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(54) Title: METHODS OF MODULATING INFLAMMATION AND COMPOSITIONS THEREFORE

(57) Abstract: The present invention provides compositions and methods useful for detecting or treating asthma or other allergic or inflammatory diseases. In one aspect, the methods of the invention include modulating an IL-13/sIL-13Rα2 complex and methods of modulating expression of various nucleotide sequences of interest including Pira1, Vannin1 and ApoA1. In an aspect, the methods of the invention allow detection of an altered IL-13/sIL-13Rα2 complex level in a subject. Further aspects of the invention allow for identification of subjects suitable for inclusion in a sIL-13Rα2 related study or inflammatory related disorder study.

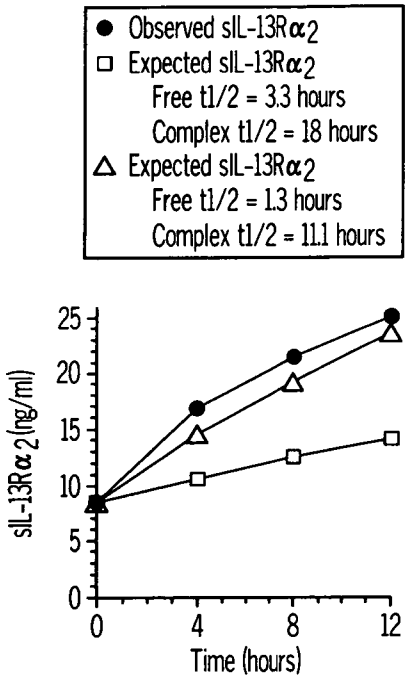

FIG. 9

WO 2008/153790 A2

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METHODS OF MODULATING INFLAMMATION AND COMPOSITIONS THEREFORE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to and benefit of U.S. Provisional Patent Application No:60/932,095, filed on May 29 2007, which is herein incorporated by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] Work for this invention was funded in whole or in part by grants from a Veteran's Administration Merit Award and NIH Grants AI052099, AI55848 and HL076383. The United States government may have certain rights in this invention.

FIELD OF THE INVENTION

[0003] The present invention is directed to compositions for and methods of modulating inflammation, more particularly to modulating inflammation related disorders. Additionally the invention is directed to compositions for and methods of modulating asthmatic disorders, allergic responses and endofibrotic disorders.

BACKGROUND OF THE INVENTION

[0004] The type 2 cytokines, IL-4, IL-5, IL-9, and IL-13 substantially participate in allergic immunopathology and host protection against helminth parasites (Urban *et al.* 1992 *Immunol. Rev.* 127:205-220; Venkayya *et al.* 2002 *Am. J. Respir. Cell Mol. Biol.* 26:202-208; Hershey, G.K. 2003 *J. Allergy Clin. Immunol.* 111:677-690, herein incorporated by reference in their entirety.) Two related cytokines, IL-4 and IL-13 bind to cell membrane receptors that contain IL-4Rα and activate the transcription factor Stat6 (Zurawski *et al.* 1995, *J.Biol. Chem.* 270:13869-13878; Kaplan *et al.* 1996 *Immunity* 4:313-319; and Finkelman *et al* 1999 *Curr Opin Immunol* 11:420-426, herein incorporated by reference in their entirety), and both have prominent effects on multiple cell types that contribute to allergic inflammation. See for example Madden *et al.* 1991 *J. Immunol.* 147:1387-1391; Doucet *et al.* 1998 *J. Clin Invest* 101:2129-2139; Zhu *et al.* 1999 *J. Clin. Invest* 103:779-788; Zhao *et al.* 2003 *J. Immunol.* 171:948-954, herein incorporated by reference in their entirety, among others. However, IL-4 and IL-13 also differ significantly. IL-13 does not signal through the type 1 IL-4 receptor (IL-4R), composed of IL-4Rα and the cytokine receptor common γ chain, γ_c, that is expressed

on lymphocytes, dendritic cells, macrophages, and mast cells (Andersson *et al* 1997 *Eur J Immunol* 27:1762-1768; Schnyder *et al* 1996 *Blood* 87:4286-4295; and He & Malek 1995 *J. Immunol.* 155:9-12; herein incorporated by reference in their entirety). This observation has led to the consensus that IL-4 is of greater importance than IL-13 in the promotion of a Th2 response. In contrast some bone marrow-derived cells, including macrophages, as well as most non-bone marrow derived cells express the type 2 IL-4R, composed of the IL4Rα and IL13Rα1 polypeptides. Both IL-4 and IL-13 activate the type 2 IL-4R (Andersson *et al* 1997 *Eur J Immunol* 27:1762-1768; Schnyder *et al* 1996 *Blood* 87:4286-4295; Doyle *et al* 1994 *Eur J Immunol* 24:1441-1445; de Vries 1998 *J Allergy Clin Immunol* 102:165-169; herein incorporated by reference in their entirety.) Signaling through the type 2 IL-4R appears to be responsible for many of the pro-allergic effects of IL-4 and IL-13. See, for example, Herbert *et al.* 2004 *Immunity* 20:623-635; Hershey, G.K. 2003 *J. Allergy Clin. Immunol.* 111:677-690; Andersson *et al* 1997 *Eur J Immunol* 27:1762-1768; Doyle *et al* 1994 *Eur J Immunol* 24:1441-1445; de Vries 1998 *J Allergy Clin Immunol* 102:165-169; Wills-Karp *et al* 1998 *Science* 282:2258-2261; Shim *et al* 2001 *Am J Physiol Lung Cell Physiol* 280:L134-140, herein incorporated by reference in their entirety. Some have suggested that IL-13 also binds and signals through a cell-membrane form of an additional IL-13 binding protein, cell membrane IL-13Rα2 that may contribute to the pro-fibrotic effects of IL-13 (Fichtner-Feigl *et al* 2006 *Nat Med* 12:99-106, herein incorporated by reference in its entirety).

[0005] IL-13Rα2 is spliced into multiple different forms including both the membrane bound form of IL-13Rα2 and a soluble form known as sIL-13Rα2 (SEQ ID NO:2). Both IL-4 and IL-13 upregulate sIL-13Rα2 gene expression (Zheng *et al.* 2003 *J. Allergy Clin Immunol* 111:720-728, herein incorporated by reference in its entirety), however this work did not address the effect on serum sIL-13Rα2 polypeptide levels. Investigators have speculated that IL-13Rα2's role is that of a sink or trap to limit dispersal of IL-13 (Chiaramonte *et al.* (2003) J Exp Med. Mar 17;197(6):687-701 and Zhang *et al* (1997) J. Biol. Chem 272:9474-9480 herein incorporated by reference in their entirety). Furthermore, commercially available recombinant sIL-13Rα2-Fc is considered "a potent soluble IL-13 antagonist" (U.S. Application No.04/0234517 Bowman et al). This bivalent polypeptide exhibits a lengthy half-life and binds two IL-13 molecules.

SUMMARY OF THE INVENTION

[0006] Compositions and methods for diagnosis and modulation of inflammation related disorders, particularly asthmatic disorders, allergic responses, and endofibrotic disorders are provided. The invention encompasses methods of modulating IL-13/sIL13Rα2 complex levels, modulating inflammation, and modulating expression of nucleotide sequences of interest. The invention also encompasses methods of detecting altered IL-13/sIL13Rα2 complex levels in a subject, detecting an allergic response in a subject, and detecting an inflammation related disorder in a subject.

