures and drawings.

[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, spirit and scope of the invention. All such modifications are intended to be within the scope of the claims appended hereto without departing from the spirit and 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.

In the claims which follow and in the preceding description of the invention, except where the context requires otherwise due to express language or necessary implication, the word "comprise" or variations such as "comprises" or "comprising" is used in an inclusive

sense, i.e. to specify the presence of the stated features but not to preclude the presence or addition of further features in various embodiments of the invention.

It is to be understood that a reference herein to a prior art document does not constitute an admission that the document forms part of the common general knowledge in the art in Australia or any other country.

The Claims defining the invention are as follows:

1. A method of modulating an immune disorder or condition in a subject, comprising administering to said subject an effective amount of an IL-33 antagonist comprising:
 - a. a binding composition derived from the antigen binding site of an antibody that specifically binds to mature human IL-33;
 - b. anti-sense nucleic acid to IL-33; or
 - c. small interference RNA (siRNA) to IL-33,
wherein the mature human IL-33 comprises residues 112-270 of SEQ ID NO:2.

2. Use of an IL-33 antagonist comprising:
 - a. a binding composition derived from the antigen binding site of an antibody that specifically binds to mature human IL-33;
 - b. anti-sense nucleic acid to IL-33; or
 - c. small interference RNA (siRNA) to IL-33,
wherein the mature human IL-33 comprises residues 112-270 of SEQ ID NO:2,in the manufacture of a medicament for modulating an immune disorder or condition in a subject.

3. The method of claim 1, or the use of claim 2, wherein the disorder or condition comprises:
 - a. asthma or allergy involving a pathogenic TH2-type response;
 - b. multiple sclerosis;
 - c. an inflammatory bowel disorder; or
 - d. arthritis.

4. The method of claim 1, or the use of claim 2, wherein the binding composition comprises:
 - a. a monoclonal antibody, or a fragment thereof;
 - b. a humanized antibody, or a fragment thereof; or
 - c. an Fab, Fv, or F(ab')₂ antibody fragment.

5. A method of modulating an immune disorder or condition in a subject, comprising administering to said subject an effective amount of an IL-33 agonist comprising:

- a. mature human IL-33 consisting of residues 112-270 of SEQ ID NO:2; or
 - b. a nucleic acid encoding mature human IL-33.
6. Use of an IL-33 agonist comprising:
- a. mature human IL-33 consisting of residues 112-270 of SEQ ID NO:2; or
 - b. a nucleic acid encoding mature human IL-33,
- in the manufacture of a medicament for modulating an immune disorder or condition in a subject.
- 7.. The method of claim 5, or the use of claim 6, wherein the immune disorder or condition comprises a TH1-type response.
8. The method of claim 5, or the use of claim 6, wherein the immune disorder or condition comprises:
- a. innate response;
 - b. infection; or
 - c. a cancer or tumor.
9. A method of modulating an immune disorder or condition in a subject comprising administering to said subject an effective amount of a composition that blocks the binding of human IL-33 (residues 112-270 of SEQ ID NO: 2) to a receptor comprising human T1/ST2, wherein the composition comprises:
- a. a binding composition derived from the antigen-binding site of an antibody that specifically binds to human IL-33;
 - b. anti-sense nucleic acid to IL-33; or
 - c. small interference RNA (siRNA) to IL-33.
10. The method of claim 9, wherein the disorder or condition comprises:
- a. asthma or allergy involving a pathogenic TH2-type response;
 - b. multiple sclerosis;
 - c. an inflammatory bowel disorder; or
 - d. arthritis.

11. The method of Claim 9, wherein the binding composition comprises:
 - a. a monoclonal antibody, or a fragment thereof;
 - b. a humanized antibody, or a fragment thereof; or
 - c. an Fab, Fv, or F(ab')₂ antibody fragment.

12. A complex comprising:
 - a. mature human IL-33 (residues 112-270 of SEQ ID NO: 2); and
 - b. human T1/ST2.

13. A method of inhibiting the activity of T1/ST2 in a subject comprising administering to said subject a therapeutically effective amount of an antagonist of IL-33 selected from the group consisting of:
 - a. a binding composition derived from the antigen-binding site of an antibody that specifically binds to human IL-33;
 - b. anti-sense nucleic acid to IL-33; and
 - c. small interference RNA (siRNA) to IL-33.

14. Use of an antagonist of IL-33 selected from the group consisting of:
 - a. a binding composition derived from the antigen-binding site of an antibody that specifically binds to human IL-33;
 - b. anti-sense nucleic acid to IL-33; and
 - c. small interference RNA (siRNA) to IL-33,in the manufacture of a medicament for inhibiting the activity of T1/T2 in a subject.

15. The method of claim 1, 5, 9 or 13, the use of claim 2, 6, or 14, or the complex of claim 12, substantially as hereinbefore described with reference to any one of the Examples.