[0007] In a first embodiment, the invention provides methods of modulating inflammation in a subject comprising the steps of providing a subject exhibiting an IL-13/ sIL-13Rα2 complex related disorder such as an inflammation related disorder, administering an IL-13/sIL-13Rα2 complex modulating agent to said subject, and monitoring an inflammation related phenotype in the subject. In aspects of the invention, the IL-13/sIL-13Rα2 complex modulating agent is isolated IL-13/sIL-13Rα2 complex or an agent such as an IL-13 like molecule, a sIL-13Rα2-like molecule, a sIL-13Rα2 agonist, and a sIL-13Rα2 binding antibody. In an aspect of the invention, the inflammation related phenotype alters. In an aspect of the invention the inflammation related phenotype increases. In other aspects of the invention the inflammation related phenotype decreases. In an aspect of the invention, the inflammation related phenotype is expression of a nucleotide sequence of interest and said expression is altered. Nucleotide sequences of interest include, but are not limited to, the nucleotide sequences set forth in SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:5. In an aspect, the IL-13/sIL-13Rα2 complex related disorder includes but is not limited to asthma, allergic responses, endofibrotic disorders, and inflammatory disorders.

[0008] In a second embodiment, the invention provides methods of modulating expression of a nucleotide sequence of interest comprising the steps of providing a subject exhibiting a nucleotide sequence of interest-related disorder and administering an IL-13/sIL-13Rα2 complex modulating agent to the subject. Particular nucleotide sequences of interest include the nucleotide sequences set forth in SEQ ID NO:3 (Pira1), SEQ ID NO:4 (Vannin1), and SEQ ID NO:5 (ApoA1). In an aspect SEQ ID NO:3 expression levels increase subsequent to administration of the IL-13/sIL-13Rα2 complex modulating agent. In an aspect SEQ ID NO:4 expression levels increase subsequent to administration of the IL-13/sIL-13Rα2

complex modulating agent. In another aspect SEQ ID NO:5 expression levels decrease subsequent to administration of the IL-13/sIL-13Rα2 complex modulating agent. In an aspect expression levels of at least two nucleotide sequences of interest are altered. In another aspect expression levels of at least three nucleotide sequences of interest are altered. In aspects of the invention, the IL-13/sIL-13Rα2 complex modulating agent is isolated IL-13/sIL-13Rα2 complex or an agent such as an IL-13 like molecule, a sIL-13Rα2-like molecule, a sIL-13Rα2 agonist, and a sIL-13Rα2 binding antibody.

[0009] In a third embodiment, the invention provides methods of determining the ratio of sIL13Rα2 and IL-13/sIL13Rα2 complex in a biological sample comprising the steps of obtaining a biological sample and incubating the biological sample with a first IL-13/sIL13Rα2 complex detecting reagent. In an aspect of the invention, the method further comprises incubating a reaction mixture comprising a biological sample and a first IL-13/sIL-13Rα2 complex detecting reagent with a second IL-13/sIL-13Rα2 complex detecting reagent. In an aspect of the invention, the IL-13/sIL-13Rα2 complex detecting reagent comprises an antibody selected from the group consisting of an antibody that binds an IL-13 polypeptide and an antibody that binds a sIL-13Rα2 polypeptide. In an aspect of the invention, the methods include the step of incubating a biological sample with isolated IL-13.

[0010] In a fourth embodiment, the invention provides methods of determining the ratio of sIL13Rα2 and IL-13/sIL13Rα2 complex in a biological sample comprising the steps of obtaining a biological sample from the subject, dividing said biological sample into a first aliquot and a second aliquot of pre-determined volume, incubating the first aliquot with an isolated IL-13 polypeptide, and incubating the first and second aliquots with a first IL-13/sIL-13Rα2 complex detecting reagent. In an aspect of the invention, the method further comprises the step of incubating a first reaction mixture comprising the first aliquot, the IL-13, and the first IL-13/sIL-13Rα2 complex detecting reagent with a second IL-13/sIL-13Rα2 complex detecting reagent. In an aspect of the invention, the method comprises the step of incubating a second reaction mixture comprising the second aliquot and the first IL-13/sIL-13Rα2 complex detecting reagent with a second IL-13/sIL-13Rα2 complex detecting reagent. In an aspect of the invention, the IL-13/sIL-13Rα2 complex detecting reagent comprises an antibody selected from the group consisting of an antibody that preferentially binds an IL-13 polypeptide and an antibody that preferentially binds a sIL-13Rα2 polypeptide.

[0011] In a fifth embodiment, the invention provides methods of detecting an allergic response in a subject comprising the steps of obtaining a biological sample from a subject, incubating the biological sample with a first IL-13/sIL-13Rα2 complex detecting reagent, determining the IL-13/sIL-13Rα2 complex level in the biological sample and comparing the IL-13/sIL-13Rα2 complex level in the sample with a standard IL-13/sIL-13Rα2 complex level. In an aspect, the first IL-13/sIL-13Rα2 complex detecting reagent comprises an antibody. In various aspects the antibody is selected from the group of antibodies that bind an IL-13 polypeptide and antibodies that bind a sIL-13Rα2 polypeptide. In an aspect of the invention the second IL-13/sIL-13Rα2 complex detecting reagent comprises an antibody. In various aspects the antibody is selected from the group of antibodies that bind an IL-13 polypeptide and antibodies that bind a sIL-13Rα2 polypeptide. In aspects of the invention the first IL-13/sIL-13Rα2 complex detecting reagent comprises an antibody that binds either IL-13 or sIL-13Rα2 and the second IL-13/sIL-13Rα2 complex detecting reagent comprises an antibody that binds the other component (IL-13 or sIL-13Rα2). In aspect of the invention the biological sample includes, but is not limited to, blood and serum.

[0012] In a sixth embodiment, the invention provides kits for detecting an allergic response in a subject, kits for detecting an altered IL-13/sIL-13Rα2 complex level in a subject, and kits for detecting an inflammatory related disorder in a subject, comprising a first IL-13/sIL-13Rα2 complex detecting reagent. In an aspect of the invention the IL-13/sIL-13Rα2 complex detecting reagent comprises an antibody selected from the group of antibodies that bind an IL-13 polypeptide and the group of antibodies that bind a sIL-13Rα2 polypeptide. In an aspect of the invention the kit further comprises a second IL-13/sIL-13Rα2 complex detecting reagent.

[0013] In a seventh embodiment, the invention provides methods of detecting an inflammatory related disorder in a subject comprising the steps of obtaining a biological sample from the subject, incubating the biological sample with a first IL-13/sIL-13Rα2 complex detecting reagent, determining the IL-13/sIL-13Rα2 complex level in the biological sample and comparing the IL-13/sIL-13Rα2 complex level in the sample with a standard IL-13/sIL-13Rα2 complex level. In an aspect of the invention, subjects with an altered IL-13/sIL-13Rα2 complex level in the sample are characterized as exhibiting an inflammatory related disorder.

[0014] In an eighth embodiment, the invention provides methods of detecting an altered IL-13/sIL-13Rα2 complex level in a subject comprising the steps of obtaining a biological sample from the subject, assaying the expression level of at least one nucleotide sequence of interest in the biological sample, and comparing the expression level of the nucleotide sequence of interest with a predetermined standard expression level. In an aspect of the invention, the subject exhibits an inflammatory related disorder. In aspects of the invention, the methods comprise assaying the expression level of at least two or at least three nucleotide sequences of interest in the biological sample. In aspects of the invention the nucleotide sequence of interest is selected from the group of nucleotide sequences of interest having a nucleotide sequence set forth in SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or a nucleotide sequence having at least 95% identity to a nucleotide sequence set forth in SEQ ID NO:3, SEQ ID NO:4, or SEQ ID NO:5. In various aspects of the invention, an increased expression level of a nucleotide sequence of interest set forth in SEQ ID NO:3 or SEQ ID NO:4 or a nucleotide sequence having at least 95% identity to a nucleotide sequence set forth in SEQ ID NO:3 or SEQ ID NO:4 indicates an increased IL-13/sIL-13Rα2 complex level in the subject. In aspects of the invention, a decreased expression level of a nucleotide sequence of interest having the nucleotide sequence set forth in SEQ ID NO:5 or a nucleotide sequence having at least 95% identity to the nucleotide sequence set forth in SEQ ID NO:5 indicates an increased IL-13/sIL-13Rα2 complex level in the subject.

[0015] In a ninth embodiment, the invention provides methods of identifying subjects suitable for inclusion in an IL-13/sIL-13Rα2 complex related study comprising the steps of obtaining a biological sample from a subject, assaying the expression level of at least one nucleotide sequence of interest in the biological sample, comparing the expression level of said at least one nucleotide sequence of interest with a predetermined standard expression level, and identifying the subject as a subject with an altered IL-13/sIL-13Rα2 complex level or a normal IL-13/sIL-13Rα2 complex level. In aspects of the invention, the methods further comprise the steps of identifying a subject with the attribute of an altered IL-13/sIL-13Rα2 complex level or normal IL-13/sIL-13Rα2 complex level, and identifying said subject as a subject having a preferred attribute for an IL-13/sIL-13Rα2 complex related study. In aspects of the invention, the methods comprise assaying the expression level of at least two or at least three nucleotide sequences of interest in the biological sample. In aspects of the invention the nucleotide sequence of interest is selected from the group of nucleotide sequences of interest having a nucleotide sequence set forth in SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or a

nucleotide sequence having at least 95% identity to a nucleotide sequence set forth in SEQ ID NO:3, SEQ ID NO:4, or SEQ ID NO:5. In aspects of the invention, the methods further comprise characterizing a subject with an increased expression level of a nucleotide sequence of interest set forth in SEQ ID NO:3 or SEQ ID NO:4 or a nucleotide sequence of interest having at least 95% identity to a nucleotide sequence set forth in SEQ ID NO:3 or SEQ ID NO:4 or a decreased expression level of a nucleotide sequence of interest set forth in SEQ ID NO:5 or a nucleotide sequence having at least 95% identity to the nucleotide sequence set forth in SEQ ID NO:5 as a subject with an elevated IL-13/sIL-13Rα1 complex level.

[0016] In a tenth embodiment, the invention provides methods of identifying subjects suitable for inclusion in an inflammatory related disorder study comprising the steps of obtaining a biological sample from a subject, assaying the expression level of at least one nucleotide sequence of interest in the biological sample, comparing the expression level of said at least one nucleotide sequence of interest with a predetermined standard expression level, and identifying the subject as a subject with an altered IL-13/sIL-13Rα2 complex level or a normal IL-13/sIL-13Rα2 complex level. In aspects of the invention, the methods comprise assaying the expression level of at least two or at least three nucleotide sequences of interest in the biological sample. In aspects of the invention the nucleotide sequence of interest is selected from the group of nucleotide sequences of interest having a nucleotide sequence set forth in SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or a nucleotide sequence having at least 95% identity to a nucleotide sequence set forth in SEQ ID NO:3, SEQ ID NO:4, or SEQ ID NO:5. In aspects of the invention, the methods further comprise characterizing a subject with an increased expression level of a nucleotide sequence of interest set forth in SEQ ID NO:3 or SEQ ID NO:4 or a nucleotide sequence of interest having at least 95% identity to a nucleotide sequence set forth in SEQ ID NO:3 or SEQ ID NO:4 or a decreased expression level of a nucleotide sequence of interest set forth in SEQ ID NO:5 or a nucleotide sequence having at least 95% identity to the nucleotide sequence set forth in SEQ ID NO:5 as a subject with an elevated IL-13/sIL-13Rα1 complex level.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] Figure 1 presents an evaluation of serum levels of sIL-13Rα2 and sIL-4Rα in normal mice (panel A) and their average percent saturation with IL-13 or IL-4, respectively (panel B). Average concentration levels are presented in ng/ml. Experimental details are described elsewhere herein. IL-4/sIL-4Rα complex levels in the naïve serum were too low to be detected, thus are not shown in the figure. IL-13/sIL-13Rα2 complex levels were higher than

IL-4/sIL-4Rα levels. Fifteen to forty percent of sIL-13Rα2 was complexed with IL-13 in naïve mouse serum. Error bars indicate standard error.

[0018] Figure 2 presents an evaluation of sIL-13Rα2 and sIL-4Rα responses to Th2 stimulation. The data in panels A and B were obtained from mice before (day 0) and after inoculation with *N. brasiliensis*. Samples were bled from the mice at the indicated day post-inoculation. Panel A indicates the average level of each receptor (ng) in serum (ml). Panel B indicates the average percent of each soluble receptor complexed with its ligand at each time point. The data in panels C and D were obtained from mice before (day 0) and after immunization with goat antimouse IgD antiserum (GaMD). Panel C indicates the average level of each receptor (ng) in serum (ml) at each time point. Panel D indicates the average percent of each soluble receptor complexed with its ligand at each time point. Solid circles indicate sIL-13Rα2 data, empty circles indicate sIL-4Rα data. Error bars indicate standard error. After exposure to *N. brasiliensis* or GaMD, the percent of IL-13/sIL-13Rα2 complex in the samples increased to a peak approaching 90%.

[0019] Figure 3 presents results obtained from an investigation of cytokine dependence. The data in the figure were obtained from normal BALB-c (wild-type, solid circles), IL-13 deficient (IL-13$^-$, squares), and IL-4/IL-13 double-deficient (IL-4$^-$ IL-13$^-$, triangles) mice. The mice were bled to obtain a 0 day time point and inoculated with *Schistosoma mansoni*. Samples were obtained at the indicated time points post-inoculation. Panel A indicates the average IL-13Rα2 level (ng) in serum (ml) obtained from each type of mice at the designated timepoints. Panel B indicates the average percent of IL-13Rα2 complexed with IL-13 in serum obtained from each mice type at the designated timepoints. Experimental details are provided elsewhere herein. Error bars indicate standard error.

[0020] Figure 4 presents results obtained from mice after rapid production of IL-4 and IL-13. The data in the figure were obtained from BALB/c mice (n=5) injected with either saline (Saline) or GaMD. The data in Panel A were obtained from GaMD injected mice subsequently challenged with saline (αIgD/Saline) or rat anti-IgE mAb (αIgD/αIgE). Panel A presents serum concentrations of sIL-4Rα (hatched bars) and sIL-13Rα2 (solid bars) and their percent saturation with the appropriate cytokine ligands. As indicated in the figure, sIL-4Rα saturation with IL-4 increased from extremely low levels to approximately 8%. As indicated in the figure, sIL-13Rα2 saturation with IL-13 increased from approximately 20% to

approximately 60%. Panel B presents data obtained from samples obtained from mice injected two hours earlier with either saline (untreated) or anti-CD3 mAb (αCD3). Panel B presents serum concentrations of sIL-4Rα (hatched bars) and sIL-13Rα2 (solid bars) and their percent saturation with their cytokine ligands. As indicated in panel B, sIL-4Rα saturation with IL-4 increased from extremely low levels to nearly 50%. As indicated in panel B, sIL-13Rα2 saturation with IL-13 increased to nearly 100%. Asterisks in Figure 4 indicate that the value for the indicated group is significantly altered ($p<0.05$) as compared with the value for an untreated, saline-treated, or vehicle-treated group.

[0021] Figure 5 presents serum concentration and percent saturation levels in mice treated with either IL-4 (Panels A and B) or IL-13 (Panels C and D). Panel B also presents data obtained from mice treated with IL-13 (IL-13, 2 hours). sIL-4Rα serum concentration and percent saturation levels are presented in Panels A and C. sIL-13Rα serum concentration and percent saturation levels are presented in Panels B and D. Additionally each panel contains data obtained from untreated animals. Samples were obtained at the indicated timepoints. As indicated in the figure, sIL-13Rα2 remained saturated with IL-13 for at least 12 hours. Asterisks in Figure 5 indicate that the value for the indicated group is significantly altered ($p<0.05$) as compared with the value for an untreated, saline-treated, or vehicle-treated group.