1/3

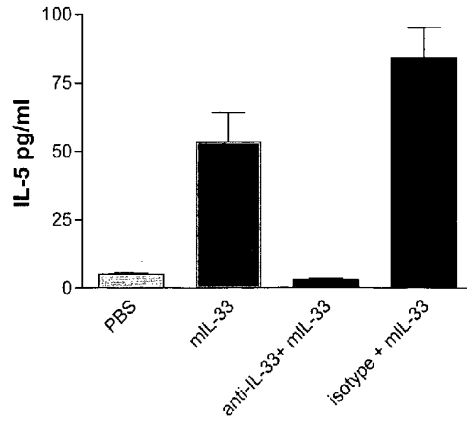


FIG. 1

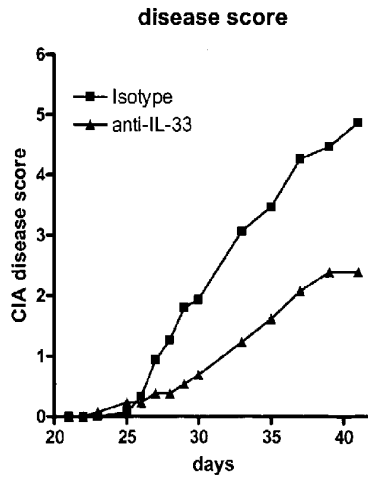


FIG. 2

2/3

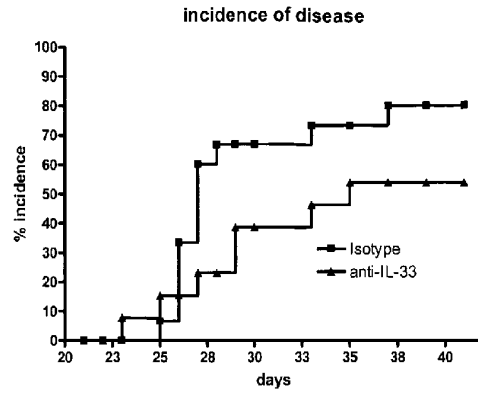


FIG. 3

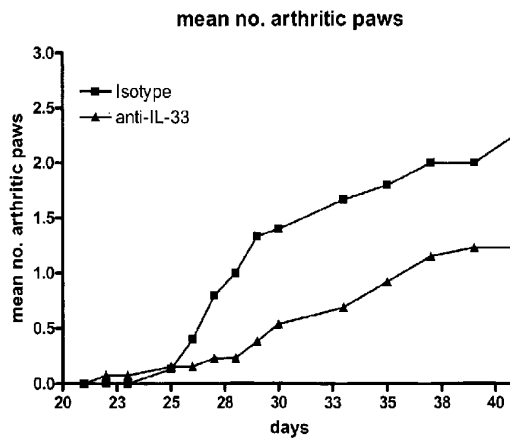


FIG. 4

3/3

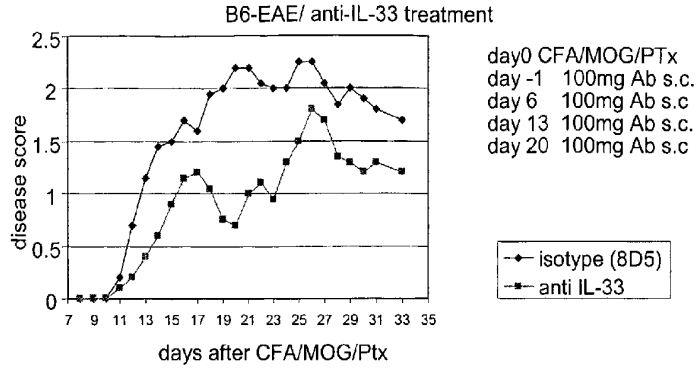


FIG. 5

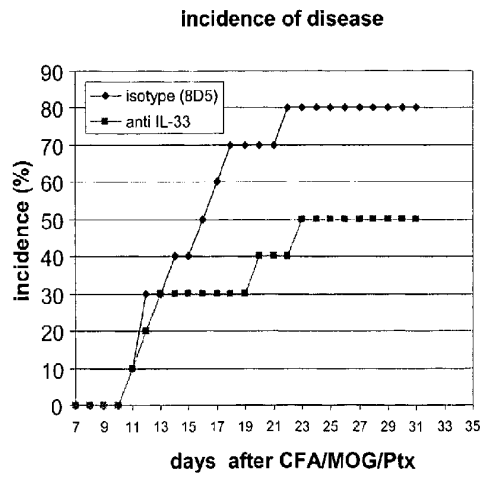


FIG. 6

SEQUENCE LISTING

<110> Schering Corporation

<120> METHODS OF MODULATING A MAMMALIAN CYTOKINE

<130> DX06173

<150> US 60/545,730

<151> 2004-02-17

<160> 12

<170> PatentIn version 3.2

<210> 1

<211> 813

<212> DNA

<213> Homo sapiens

<400> 1

atgaagccta	aatgaagta	ttcaaccaac	aaaatttcca	cagcaaagtg	gaagaacaca	60
gcaagcaaag	ccttgtgttt	caagctggga	aatcccaac	agaaggccaa	agaagtttgc	120
cccatgtact	ttatgaagct	ccgctctggc	cttatgataa	aaaaggaggc	ctgttacttt	180
aggagagaaa	ccaccaaaaag	gccttcactg	aaaacaggta	gaaagcacia	aagacatctg	240
gtactcgtcg	cctgtcaaca	gcagtctact	gtggagtgct	ttgcctttgg	tatatcaggg	300
gtccagaaat	atactagagc	acttcatgat	tcaagtatca	caggaatttc	acctattaca	360
gagtatcttg	cttctctaag	cacatacaat	gatcaatcca	ttacttttgc	tttgagggat	420
gaaagttagt	agatatatgt	tgaagacttg	aaaaaagatg	aaaagaaaga	taagggtgta	480
ctgagttact	atgagtctca	acaccctca	aatgaatcag	gtgacggtgt	tgatggtaag	540
atgttaatgg	taaccctgag	tccatacaaaa	gacttctggt	tgcatgccaa	caacaaggaa	600
cactctgtgg	agctccataa	gtgtgaaaaa	ccactgccag	accaggcctt	ctttgtcctt	660
cataaatatgc	actccaactg	tgtttcattt	gaatgcaaga	ctgatcctgg	agtgtttata	720
gggtgaaagg	ataatcatct	tgctctgatt	aaagtagact	cttctgagaa	tttgtgtact	780
gaaaatatct	tgtttaagct	ctctgaaact	tag			813