[0022] Figure 6 presents sIL-13Rα2 serum concentration (ng sIL-13Rα2/ml serum) data from multiple strains of mice. The results in Panel A were obtained from normal BALB/c (Wild-type) mice one day after injection with either saline or IL-4C. The results in Panel B were obtained from mice injected with IL-4C on day 0 (solid bars) or day 3 (hatched bars). IL-4C was injected into BALB/c (Wild-type), IL-4Rα deficient (IL-4Rα$^-$), and Stat6 deficient (Stat6$^-$) mice. The results in Panel C were obtained from mice injected with IL-13 (hatched bars) and untreated mice (solid bars). IL-13 was injected in normal BALB/c (Wild-type), IL-4Rα deficient (IL-4Rα$^-$), Stat6 deficient (Stat6$^-$) mice and IL-13Rα2 deficient (IL-13Rα2$^-$) mice. Asterisks in Figure 6 indicate that the value for the indicated group is significantly altered ($p<0.05$) as compared with the value for an untreated, saline-treated, or vehicle-treated group. Experimental details are provided elsewhere herein.

[0023] Figure 7 presents data obtained from experiments investigating the serum half-life of free sIL-13Rα2 and sIL-4Rα and cytokine-complexed sIL-13Rα2 and sIL-4Rα. The data in panel A were obtained from IL-4Rα deficient mice that were injected with concentrated sera

from normal mice (Serum) or concentrated sera from normal mice that was enriched with IL-4 (Serum + IL-4). Mice were bled at the indicated timepoints. Total sIL-4Rα levels are indicated with solid bars and serum levels of IL-4/sIL-4Rα complex are indicated with hatched bars. The receptor and receptor-complex levels are presented in pg/ml of serum on a log scale. Panel B presents results from two independent experiments. Concentrated serum from *N. brasiliensis*-infected wild-type (triangles and diamonds) or IL-13-deficient BALB/c (circles and squares) mice was injected into IL-13/IL-13Rα2 double deficient mice. The double deficient mice were bled at the indicated times and the free sIL-13Rα2 and IL-13/sIL-13Rα2 complex levels were determined. Panel C presents half-life curves (dashed lines) calculated from the mean values of the sets of independent experiments presented in Panel B. Circles represent the mean free IL-13Rα2 data and squares represent the mean IL-13/sIL-13Rα2 complex data. Panel D presents data obtained from IL-13/IL-13Rα2 double deficient mice injected with concentrated IL-13 rich serum from IL-13Rα2-deficient mice. Blood (circles, serum) and urine (squares, urine) samples were taken at the indicated timepoints. IL-13 levels in pg IL-13/ ml sample were determined by ELISA.

[0024] Figure 8 presents sIL-13Rα2 (IL-13Rα2) levels in ng/ml. The left column of graphs contains information obtained from serum samples. The right column of graphs contains information obtained from urine samples. IL-13/sIL-13Rα2 complex levels are indicated with solid bars. Total sIL-13Rα2 levels are indicated with hatched bars. IL-13/sIL-13Rα2 complex was not detected in the urine samples. Percentages shown in the panels indicate the urine concentration relative to serum concentration of total sIL-13Rα2. The upper graphs (Panel A) present data obtained from wild-type BALB/c mice injected with saline (saline) or recombinant mouse IL-13 (IL-13). The middle graphs (Panel B) present data obtained from BALB/c mice injected with saline (BALB/c + saline) or IL-4C (BALB/c + IL-4C); and from IL-13Rα2 deficient mice injected with saline (IL-13Rα2$^-$ + saline). The lower graphs (Panel C) present data obtained from IL-13 deficient BALB/c mice injected with saline (IL-13$^-$ + saline) or IL-4C (IL-13$^-$ + IL-4C).

[0025] Figure 9 presents observed and expected sIL-13Rα2 levels (ng sIL-13Rα2/ ml sample) in IL-4Rα deficient mice injected with 1 μg IL13. Samples were collected from the injected mice for an initial bleed (0 hours) and at 4, 8, and 12 hours post injection. Total sIL-13Rα2 levels in the samples were determined (circles). These levels are compared to the expected levels based on no change in the rate of sIL-13Rα2 secretion and either an increase in serum sIL-13Rα2 half-life from 3.3 hours for free sIL-13Rα2 to 18 hours for IL-13/sIL-13Rα2

complex (squares, based on the last segment of each curve in Figure 7, panel C) or an increase in serum sIL-13Rα2 half-life from 1.3 hours for free sIL-13Rα2 to 11.1 hours for IL-13/sIL-13Rα2 complex (triangles, based on the entire curves in panel Figure 7, panel C).

[0026] Figure 10 presents the results of real time PCR analysis of sIL-13Rα2 mRNA relative to 18S RNA mRNA levels. Panel A depicts the relative sIL-13Rα2 expression levels in normal BALB/c (Wild-type) or IL-13 deficient BALB/c (IL-13-) mice three days after injection with vehicle (solid bars) or IL-4C (hatched bars). Panel B depicts the relative sIL-13Rα2 expression levels in normal BALB/c (Wild-type) or IL-4Rα- deficient BALB/c (IL-4Rα-) one day after injection with vehicle (solid bars) or IL-13 (hatched bars). Panel C depicts the relative sIL-13Rα2 expression levels in IL-4Rα deficient BALB/c mice one day after injection with vehicle, 3 μg IL-13, or 3 μg IL-13 plus 9 μg sIL-13Rα2-Fc. Asterisks in Figure 10 indicate that the value for the indicated group is significantly altered ($p<0.05$) as compared with the value for an untreated, saline-treated, or vehicle-treated group.

[0027] Figure 11 presents a graph indicating the amount of IL-13 (pg/ml) released from recombinant sIL-13Rα2-IgGFc fusion protein under the indicated pH conditions. IL-13 remains bound to the recombinant sIL-13Rα2-IgGFc fusion protein over a broad range of pH values. Exposure of the complex to 3.5M $MgCl_2$ resulted in significant dissociation of IL-13 from sIL-13Rα2-IgGFc fusion protein.

[0028] Figure 12 presents the normalized gene expression results pooled from two identical experiments. Panel A presents data obtained from real time PCR analysis of the Pira1 (SEQ ID NO:3), Vnn1 (SEQ ID NO:4), and ApoA1 (SEQ ID NO:5) expression levels relative to β-actin expression levels in pulmonary tissue of IL-13/IL13Rα2 double deficient mice inoculated with PBS (solid bars), serum from *N. brasiliensis* infected wild-type mice (hatched bars, Wild-type Serum), or serum from *N. brasiliensis* infected IL-13/IL-13Rα2 double deficient mice (cross-hatched bars, IL-13$^-$/sIL-13Rα2$^-$ Serum). Differences between values for lungs inoculated with serum that contained IL-13 and sIL-13Rα2 are all significantly different from values for lungs inoculated with IL-13/IL-13Rα2 double deficient serum or PBS ($p<0.05$). Numbers to the right of the hatched bars show the ratio of expression in lungs from mice treated with IL-13/sIL-13Rα2-containing serum vs IL-13/IL-13Rα2 deficient serum.

[0029] Panel B presents data obtained from real time PCR analysis of the Pira1 (SEQ ID NO:3), Vnn1 (SEQ ID NO:4), and ApoA1 (SEQ ID NO:5) expression levels relative to β-actin expression levels in pulmonary tissue of IL-13/IL13Rα2 double deficient mice inoculated with serum from *N. brasiliensis* infected wild-type mice that had been either absorbed with anti-IL-13Rα2 Ab-agarose (anti-IL-13Rα2 Absorbed, solid bars) or control Ab-agarose (Mock Absorbed, hatched bars) prior to inoculation. Numbers to the right show the ratio of expression in lungs from mice treated with IL-13/sIL-13Rα2-containing serum vs. IL-13/IL-13Rα2 deficient serum. Differences between values for Pira1 and Vnn1 were significantly greater ($p<0.05$) for mice that had received the mock absorbed serum.

DETAILED DESCRIPTION OF THE INVENTION

[0030] The invention provides compositions and methods for modulating inflammation in a subject and modulating expression of a nucleotide sequence of interest such as Pira1, Vannin1, or ApoA1. Additionally, the invention provides methods of modulating IL-13/sIL-13Rα2 complex related disorders and inflammation related disorders such as asthma or allergic responses. Further the invention provides methods of determining the ratio of IL-13/sIL-13Rα2 and uncomplexed sIL-13Rα2 in a biological sample, detecting an allergic response in a subject, and detecting an inflammation related disorder in a subject. Also, the invention provides methods of detecting an altered IL-13/sIL-13Rα2 complex level in a subject, identifying subjects suitable for inclusion in an IL-13/sIL-13Rα2 complex related study, and identifying subjects suitable for inclusion in an inflammatory disorder related study. The invention further provides kits for performing the methods of the invention. The compositions and methods of the invention were developed from investigations that revealed that IL-13/sIL-13Rα2 complex possesses a biological activity that increases expression of two genes, Pira1 and Vannin-1, and decreases ApoA1 expression. Prior to this work sIL-13Rα2 was considered a containment protein for IL-13 and an IL-13 antagonist (Zhang *et al* (1997), *J. Biol. Chem.* 272:9474-9480 and Zheng *et al* (2003) *J. Allergy Clin Immunol* 111:721-728, herein incorporated by reference in their entirety); thus the results of the investigations described herein were unexpected. The observation that sIL-13Rα2 associated with IL-13 exhibits a significantly increased half-life compared to the half-life of sIL-13Rα2 alone contributed to the inventions described herein.

[0031] Various embodiments of the invention pertain to methods of determining the ratio of sIL-13Rα2 and IL-13/sIL-13Rα2 in a biological sample comprising the steps of obtaining a

biological sample and incubating the biological sample with a first IL-13/sIL-13Rα2 complex detecting reagent. By "determining" is intended identifying, measuring, evaluating, assaying, obtaining a value, discovering, ascertaining, finding out, or discerning. The ratio of two groups is a relation of the quantity of one of the groups to the quantity of the other group. It is recognized that a ratio of the quantities of two groups (A and B) encompasses multiple arrangements. Such arrangements include, but are not limited to, the relation of A to B, the relation of B to A, the relation of A to A+B, the relation of B to A+B, the relation of A+B to A, and the relation of A+B to B. Of particular interest to the invention is the ratio of the quantity of sIL-13Rα2 associated with IL-13 in relation to the quantity of sIL-13Rα2 not associated with IL-13. Means of describing the ratio of sIL-13Rα2 and IL-13/sIL-13Rα2 include, but are not limited to, percent saturation values. Percent saturation values describe the ratio of IL-13/sIL-13Rα2 to the total sIL-13Rα2 present.

[0032] By sIL-13Rα2 is intended a polypeptide having the amino acid sequence set forth in SEQ ID NO:2 and fragments and variants thereof. Native sIL-13Rα2 is a soluble form of IL-13Rα2 thought, while not bound to mechanism, to result from alternative splicing of IL-13Rα2. The IL-13Rα2 genomic sequence is set forth in SEQ ID NO:1. IL-13 is a polypeptide having the amino acid sequence set forth in SEQ ID NO:6 and fragments and variants thereof. Antigenic fragments of IL-13 are known in the art. Fragments and variants of the sIL-13Rα2 (SEQ ID NO:2) and IL-13 (SEQ ID NO:6) polypeptides are also encompassed by the present invention. By "fragment" is intended a portion of the amino acid sequence and hence polypeptide. Fragments of a nucleotide sequence may encode protein fragments that retain the biological activity of the native protein and hence exhibit a sIL-13Rα2 or IL-13 activity.

[0033] A biologically active fragment of a polypeptide of interest will consist of at least 10, 15, 25, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 339, 340, 350, 360, 370, 380, or 383 contiguous amino acids, or up to the total number of amino acids present in the full-length sIL-13Rα2 protein (SEQ ID NO:2) or at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85,86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114,

115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, or 131 contiguous amino acids, or up to the total number of amino acids present in the full-length IL-13 protein (SEQ ID NO:6). A biologically active fragment of a polypeptide of interest can be prepared by isolating a portion of a nucleotide sequence that encodes said polypeptide, expressing the encoded portion of the IL-13 or sIL-13Rα2 protein (e.g., by recombinant expression *in vitro*), and assessing an activity of the encoded portion of the sIL-13Rα2 or IL-13 protein. Activities of the IL-13 protein include, but are not limited to, sIL-13Rα2 binding, IL-4R binding, IL-13Rα1 binding, and antigen formation. Any antigenic fragments of IL-13 known in the art are encompassed. Activities of the sIL-13Rα2 protein include but are not limited to IL-13 binding, solubility, and antigen formation.

[0034] By "variants" is intended substantially similar sequences. By "variant" protein is intended a protein derived from the native protein by deletion (so-called truncation) or addition of one or more amino acids to the N-terminal and/or C-terminal end of the native protein; deletion or addition of one or more amino acids at one or more sites in the native protein; or substitution of one or more amino acids at one or more sites in the native protein. Variant proteins encompassed by the present invention are biologically active, that is they continue to possess the desired biological activity of the native protein, that is, an IL-13 or sIL-13Rα2 activity as described herein. Such variants may result from, for example, genetic polymorphism or from human manipulation. Biologically active variants of a native IL-13 or sIL-13Rα2 of the invention will have at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, and more preferably at least about 98%, 99% or more sequence identity to the amino acid sequence for the native protein as determined by sequence alignment programs described elsewhere herein using default parameters. A biologically active variant of a protein of the invention may differ from that protein by as few as 1-15 amino acid residues, as few as 1-10, such as 6-10, as few as 5, as few as 4, 3, 2, or even 1 amino acid residue.

[0035] Computer implementations of mathematical algorithms can be utilized for comparison of sequences to determine sequence identity. For purposes of the present invention, comparison of nucleotide or protein sequences for determination of percent sequence identity to the sequences disclosed herein is preferably made using the GCG program GAP (Version 10.00 or later) with its default parameters or any equivalent program. By "equivalent program" is intended any sequence comparison program that, for any two sequences in question, generates an alignment having identical nucleotide or amino acid residue matches

and an identical percent sequence identity when compared to the corresponding alignment generated by the preferred program. Alignment may also be performed manually by inspection.

[0036] sIL-13Rα2 interacts with IL-13 to form an IL-13/sIL-13Rα2 complex. While not limited by mechanism, sIL-13Rα2 and IL-13 molecules associate with or bind to each other. An IL-13/sIL-13Rα2 complex consists of an IL-13 polypeptide associated with a sIL-13Rα2 polypeptide. The IL-13/sIL-13Rα2 complex is stable over a broad range of pH levels including but not limited to pH 7 to pH 6, pH 6 to pH 5, pH 5 to pH 4.1. The IL-13/sIL-13Rα2 complex is stable at pH levels at least as low as 4.1.

[0037] Any means of assaying protein protein interactions may be utilized in the methods of the invention. Methods of assaying protein protein interactions are known in the art and include, but are not limited to, cross-linking analysis, yeast two hybrid, immunoassays, gel exclusion assays, X-ray crystallography, NMR, dissociation constant determinations, filter binding assays, dissociation curves, affinity chromatography, saturation binding assays, gel filtration chromatography, coimmunoprecipitation, FRET, fluorescence quenching, phage expression systems, bimolecular fluorescence complementation analysis, Far Western analysis, sedimentation velocity analysis, spectroscopy, and mass spectrometry. See for example Coligan et al Ed. 2007 *Current Protocols in Protein Science*, John Wiley & Sons and Walker Ed 2002 *Protein Protocols Handbook* Humana Press Totowa NJ herein incorporated by reference in their entirety.