<210> 2

<211> 270

<212> PRT

<213> Homo sapiens

<400> 2

Met	Lys	Pro	Lys	Met	Lys	Tyr	Ser	Thr	Asn	Lys	Ile	Ser	Thr	Ala	Lys
1			5						10					15	

- 2 -

Trp Lys Asn Thr Ala Ser Lys Ala Leu Cys Phe Lys Leu Gly Lys Ser
 20 25 30

Gln Gln Lys Ala Lys Glu Val Cys Pro Met Tyr Phe Met Lys Leu Arg
 35 40 45

Ser Gly Leu Met Ile Lys Lys Glu Ala Cys Tyr Phe Arg Arg Glu Thr
 50 55 60

Thr Lys Arg Pro Ser Leu Lys Thr Gly Arg Lys His Lys Arg His Leu
 65 70 75 80

Val Leu Ala Ala Cys Gln Gln Gln Ser Thr Val Glu Cys Phe Ala Phe
 85 90 95

Gly Ile Ser Gly Val Gln Lys Tyr Thr Arg Ala Leu His Asp Ser Ser
 100 105 110

Ile Thr Gly Ile Ser Pro Ile Thr Glu Tyr Leu Ala Ser Leu Ser Thr
 115 120 125

Tyr Asn Asp Gln Ser Ile Thr Phe Ala Leu Glu Asp Glu Ser Tyr Glu
 130 135 140

Ile Tyr Val Glu Asp Leu Lys Lys Asp Glu Lys Lys Asp Lys Val Leu
 145 150 155 160

Leu Ser Tyr Tyr Glu Ser Gln His Pro Ser Asn Glu Ser Gly Asp Gly
 165 170 175

Val Asp Gly Lys Met Leu Met Val Thr Leu Ser Pro Thr Lys Asp Phe
 180 185 190

Trp Leu His Ala Asn Asn Lys Glu His Ser Val Glu Leu His Lys Cys
 195 200 205

Glu Lys Pro Leu Pro Asp Gln Ala Phe Phe Val Leu His Asn Met His
 210 215 220

Ser Asn Cys Val Ser Phe Glu Cys Lys Thr Asp Pro Gly Val Phe Ile
 225 230 235 240

Gly Val Lys Asp Asn His Leu Ala Leu Ile Lys Val Asp Ser Ser Glu
 245 250 255

Asn Leu Cys Thr Glu Asn Ile Leu Phe Lys Leu Ser Glu Thr
 260 265 270

<210> 3
 <211> 801
 <212> DNA
 <213> Mus musculus

<400> 3
 atgagaccta gaatgaagta ttccaactcc aagatttccc cggcaaagtt cagcagcacc 60
 gcaggggaaac gctcgggtccc gccttgcaaa ataagaagat cccaacagaa gaccaaaagaa 120
 ttctgccatg tctactgcat gagactccgt tctggcctca ccataagaaa ggagactagt 180
 tatttttagga aagaaccac gaaaagatat tcaactaaaat cggglaccaa gcatgaagag 240
 aacttctctg cctatccacg ggattctagg aagagatcct tgcttggcag tatccaagca 300
 ttgtctcgt ctgttgacac attgagcacc caaggaactt cacttttaac acagtctcct 360
 gcctcctga gtacatacaa tgaccaatct gttagttttg ttttggagaa tggatggtat 420
 gtgatcaatg ttgacgactc tggaaaagac caagagcaag accaggtgct actacgctac 480
 tatgagtctc cctgtcctgc aagtcaatca ggcgacggtg tggatgggaa gaagctgatg 540
 gtgaacatga gttccatcaa agacacagac atctggctgc atgccaacga caaggactac 600
 tccgtggagc ttcaaagggg tgacgtctcg cctccggaac aggcttctt cgtccttcac 660
 aaaaagtcct cggactttgt ttcatttgaa tgcaagaatc ttctggcacc ttacatagga 720
 gtgaaggaca accagctggc tctagtggag gaaaaagatg agagctgcaa caatattatg 780
 tttaaagtct cgaaaaatcta a 801

<210> 4
 <211> 266
 <212> PRT
 <213> Mus musculus

<400> 4

Met Arg Pro Arg Met Lys Tyr Ser Asn Ser Lys Ile Ser Pro Ala Lys
 1 5 10 15
 Phe Ser Ser Thr Ala Gly Glu Ala Leu Val Pro Pro Cys Lys Ile Arg
 20 25 30
 Arg Ser Gln Gln Lys Thr Lys Glu Phe Cys His Val Tyr Cys Met Arg
 35 40 45
 Leu Arg Ser Gly Leu Thr Ile Arg Lys Glu Thr Ser Tyr Phe Arg Lys
 50 55 60

Glu Pro Thr Lys Arg Tyr Ser Leu Lys Ser Gly Thr Lys His Glu Glu
 65 70 75 80

Asn Phe Ser Ala Tyr Pro Arg Asp Ser Arg Lys Arg Ser Leu Leu Gly
 85 90 95

Ser Ile Gln Ala Phe Ala Ala Ser Val Asp Thr Leu Ser Ile Gln Gly
 100 105 110

Thr Ser Leu Leu Thr Gln Ser Pro Ala Ser Leu Ser Thr Tyr Asn Asp
 115 120 125

Gln Ser Val Ser Phe Val Leu Glu Asn Gly Cys Tyr Val Ile Asn Val
 130 135 140

Asp Asp Ser Gly Lys Asp Gln Glu Gln Asp Gln Val Leu Leu Arg Tyr
 145 150 155 160

Tyr Glu Ser Pro Cys Pro Ala Ser Gln Ser Gly Asp Gly Val Asp Gly
 165 170 175

Lys Lys Leu Met Val Asn Met Ser Pro Ile Lys Asp Thr Asp Ile Trp
 180 185 190

Leu His Ala Asn Asp Lys Asp Tyr Ser Val Glu Leu Gln Arg Gly Asp
 195 200 205

Val Ser Pro Pro Glu Gln Ala Phe Phe Val Leu His Lys Lys Ser Ser
 210 215 220

Asp Phe Val Ser Phe Glu Cys Lys Asn Leu Pro Gly Thr Tyr Ile Gly
 225 230 235 240

Val Lys Asp Asn Gln Leu Ala Leu Val Glu Glu Lys Asp Glu Ser Cys
 245 250 255

Asn Asn Ile Met Phe Lys Leu Ser Lys Ile
 260 265

<210> 5
 <211> 1671
 <212> DNA
 <213> Homo sapiens

<400> 5
 atggggtttt ggatccttagc aattotcaca attotcatgt attccacagc agcaaagttt 60

agtaaacaat catggggcct ggaaaatgag gctttaattg taagatgtcc tagacaagga 120
 aaacctagtt acaccgtgga ttggtattac tcacaaaca acaaaagtat tcccactcag 180
 gaaagaaatc gtgtgtttgc ctcaggcaca cttctgaagt ttctaccagc tgaagttgct 240
 gattctggtt tttataacctg tattgtcaga agtcccacat tcaataggac tggatatgctg 300
 aatgtcacca tatataaaaa acaatcagat tgcaatgttc cagattatct gatgtattca 360
 acagtatctg gatcagaaaa aaattccaaa atttattgtc ctaccattga cctctacaac 420
 tggacagcac ctcttgagtg gtttaagaat tgtcaggctc ttcaaggatc aaggtacagg 480
 gcgcacaagt ctttttggc cattgataat gtgatgactg aggacgcagg tgattacacc 540
 tghtaaatga tacacaatga aaatggagcc aattatagtg tgacggcgac caggctcttc 600
 acggtcaagg atgagcaagg cttttctctg ttccagtaa tggagcccc tcacaaaaat 660
 gaaataaagg aagtggaaat tggaaaaaac gcaaacctaa ctgctctgctc ttgttttggg 720
 aaaggcactc agttcttggc tgcctctctg tggcagctta atggaacaaa aattacagac 780
 ttggtgtaac caagaattca acaagaggaa gggcaaaatc aaagtttcag caatgggctg 840
 gcttctctag acatggtttt aagaatagct gacgtgaagg aagaggatct attgctgcag 900
 tacgactgtc tggccctgaa tttgcatggc ttgagaaggc acaccgtaag actaagtagg 960
 aaaaatccaa ttgatcatca tagcatctac tgcataattg cagtatgtag tgtattttta 1020
 atgctaataca atgtcctggt tatcatccta aaaatgttct ggattgaggc cactctgctc 1080
 tggagagaca tagctaaacc ttacaagact aggaatgatg gaaagctcta tgatgcttat 1140
 gttgtctacc cacggaacta caaatccagt acagatgggg ccagtcgtgt agagcacttt 1200
 gttcaccaga ttctgcctga tgttcttgaa aataaatgtg gctatacctt atgcatttat 1260
 gggagagata tgctacctgg agaagatgta gtcaactgcag tggaaaccaa catacgaag 1320
 agcaggcggc acattttcat cctgacccct cagatcactc acaataagga gtttgcctac 1380
 gagcaggagg ttgccctgca ctgtgccctc atccagaacg acgccaaggt gatacttatt 1440
 gagatggagg ctctgagcga gctggacatg ctgcaggctg aggcgcttca ggactcctc 1500
 cagcatctta tgaagtaca ggggaccatc aagtggaggg aggaccacat tgccaataaa 1560
 aggtccctga attccaaatt ctggaagcac gtgaggtacc aaatgctctg gccaaagaaa 1620
 attccagaa aggcctctag tttgactccc ttggctgccc agaagcaata g 1671