[0038] By "biological sample" is intended a sample collected from a subject including, but not limited to, whole blood, serum, tissue, cells, mucosa, fluid, scrapings, hairs, cell lysates, urine, and secretions. Biological samples such as blood samples can be obtained by any method known to one skilled in the art. Further, biological samples can be enriched, purified, isolated, or stabilized by any method known to one skilled in the art. Such enrichment, purification, isolation, or stabilization procedures can be performed at any time during the methods of the invention including but not limited to, prior to incubating the biological sample with a first IL-13/sIL-13Rα2 complex detecting reagent, concurrent with incubating the biological sample with a first IL-13/sIL-13Rα2 complex detecting reagent, subsequent to incubating the biological sample with a first IL-13/sIL-13Rα2 complex detecting reagent, prior to incubating the biological sample with a second IL-13/sIL-13Rα2 complex detecting

reagent, concurrent with incubating the biological sample with a second IL-13/sIL-13Rα2 complex detecting reagent, or subsequent to incubating the biological sample with a second IL-13/sIL-13Rα2 complex detecting reagent. The invention encompasses isolated or substantially purified nucleic acid or protein compositions.

[0039] An "isolated" or substantially "purified" nucleic acid molecule, polypeptide, or biologically active portion thereof, is substantially free of other cellular material, or culture medium when produced by recombinant techniques or substantially free of chemical precursors or other chemicals when chemically synthesized. An isolated or substantial purified nucleic acid molecule, polypeptide, or biologically active portion thereof, may be suspended in solution, combined with a buffering agent, or otherwise utilized in a combination.

[0040] By "incubating" is intended maintaining environmental conditions favorable to a desired outcome for a period of time. The methods of the invention require incubation of at least a first IL-13/sIL-13Rα2 complex detecting reagent with a biological sample. The indicated components are combined and incubated. Frequently the incubation includes additional substances that facilitate the desired outcome of the incubation. Incubating an IL-13/sIL-13Rα2 complex detecting reagent with a biological sample may be performed under a variety of temperature or reaction conditions. Incubation temperatures may range from 0°C to 100°C depending on the components being incubated and the desired outcome of the incubation. Multiple temperatures may be used during the incubation period. Multiple buffers may be used during the incubation period. Incubation temperatures and conditions for the various components and the desired outcome of the incubations are known in the art. Duration of an incubation may range from 10, 20, 30, 40, 50, to 60 seconds; 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, to 60 minutes; 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 7, 8, 9, 10, 11, 12, 24, 36, 48, to 60 hours.

[0041] By "IL-13/sIL-13Rα2 complex detecting reagent" is intended a composition comprising any agent, compound, complex, or molecule capable of preferentially interacting with an IL-13/sIL-13Rα2 complex. By "preferentially interacting" is intended that the detecting reagent interacts with an IL-13/sIL-13Rα2 complex or an IL-13/sIL-13Rα2 complex component at levels at least 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% greater than a non-IL-13/sIL-13Rα2 complex or complex component. In

multiple embodiments of the invention an IL-13/sIL-13Rα2 complex detecting reagent also is capable of preferentially interacting with IL-13 or with sIL-13Rα2. It is recognized that an IL-13/sIL-13Rα2 complex detecting reagent that is also capable of preferentially interacting with a complex component such as IL-13 or sIL-13Rα2 may interact with a higher affinity with a complex component than with the complex itself. Detecting reagents may indicate the presence of the IL-13/sIL-13Rα2 complex directly or indirectly. IL-13/sIL-13Rα2 detecting reagents include but are not limited to antibodies, monoclonal antibodies, polyclonal antibodies, recombinant antibodies, polypeptides, small molecules, nucleic acids, IL-13 binding antibodies, sIL-13Rα2 binding antibodies, IL-13/sIL-13Rα2 binding antibodies, peptidomimetics, humanized antibodies, antibody fragments such as Fab fragments, single-chain antibodies, and peptides. An antibody is an immunoglobulin molecule produced in response to a unique antigen. Interaction of the detecting reagent itself may be detected directly or indirectly, for example through an interaction with a conjugated antibody. It is recognized that a complex detecting reagent may also comprise other components such as, but not limited to, buffers, buffering agents, chelating agents, diluents, fetal bovine serum, glycerol, diluents, suspension solutions, and serums.

[0042] Any method of obtaining a biological sample from a subject known in the art may be used in the methods of the invention. By "subject" is intended a mammal, e.g., a human, or an experimental or animal or disease model or mammalian tissue or mammalian cells. Suitable subjects include mammals, particularly humans and mice. The subject can also be a non-human mammal such as, but not limited to, a mouse, horse, hamster, guinea pig, rabbit, dog, pig, goat, bovine, rat, rodent, feline, monkey, chimpanzee, sheep, or other domestic animal. It is recognized that the preferred methods of obtaining a biological sample for use in the methods of the invention vary depending on factors including, but not limited to, the biological sample type, the subject, the subject's age, and the subject's health. A person skilled in the art of obtaining biological samples would recognize various benefits and drawbacks associated with the different methodologies and would choose accordingly.

[0043] In various embodiments of the invention, the methods comprise the step of providing a first aliquot of the biological sample and providing a second aliquot of the biological sample. By "aliquot" is intended a portion, sub-sample, amount, sub-set, or part of the total biological sample. Various enrichment, purification, stabilization, or storage processes may be performed on the total biological sample before or after the first and second aliquots are

provided. In aspects of the invention, the amount, volume, or portion of the first and second aliquots is predetermined. The volume of the first aliquot may be less than, equal to, or greater than the volume of the second aliquot. The total volume of the first and second aliquots may be less than or equal to the total volume of the biological sample. It is recognized that substances including but not limited to stabilizing agents, buffering agents, salts, diluents, and proteinase inhibitors may be added to the first aliquot, the second aliquot, or the first and second aliquot.

[0044] The invention encompasses various methods of identifying an allergic response in a subject, identifying an inflammation related disorder in a subject, identifying an altered IL-13/sIL-13Rα2 complex level in a subject, and identifying subjects suitable for inclusion in pertinent studies. Identifying involves detecting, evaluating, assaying, recognizing or discovering the characteristic of interest such as, but not limited to, an allergic response, an inflammation related disorder, an altered IL-13/sIL-13Rα2 complex level, or subjects suitable for inclusion in a particular study.

[0045] Allergic responses are inappropriate immune reactions to an allergen. They affect about 20% of the American public. Symptoms of allergic responses include, but are not limited to, inflammation, mucus production, watery eyes, itching, rashes, tissue swelling, nasal inflammation, bronchospasm, stridor, shock, vasculitis, systemic anaphylaxis, laryngeal edema, transfusion reactions, angioedema, urticaria, eczematous dermatitis, rhinitis, conjunctivitis, abdominal cramps, gastrointestinal stress, asthma, wheezing, coughing, shortness of breath, perspiration, confusion, lethargy, upregulation of serum IgE, eosinophilia, airway hyper responsiveness, and cyanosis.

[0046] Inflammation related disorders amenable to the present invention include but are not limited to, asthma, airway hyper responsiveness, chronic airway remodeling, chronic obstructive pulmonary disease, arthritis, fibrotic disorders, non-allergic asthma, cystic fibrosis, liver fibrosis, and pulmonary fibrosis.

[0047] Symptoms of asthma include, but are not limited to, wheezing, shortness of breath, bronchoconstruction, airway hyper reactivity, decreased lung capacity, fibrosis, airway inflammation, and mucus production.