<210> 6
 <211> 556
 <212> PRT
 <213> Homo sapiens

<400> 6

Met Gly Phe Trp Ile Leu Ala Ile Leu Thr Ile Leu Met Tyr Ser Thr
 1 5 10 15

Ala Ala Lys Phe Ser Lys Gln Ser Trp Gly Leu Glu Asn Glu Ala Leu
 20 25 30

Ile Val Arg Cys Pro Arg Gln Gly Lys Pro Ser Tyr Thr Val Asp Trp
 35 40 45

Tyr Tyr Ser Gln Thr Asn Lys Ser Ile Pro Thr Gln Glu Arg Asn Arg
 50 55 60

Val Phe Ala Ser Gly Gln Leu Leu Lys Phe Leu Pro Ala Glu Val Ala
 65 70 75 80

Asp Ser Gly Ile Tyr Thr Cys Ile Val Arg Ser Pro Thr Phe Asn Arg
 85 90 95

Thr Gly Tyr Ala Asn Val Thr Ile Tyr Lys Lys Gln Ser Asp Cys Asn
 100 105 110

Val Pro Asp Tyr Leu Met Tyr Ser Thr Val Ser Gly Ser Glu Lys Asn
 115 120 125

Ser Lys Ile Tyr Cys Pro Thr Ile Asp Leu Tyr Asn Trp Thr Ala Pro
 130 135 140

Leu Glu Trp Phe Lys Asn Cys Gln Ala Leu Gln Gly Ser Arg Tyr Arg
 145 150 155 160

Ala His Lys Ser Phe Leu Val Ile Asp Asn Val Met Thr Glu Asp Ala
 165 170 175

Gly Asp Tyr Thr Cys Lys Phe Ile His Asn Glu Asn Gly Ala Asn Tyr
 180 185 190

Ser Val Thr Ala Thr Arg Ser Phe Thr Val Lys Asp Glu Gln Gly Phe
 195 200 205

Ser Leu Phe Pro Val Ile Gly Ala Pro Ala Gln Asn Glu Ile Lys Glu
 210 215 220

Val Glu Ile Gly Lys Asn Ala Asn Leu Thr Cys Ser Ala Cys Phe Gly
 225 230 235 240

Lys Gly Thr Gln Phe Leu Ala Ala Val Leu Trp Gln Leu Asn Gly Thr
 245 250 255

Lys Ile Thr Asp Phe Gly Glu Pro Arg Ile Gln Gln Glu Glu Gly Gln
 260 265 270

Asn Gln Ser Phe Ser Asn Gly Leu Ala Cys Leu Asp Met Val Leu Arg
 275 280 285

Ile Ala Asp Val Lys Glu Glu Asp Leu Leu Leu Gln Tyr Asp Cys Leu
 290 295 300

Ala Leu Asn Leu His Gly Leu Arg Arg His Thr Val Arg Leu Ser Arg
 305 310 315 320

Lys Asn Pro Ile Asp His His Ser Ile Tyr Cys Ile Ile Ala Val Cys
 325 330 335

Ser Val Phe Leu Met Leu Ile Asn Val Leu Val Ile Ile Leu Lys Met
 340 345 350

Phe Trp Ile Glu Ala Thr Leu Leu Trp Arg Asp Ile Ala Lys Pro Tyr
 355 360 365

Lys Thr Arg Asn Asp Gly Lys Leu Tyr Asp Ala Tyr Val Val Tyr Pro
 370 375 380

Arg Asn Tyr Lys Ser Ser Thr Asp Gly Ala Ser Arg Val Glu His Phe
 385 390 395 400

Val His Gln Ile Leu Pro Asp Val Leu Glu Asn Lys Cys Gly Tyr Thr
 405 410 415

Leu Cys Ile Tyr Gly Arg Asp Met Leu Pro Gly Glu Asp Val Val Thr
 420 425 430

Ala Val Glu Thr Asn Ile Arg Lys Ser Arg Arg His Ile Phe Ile Leu
 435 440 445

Thr Pro Gln Ile Thr His Asn Lys Glu Phe Ala Tyr Glu Gln Glu Val
 450 455 460

Ala Leu His Cys Ala Leu Ile Gln Asn Asp Ala Lys Val Ile Leu Ile
 465 470 475 480

Glu Met Glu Ala Leu Ser Glu Leu Asp Met Leu Gln Ala Glu Ala Leu
 485 490 495

Gln Asp Ser Leu Gln His Leu Met Lys Val Gln Gly Thr Ile Lys Trp
 500 505 510

Arg Glu Asp His Ile Ala Asn Lys Arg Ser Leu Asn Ser Lys Phe Trp
 515 520 525

Lys His Val Arg Tyr Gln Met Pro Val Pro Ser Lys Ile Pro Arg Lys
 530 535 540

Ala Ser Ser Leu Thr Pro Leu Ala Ala Gln Lys Gln
 545 550 555

<210> 7
 <211> 1704
 <212> DNA
 <213> Mus musculus

<400> 7
 atgattgaca gacagagaat gggactttgg gctttggcaa ttctgacact tcccatgtat 60
 ttgacagtta cggagggcag taaatcgtcc tggggtctgg aaaatgagge tttaattgtg 120
 agatgcccc aaagaggacg ctcgacttat cctgtggaat ggtattactc agatacaaat 180
 gaaagtattc ctactcaaaa aagaaatcgg atctttgtct caagagatcg tctgaagttt 240
 ctaccagcca gagtgaaga ctctgggatt tatgcttgtg ttatcagaag ccccaacttg 300
 aataagactg gatacttgaa tgtcaccata cataaaaagc cgccaagctg caatatccct 360
 gattatttga tgtactcgac agtacgtgga tcagataaaa atttcaagat aacgtgtcca 420
 acaattgacc tgtataattg gacagcacct gtccagtgg ttaagaactg caaagctctc 480
 caagagccaa ggttcagggc acacaggtcc tacttgttca ttgacaacgt gactcatgat 540
 gatgaaggtg actacacttg tcaattcaca cacgaggaga atggaaccaa ctacatcgtg 600
 acggccacca gatcattcac agttgaagaa aaaggctttt ctatgtttcc agtaattaca 660
 aatcctccat acaaccacac aatggaagtg gaaataggaa aaccagcaag tattgcctgt 720
 tcagcttget ttggcaaagg ctctcacttc ttggctgatg tcctgtggca gattaacaaa 780
 acagtagttg gaaattttgg tgaagcaaga attcaagaag aggaaggtcg aatgaaagt 840
 tccagcaatg acatggattg ttaacctca gtgtaagga taactggtgt gacagaaaag 900
 gacctgtccc tggaatatga ctgtctggcc ctgaaccttc atggcatgat aaggcacacc 960
 ataaggctga gaaggaaca accaattgat caccgaagca tctactacat agttgctgga 1020
 tgtagtttat tgctaattgt tatcaatgtc ttggtgatag tottaaaagt gttctggatt 1080

gaggttgctc tgttctggag agatatagtg acaccttaca aaacccggaa cgatggcaag 1140
 ctctacgatg cgtacatcat ttaccctcgg gtcttccggg gcagcgcggc ggaacccac 1200
 tctgtggagt actttgttca ccacactctg cccgacgttc ttgaaaataa atgtggctac 1260
 aaattgtgca tttatgggag agacctgtta cctgggcaag atgcagccac cgtggtggaa 1320
 agcagtatcc agaatagcag aagacaggtg tttgttctgg cccctcacat gatgcacagc 1380
 aaggaatttg cctacgagca ggagattgct ctgcacagcg cccctcatcca gaacaactcc 1440
 aaggtgattc ttattgaaat ggagcctctg ggtgaggcaa gccgactaca ggttggggac 1500
 ctgcaagatt ctctccagca tcttgtgaaa attcagggga ccatcaagtg gaggggaagat 1560
 catgtggccg acaagcagtc tctaagtctc aaattctgga agcatgtgag gtaccaaagt 1620
 ccagtgccag aaagagcctc caagacggca tctgttgccg ctccggtgag tggcaaggca 1680
 tgcttagacc tgaaacactt ttga 1704

<210> 8
 <211> 567
 <212> PRT
 <213> Mus musculus

<400> 8

Met Ile Asp Arg Gln Arg Met Gly Leu Trp Ala Leu Ala Ile Leu Thr
 1 5 10 15

Leu Pro Met Tyr Leu Thr Val Thr Glu Gly Ser Lys Ser Ser Trp Gly
 20 25 30

Leu Glu Asn Glu Ala Leu Ile Val Arg Cys Pro Gln Arg Gly Arg Ser
 35 40 45

Thr Tyr Pro Val Glu Trp Tyr Tyr Ser Asp Thr Asn Glu Ser Ile Pro
 50 55 60

Thr Gln Lys Arg Asn Arg Ile Phe Val Ser Arg Asp Arg Leu Lys Phe
 65 70 75 80

Leu Pro Ala Arg Val Glu Asp Ser Gly Ile Tyr Ala Cys Val Ile Arg
 85 90 95

Ser Pro Asn Leu Asn Lys Thr Gly Tyr Leu Asn Val Thr Ile His Lys
 100 105 110

Lys Pro Pro Ser Cys Asn Ile Pro Asp Tyr Leu Met Tyr Ser Thr Val
 115 120 125

Arg Gly Ser Asp Lys Asn Phe Lys Ile Thr Cys Pro Thr Ile Asp Leu
 130 135 140

Tyr Asn Trp Thr Ala Pro Val Gln Trp Phe Lys Asn Cys Lys Ala Leu
 145 150 155 160

Gln Glu Pro Arg Phe Arg Ala His Arg Ser Tyr Leu Phe Ile Asp Asn
 165 170 175

Val Thr His Asp Asp Glu Gly Asp Tyr Thr Cys Gln Phe Thr His Ala
 180 185 190

Glu Asn Gly Thr Asn Tyr Ile Val Thr Ala Thr Arg Ser Phe Thr Val
 195 200 205

Glu Glu Lys Gly Phe Ser Met Phe Pro Val Ile Thr Asn Pro Pro Tyr
 210 215 220

Asn His Thr Met Glu Val Glu Ile Gly Lys Pro Ala Ser Ile Ala Cys
 225 230 235 240

Ser Ala Cys Phe Gly Lys Gly Ser His Phe Leu Ala Asp Val Leu Trp
 245 250 255

Gln Ile Asn Lys Thr Val Val Gly Asn Phe Gly Glu Ala Arg Ile Gln
 260 265 270

Glu Glu Glu Gly Arg Asn Glu Ser Ser Ser Asn Asp Met Asp Cys Leu
 275 280 285

Thr Ser Val Leu Arg Ile Thr Gly Val Thr Glu Lys Asp Leu Ser Leu
 290 295 300

Glu Tyr Asp Cys Leu Ala Leu Asn Leu His Gly Met Ile Arg His Thr
 305 310 315 320

Ile Arg Leu Arg Arg Lys Gln Pro Ile Asp His Arg Ser Ile Tyr Tyr
 325 330 335

Ile Val Ala Gly Cys Ser Leu Leu Leu Met Phe Ile Asn Val Leu Val
 340 345 350

Ile Val Leu Lys Val Phe Trp Ile Glu Val Ala Leu Phe Trp Arg Asp
 355 360 365

Ile Val Thr Pro Tyr Lys Thr Arg Asn Asp Gly Lys Leu Tyr Asp Ala
 370 375 380

Tyr Ile Ile Tyr Pro Arg Val Phe Arg Gly Ser Ala Ala Gly Thr His
 385 390 395 400

Ser Val Glu Tyr Phe Val His His Thr Leu Pro Asp Val Leu Glu Asn
 405 410 415

Lys Cys Gly Tyr Lys Leu Cys Ile Tyr Gly Arg Asp Leu Leu Pro Gly
 420 425 430

Gln Asp Ala Ala Thr Val Val Glu Ser Ser Ile Gln Asn Ser Arg Arg
 435 440 445

Gln Val Phe Val Leu Ala Pro His Met Met His Ser Lys Glu Phe Ala
 450 455 460

Tyr Glu Gln Glu Ile Ala Leu His Ser Ala Leu Ile Gln Asn Asn Ser
 465 470 475 480

Lys Val Ile Leu Ile Glu Met Glu Pro Leu Gly Glu Ala Ser Arg Leu
 485 490 495

Gln Val Gly Asp Leu Gln Asp Ser Leu Gln His Leu Val Lys Ile Gln
 500 505 510

Gly Thr Ile Lys Trp Arg Glu Asp His Val Ala Asp Lys Gln Ser Leu
 515 520 525

Ser Ser Lys Phe Trp Lys His Val Arg Tyr Gln Met Pro Val Pro Glu
 530 535 540

Arg Ala Ser Lys Thr Ala Ser Val Ala Ala Pro Leu Ser Gly Lys Ala
 545 550 555 560

Cys Leu Asp Leu Lys His Phe
 565

<210> 9
 <211> 1233
 <212> DNA
 <213> Homo sapiens

<400> 9
 atgccaggtg tctgtgatag ggccctgac ttctctccc cgtctgaaga ccaggtgctg 60
 aggcctgcct tgggcagctc agtggctctg aactgcaagg cttgggtagt ctctgggccc 120