[0048] An embodiment of the invention is a method of detecting an IL-13/sIL-13Rα2 complex level abnormality such as an allergic response or an inflammation related disorder. The method comprises obtaining a sample and assaying the IL-13/sIL-13Rα2 complex level in the sample. An increase or decrease in complex level compared to standard complex levels in a similar sample obtained from a healthy subject, either directly or indirectly (for example, a predetermined standard IL-13/sIL-13Rα2 complex level) indicates an IL-13/sIL-13Rα2 complex level abnormality or an altered IL-13/sIL-13Rα2 complex level. In an embodiment the IL-13/sIL-13Rα2 complex level is expressed as a relation of the IL-13/sIL-13Rα2 complex level and the free or non-bound sIL-13Rα2 complex level. IL-13/sIL-13Rα2 complex levels can be expressed in various ways including but not limited to, mass units of complex per volume units of sample, (for example pg/ml, ng/ml, μg/ml, mg/ml); percent saturation, raw mass units, weight volume, and volume volume. The invention provides multiple methods of detecting an altered IL-13/sIL-13Rα2 complex level. In an embodiment the invention provides methods of detecting an altered IL-13/sIL-13Rα2 complex level that involve assaying the IL-13/sIL-13Rα2 complex level. In another embodiment the invention provides methods of detecting an altered IL-13/sIL-13Rα2 complex level that involve assaying the expression level of a nucleotide sequence of interest.

[0049] Aspects of the invention include kits for performing the methods of the invention. The kits comprise a first IL-13/sIL-13Rα2 complex detecting reagent and may comprise a second IL-13/sIL-13Rα2 complex detecting reagent. In various embodiments, the first IL-13/sIL-13Rα2 complex detecting reagent is an antibody that binds an IL-13 polypeptide or an antibody that binds a sIL-13Rα2 polypeptide. In various embodiments, the second IL-13/sIL-13Rα2 complex detecting reagent is an antibody that binds an IL-13 polypeptide or an antibody that binds a sIL-13Rα2 polypeptide. Preferredly either the first IL-13/sIL-13Rα2 complex detecting reagent is an anti-IL-13 antibody and the second IL-13/sIL-13Rα2 complex detecting reagent is an anti-sIL-13Rα2 antibody, or the first IL-13/sIL-13Rα2 complex detecting reagent is an anti- sIL-13Rα2 antibody and the second IL-13/sIL-13Rα2 complex detecting reagent is an anti-IL-13 antibody. In various embodiments, the IL-13/sIL-13Rα2 complex detecting reagent is a molecule that preferentially interacts with IL-13 or sIL-13Rα2 and is not an antibody. In various embodiments, the first IL-13/sIL-13Rα2 complex detecting reagent preferentially interacts with IL-13 and the second IL-13/sIL-13Rα2 complex detecting reagent preferentially interacts with sIL-13Rα2. In an embodiment, the first IL-13/sIL-13Rα2 complex detecting reagent preferentially interacts with sIL-13Rα2 and

the second IL-13/sIL-13Rα2 complex detecting reagent preferentially interacts with IL-13. Embodiments of the invention encompass the use of any anti-IL-13 antibody or anti-sIL-13Rα2 antibody known in the art. Anti-IL-13 antibodies known in the art include, but are not limited to, affinity-purified goat anti-mouse IL-13 (R&D Systems) and C531, rat IgG anti-mouse IL-13 (Centocor). Anti-sIL-13Rα2 antibodies known in the art include, but are not limited to, affinity-purified rabbit anti-mouse sIL-13Rα2 (Zhang *et al* (1997) *J. Biol. Chem.* 272:9474-9480, herein incorporated by reference in its entirety).

[0050] An embodiment of the invention is a method of identifying an altered IL-13/sIL-13Rα2 complex level. The method comprises obtaining a sample and assaying the expression level of at least one nucleotide sequence of interest in the sample. An increase or decrease in expression level compared to standard expression levels of the nucleotide sequence of interest in a similar sample obtained from a healthy subject, either directly or indirectly (for example, a predetermined standard) indicates an altered IL-13/sIL-13Rα2 complex level. Predetermined standard expression level includes but is not limited to the expression level of the nucleotide sequence of interest in a pre-identified individual or the average expression level of the nucleotide sequence of interest in a group of healthy individuals. Thus, comparisons between a query subject and a baseline subject are encompassed by the invention. Altered expression levels of three nucleotide sequences of interest are observed upon introduction of IL-13/sIL-13Rα2 complex into IL-13 and IL-13Rα2 double deficient mice. The nucleotide sequences of interest are Pira1 (SEQ ID NO:3), Vannin1 (SEQ ID NO:4), and ApoA1 (SEQ ID NO:5). In aspects of the invention, the expression level of at least two nucleotide sequences of interest is determined. In aspects of the invention, the expression level of at least three nucleotide sequences of interest is determined.

[0051] The state of the art suggests that the six mouse *Pira* genes are homologous to human leukocyte immunoglobulin-like receptors and encode cell membrane proteins that couple with a homodimer of the Fc receptor common γ chain and bind MHC class I tetramers. While not being bound by mechanism, binding of the PIR-A gene products by self MHC class I activates Fc receptor γ ITAMs and increases the basal activation state of the mast cells, macrophages, neutrophils, and dendritic cells that express these genes. The stimulatory effects of the *Pira* gene products are thought to be balanced by the single *Pirb* gene, which encodes an MHC class I binding protein with extracellular domains similar to those encoded

by *Pira* genes and is expressed on the same cell types. The PIR-B polypeptide, however, is associated with inhibitory ITIM motifs and downregulates the basal level of mast cell, macrophage, neutrophil, and dendritic cell activation. See, for example, Takai, T. (2005) *Immunology* 115:433-440, herein incorporated by reference in its entirety. An increased IL-13/sIL-13Rα2 complex level results in increased *Pira1* (SEQ ID NO:3) expression levels. While not being bound by mechanism, an increase in *pira1* expression without a corresponding increase in *pirb* expression is likely to promote inflammation by increasing the activation state of inflammatory and antigen presenting cells.

[0052] Vanin-1 (*Vnn1*, SEQ ID NO:4) encodes a pantetheinase that releases cysteamine from pantetheine. It is thought that cysteamine promotes the production of chemokines that attract neutrophils and inhibits γ-glutamylcysteine synthetase, which appears necessary to synthesize the natural reducing agent glutathione. Vanin-1 deficient mice appear to exhibit decreased inflammation, decreased iNOS expression, and increased arginase expression upon infection. See, for example, Pitari *et al.* 2000 *FEBS Lett* 483:149-154; Berruyer *et al* 2004 *Mol Cell Biol* 24:7214-7224; Martin *et al* 2004 *J. Clin. Invest* 113:591-597, herein incorporated by reference in their entirety. While not being bound by mechanism, this suggests that increased Vanin-1 is likely to exacerbate inflammation dependent on neutrophils and oxidation, promote classical macrophage activation, and inhibit alternative or allergic macrophage activation. An increased IL-13/sIL-13Rα2 complex level results in increased *Vnn1* (SEQ ID NO:4) expression levels.

[0053] It is thought that ApoA-1 suppresses LPS-induced acute lung injury, inflammation, and inflammatory cytokine production; inflammatory cytokine production by macrophages in direct contact with stimulated T cells; and fMLP and PMA induced neutrophil adhesion, oxidative burst, degranulation, and promotes removal of damaged and apoptotic cells. An increased IL-13/sIL-13Rα2 complex level results in decreased *ApoA1* (SEQ ID NO:5) expression levels. While not being bound by mechanism, decreases in ApoA1 should promote inflammation, especially inflammation associated with neutrophils and classically activated macrophages. In an aspect of the invention and while not being bound by mechanism, the complexing of IL-13 with sIL-13Rα2 may simultaneously suppress the allergic inflammation that is classically associated with IL-13 and stimulate inflammation of the type associated with Th1 cytokines.

[0054] Methods of determining expression levels of a nucleotide sequence of interest are known in the art and include, but are not limited to, qualitative Western blot analysis, immunoprecipitation, radiological assays, polypeptide purification, spectrophotometric analysis, Coomassie staining of acrylamide gels, ELISAs, RT-PCR, 2-D gel electrophoresis, microarray analysis, *in situ* hybridization, chemiluminescence, silver staining, enzymatic assays, ponceau S staining, multiplex RT-PCR, immunohistochemical assays, radioimmunoassay, colorimetric analysis, immunoradiometric assays, positron emission tomography, Northern blotting, fluorometric assays and SAGE. See, for example, Ausubel *et al*, eds. (2002) Current Protocols in Molecular Biology, Wiley-Interscience, New York, New York; Coligan *et al* (2002) Current Protocols in Protein Science, Wiley-Interscience, New York, New York; and Sun *et al.* (2001) *Gene Ther.* 8:1572-1579, herein incorporated by reference. Any method of assaying expression known in the art is suitable for use in the methods of the invention. Methods of determining expression levels of a nucleotide sequence of interest may be used to determine the relative expression level of the nucleotide sequence of interest to the expression level of a second nucleotide sequence of interest.