cactgctccc tgccttcagt ccagtggtg aaagacgggc ttccattggg aattgggggc 180
 cactacagcc tccacgagta ctctggggtc aaggccaacc tgtcagaggt gcttgtgtcc 240
 agtgtcctgg gggtaaacgt gaccagcact gaagtctatg gggccttcac ctgctccatc 300
 cagaacatca gcttctctc ctteactctt cagagagctg gccctacaag ccacgtggct 360
 ggggtgctgg cctccctcct ggtcctgctg gccctgctgc tggcggccct gctctatgto 420
 aagtgcctgc tcaacgtgct gctctggtag caggacgctg atggggaggt ggagataaac 480
 gacgggaagc tctacgagc ctacgtctcc tacagcagct gccccgagga ccgcaagtcc 540
 gtgaacttca tcctaaagcc gcagctggag cggcgtcggg gctacaagct ctctcggac 600
 gaccgcgacc tctgcccgg cgctgagccc tccgcccacc tcttggtgaa cctgagccgc 660
 tgcgcagccc tcatcgtggt gctttcggac gccttcctga gccgggcctg gtgcagccac 720
 agcttccggg agggcctgtg ccgctgctg gagctcacc gcagaccat ctctcacc 780
 ttctaggggc agaggcgcga ccccgcgac ccggcgtcc gcctgctgag ccagcacgcg 840
 cacctgggtg ccttgtgct ctggaggccc ggctccgtga ctctctctc cgatttttgg 900
 aaagaagtgc agctggcgt gcccggaag gtgcggtaca gcccggtgga aggagacccc 960
 cagacgcagc tgcaggacga caaggacccc atgctgattc ttctaggccg agtccctgag 1020
 ggcggggccc tggactcaga ggtggacccg gaccctgagg gcgacctggg tgtccggggg 1080
 cctgtttttg gagagccatc agctccaccg cacaccagtg gggctctcgt gggagagagc 1140
 cggagcagcg aagtggacgt ctccgatctc ggctcgcgaa actacagtgc ccgcacagac 1200
 ttctactgcc tgggttccaa ggatgatatg tag 1233

<210> 10
 <211> 410
 <212> PRT
 <213> Homo sapiens

<400> 10

Met Pro Gly Val Cys Asp Arg Ala Pro Asp Phe Leu Ser Pro Ser Glu
 1 5 10 15

Asp Gln Val Leu Arg Pro Ala Leu Gly Ser Ser Val Ala Leu Asn Cys
 20 25 30

Thr Ala Trp Val Val Ser Gly Pro His Cys Ser Leu Pro Ser Val Gln
 35 40 45

Trp Leu Lys Asp Gly Leu Pro Leu Gly Ile Gly Gly His Tyr Ser Leu
 50 55 60

His Glu Tyr Ser Trp Val Lys Ala Asn Leu Ser Glu Val Leu Val Ser
 65 70 75 80

Ser Val Leu Gly Val Asn Val Thr Ser Thr Glu Val Tyr Gly Ala Phe
 85 90 95

Thr Cys Ser Ile Gln Asn Ile Ser Phe Ser Ser Phe Thr Leu Gln Arg
 100 105 110

Ala Gly Pro Thr Ser His Val Ala Ala Val Leu Ala Ser Leu Leu Val
 115 120 125

Leu Leu Ala Leu Leu Leu Ala Ala Leu Leu Tyr Val Lys Cys Arg Leu
 130 135 140

Asn Val Leu Leu Trp Tyr Gln Asp Ala Tyr Gly Glu Val Glu Ile Asn
 145 150 155 160

Asp Gly Lys Leu Tyr Asp Ala Tyr Val Ser Tyr Ser Asp Cys Pro Glu
 165 170 175

Asp Arg Lys Phe Val Asn Phe Ile Leu Lys Pro Gln Leu Glu Arg Arg
 180 185 190

Arg Gly Tyr Lys Leu Phe Leu Asp Asp Arg Asp Leu Leu Pro Arg Ala
 195 200 205

Glu Pro Ser Ala Asp Leu Leu Val Asn Leu Ser Arg Cys Arg Arg Leu
 210 215 220

Ile Val Val Leu Ser Asp Ala Phe Leu Ser Arg Ala Trp Cys Ser His
 225 230 235 240

Ser Phe Arg Glu Gly Leu Cys Arg Leu Leu Glu Leu Thr Arg Arg Pro
 245 250 255

Ile Phe Ile Thr Phe Glu Gly Gln Arg Arg Asp Pro Ala His Pro Ala
 260 265 270

Leu Arg Leu Leu Arg Gln His Arg His Leu Val Thr Leu Leu Leu Trp
 275 280 285

Arg Pro Gly Ser Val Thr Pro Ser Ser Asp Phe Trp Lys Glu Val Gln
 290 295 300

Leu Ala Leu Pro Arg Lys Val Arg Tyr Arg Pro Val Glu Gly Asp Pro
 305 310 315 320

Gln Thr Gln Leu Gln Asp Asp Lys Asp Pro Met Leu Ile Leu Arg Gly
 325 330 335

Arg Val Pro Glu Gly Arg Ala Leu Asp Ser Glu Val Asp Pro Asp Pro
 340 345 350

Glu Gly Asp Leu Gly Val Arg Gly Pro Val Phe Gly Glu Pro Ser Ala
 355 360 365

Pro Pro His Thr Ser Gly Val Ser Leu Gly Glu Ser Arg Ser Ser Glu
 370 375 380

Val Asp Val Ser Asp Leu Gly Ser Arg Asn Tyr Ser Ala Arg Thr Asp
 385 390 395 400

Phe Tyr Cys Leu Val Ser Lys Asp Asp Met
 405 410

<210> 11
 <211> 1229
 <212> DNA
 <213> Mus musculus

<400> 11
 atggcaggtg tctgtgacat ggcccctaatt ttcctttccc catctgaaga ccaggccttg 60
 ggtcttgccc ttggcagaga agttgctttg aattgcacag cttgggtggt ctctaggccc 120
 cagtgtcccc agccatcagt gcagtggctg aaagatggtc tggcattggg caatggaagc 180
 cacttcagcc tccatgagga cttctgggtc agcgccaact tctcagagat tgtgtccagt 240
 gtctctggtg tcaacttgac caatgcagag gactatggaa ccttcacctg ttctgtctgg 300
 aatgtcagct cccattcctt cactctttgg cgagctggcc ctgctggcca tgtggctgca 360
 gtaactggctt ccctcctggt cctgggtggt ctgctgctgg tggccctgct ctatgttaag 420
 tgtcggctga acatgctgct ttggtaccaa gacacttacg gggaggtyga gatgaacgat 480
 gggaaagttat acgatgccta cgtgtcctat agcgactgcc cagaggaccg caaatttcta 540
 aattttatc tgaagcctca gttggagcgg cgtcgggat acaaactctt cctagaggac 600
 cgcgacctct tgccctgcgc ggagccctct gccgacctt tggatgaacct gactcgtgt 660
 cggcgtctca tcgtggttct ttcagatgcc ttcctaagcc ggcctggtg tagccagagc 720

- 15 -

ttccgggagg gactgtgccg cctactggag ctcacccgca gacctatctt catcaccttt 780
 gagggccaga ggcgtgagcc catacacctt gctctccggc tectgcgcca gcaccgccac 840
 ctctgacccc tgggtctttg gaagcctggc tccgtgactc ctctctctga tttttggaaa 900
 gagctacagc tagcactgcc acgaaggtgc agtacaggcc ggtggagggg gaccctcaaa 960
 cccgacttca ggatgacaaa gatcccatgc taatcgtgag aggacgtgct gccaggggcc 1020
 ggggcatgga gtcagagctg gatccagacc ctgagggaga cctgggtgtc cgtggacctg 1080
 tttttgggga gccaccaact ccaactgcagg aaaccaggat ctgcatagga gagagccacg 1140
 gcagtgaaat ggatgtctct gacctcggct ctcgaaacta cagtgcacgg acagacttct 1200
 actgcctcgt gtctgaggat gatgtgtag 1229

<210> 12
 <211> 409
 <212> PRT
 <213> Mus musculus

<400> 12

Met Ala Gly Val Cys Asp Met Ala Pro Asn Phe Leu Ser Pro Ser Glu
1 5 10 15

Asp Gln Ala Leu Gly Leu Ala Leu Gly Arg Glu Val Ala Leu Asn Cys
20 25 30

Thr Ala Trp Val Phe Ser Arg Pro Gln Cys Pro Gln Pro Ser Val Gln
35 40 45

Trp Leu Lys Asp Gly Leu Ala Leu Gly Asn Gly Ser His Phe Ser Leu
50 55 60

His Glu Asp Phe Trp Val Ser Ala Asn Phe Ser Glu Ile Val Ser Ser
65 70 75 80

Val Leu Val Leu Asn Leu Thr Asn Ala Glu Asp Tyr Gly Thr Phe Thr
85 90 95

Cys Ser Val Trp Asn Val Ser Ser His Ser Phe Thr Leu Trp Arg Ala
100 105 110

Gly Pro Ala Gly His Val Ala Ala Val Leu Ala Ser Leu Leu Val Leu
115 120 125

Val Val Leu Leu Leu Val Ala Leu Leu Tyr Val Lys Cys Arg Leu Asn
130 135 140

Met Leu Leu Trp Tyr Gln Asp Thr Tyr Gly Glu Val Glu Met Asn Asp
 145 150 155 160

Gly Lys Leu Tyr Asp Ala Tyr Val Ser Tyr Ser Asp Cys Pro Glu Asp
 165 170 175

Arg Lys Phe Val Asn Phe Ile Leu Lys Pro Gln Leu Glu Arg Arg Arg
 180 185 190

Gly Tyr Lys Leu Phe Leu Glu Asp Arg Asp Leu Leu Pro Arg Ala Glu
 195 200 205

Pro Ser Ala Asp Leu Leu Val Asn Leu Ser Arg Cys Arg Arg Leu Ile
 210 215 220

Val Val Leu Ser Asp Ala Phe Leu Ser Arg Pro Trp Cys Ser Gln Ser
 225 230 235 240

Phe Arg Glu Gly Leu Cys Arg Leu Leu Glu Leu Thr Arg Arg Pro Ile
 245 250 255

Phe Ile Thr Phe Glu Gly Gln Arg Arg Glu Pro Ile His Pro Ala Leu
 260 265 270

Arg Leu Leu Arg Gln His Arg His Leu Val Thr Leu Val Leu Trp Lys
 275 280 285

Pro Gly Ser Val Thr Pro Ser Ser Asp Phe Trp Lys Glu Leu Gln Leu
 290 295 300

Ala Leu Pro Arg Lys Val Gln Tyr Arg Pro Val Glu Gly Asp Pro Gln
 305 310 315 320

Thr Arg Leu Gln Asp Asp Lys Asp Pro Met Leu Ile Val Arg Gly Arg
 325 330 335

Ala Ala Gln Gly Arg Gly Met Glu Ser Glu Leu Asp Pro Asp Pro Glu
 340 345 350

Gly Asp Leu Gly Val Arg Gly Pro Val Phe Gly Glu Pro Pro Thr Pro
 355 360 365

Leu Gln Glu Thr Arg Ile Cys Ile Gly Glu Ser His Gly Ser Glu Met
 370 375 380

Asp Val Ser Asp Leu Gly Ser Arg Asn Tyr Ser Ala Arg Thr Asp Phe
385 390 395 400

Tyr Cys Leu Val Ser Glu Asp Asp Val
405