[0055] Methods of evaluating relative expression of gene sequences are well known in the art. See for example Bittner, *et al.* "Expression Analysis of RNA", *DNA Microarrays a Molecular Cloning Manual* 2003 ed. Bowtell & Sambrook, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, herein incorporated by reference in its entirety. Relative expression levels may be determined in numerous ways and the data obtained therefrom may be analyzed using a mathematical algorithm. Suitable algorithms include, but are not limited to MAS5 Statistical, Probe Logarithmic Intensity Error Estimation (PLIER), and Robust Multichip Analysis (RMA). Computer implementations of these mathematical algorithms can be utilized to for evaluation of relative expression levels. See for example Ball, *et al* "An Introduction to Microarray Bioinformatics", *DNA Microarrays a Molecular Cloning Manual* 2003 ed. Bowtell & Sambrook, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, herein incorporated by reference in its entirety. Examples of computer implementations of these mathematical algorithms include, but are not limited to, ImaGene (available from BioDiscovery), GenePix Pro 6.0 (available from Axon Instruments), ScanAlyze (available from EisenLab Stanford University), Spotfinder (TIGR), Imaxia (ArrayFox), F-Scan (Analytical Biostatistics Section NIH), GeneSpotter (MicroDiscovery), CLONDIAG (IconoClust), Koada Technology (Koadarray), Vigene Tech (Micro Vigene), Nonlinear Dynamics (Phoretix), CSIRO Mathematical and Information Sciences (SPOT), Niles

Scientific (SpotReader), Applied Maths (GeneMaths XT); Array Genetics (AffyMate), Axon Instruments (Acuity 4.0), BioDiscovery (GeneSight), BioSieve (ExpressionSeive), CytoGenomics (SilicoCyte), Microarray Data Analysis (GeneSifter), MediaCybernetics (ArrayPro Analyzer), Microarray Fuzzy Clustering (BioRainbow), Molmine (J-Express Pro), Optimal Design (ArrayMiner), Partek (Partek Pro), Predictive Patterns Software (GeneLinker), SAS Microarray, Silicon Genetics (GeneSpring), Spotfire (Spotfire), Strand Genomics (Avadis), Vialogy Corp, Affymetrix (GeneChip®), Affymetrix Expression Console, ArrayStar v.20 (DNASTAR), XRay (Biotique System), Expressionist (Genedata), and ChipInspector (Genomatix).

[0056] An increase in expression level or relative expression level is an increase in the range of 1% to 1000%, particularly 5% to 500%, more particularly 5% to 250%. A decrease in expression level or relative expression level is a decrease in the range of 1% to 100%, particularly 5% to 100%, more particularly 10% to 100%, yet more particularly 20% to 100%, yet still more particularly 30% to 100%. In an embodiment relative expression of SEQ ID NO:3 increases in the range of 1 fold to 10 fold, particularly 1 fold to 5 fold, yet more particularly 1 fold to 3 fold. In an embodiment relative expression of SEQ ID NO:4 increases in the range of 1 fold to 10 fold, particularly 1 fold to 5 fold, yet more particularly 1 fold to 3 fold. In an embodiment relative expression of SEQ ID NO:5 decreases by a factor in the range of 1 to 100, particularly 1 to 50, more particularly 1 to 25, yet more particularly 1 to 10, yet still more particularly 1 to 3.

[0057] In aspects of the methods of identifying an altered IL-13/sIL-13Rα2 complex level in a subject, the subject exhibits an inflammation related disorder or one or more symptoms associated with an inflammation related disorder. IL-13/sIL-13Rα2 related disorders include, but are not limited to, allergic responses, asthma, allergic asthma, non-allergic asthma, endofibrotic disorders, and inflammatory disorders.

[0058] The invention provides methods of identifying subjects suitable for inclusion in various studies, including but not limited to an IL-13/sIL-13Rα2 complex related study and an inflammatory disorder related study. By study is intended an investigation, research project, research proposal, clinical trial, observational assessment of one or more groups, or case control study. The object of the study determines the preferred attributes of subjects included in the study. Preferred attributes include but are not limited to, a specific

physiology, disorder, symptom, phenotype, genotype, health status, age, or gender. Further a preferred attribute may be one of these characteristics that falls within a specific range depending upon the subject design. Subjects suitable for inclusion in a study possess or exhibit one or more of the preferred attributes. The methods of the invention allow identification of subjects with the attribute of either an altered IL-13/sIL-13Rα2 complex level or a normal IL-13/sIL-13Rα2 level. Any method of identifying an altered IL-13/sIL-13Rα2 complex level described herein can be used to identify subjects with the attribute of either an altered IL-13/sIL-13R2 complex or a normal IL-13/sIL-13Rα2 complex level. Studies of particular interest, include but are not limited to, IL-13/sIL-13Rα2 complex related studies and inflammatory disorder related studies. Depending upon the goals and strategy of a study such as an IL-13/sIL-13Rα2 complex related study or an inflammatory disorder related study, a preferred attribute may be either an altered IL-13/sIL-13Rα2 complex level or a normal IL-13/sIL-13Rα2 complex level. In an aspect of the invention, subjects suitable for inclusion in a study are identified by providing a biological sample from the subject, assaying the expression level of at least one nucleotide sequence of interest in the biological sample, comparing the expression level of the nucleotide sequence of interest with a predetermined standard expression level and identifying the subject as a subject with either an altered IL-13/sIL-13Rα2 complex level or a normal IL-13/sIL-13Rα2 complex level.

[0059] Various embodiments of the invention pertain to methods of modulating inflammation in a subject exhibiting a sIL-13Rα2 related disorder and methods of modulating expression of at least one nucleotide sequence of interest selected from the group consisting of a nucleotide sequence set forth in SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:5. By "modulating" is intended increasing or decreasing inflammation or expression of a nucleotide sequence of interest by at least 1%, 5%, preferably 10%, 20%, more preferably 30%, 40%, 50%, 60%, yet more preferably 70%, 80%, 90%, or 100% as compared to an untreated or placebo treatment effect.

[0060] Modulation may be an increase or decrease in inflammation in one or more samples from the subject. Modulation of inflammation may occur in only one tissue or it may occur in multiple tissues. Methods of assaying inflammation include but are not limited to visual inspection, photometric assays, temperature evaluation, white blood cell concentrations, erythrocyte sedimentation rate assays, C-reactive protein level assays, pain assays, gallium imaging, indium imaging, and gene expression assays such as those described elsewhere

herein. Any method of assaying inflammation known in the art may be used in the practice of the invention.

[0061] Modulation may be an increase or decrease in expression of the nucleotide sequence of interest in one or more samples from a subject. Modulation of expression of a nucleotide sequence of interest may occur in only one tissue or it may occur in multiple tissues. Methods for assaying expression of nucleotide sequences of interest are described elsewhere herein. Any method of assaying expression of a nucleotide sequence of interest known in the art may be used to monitor the effects of the compound of interest on a subject.

[0062] Inflammation is a physiological response usually triggered by injury, infection, or allergy but also triggered both acutely and chronically in certain inflammatory related disorders. Inflammation related disorders include but are not limited to autoimmune disorders, colitis, asthma, chronic obstructive pulmonary disorder, airway hyper responsiveness, chronic airway remodeling, arthritis, and inflammatory disorders. Inflammation related phenotypes include, but are not limited to, edema, altered cytokine production, pain, discomfort, vasodilation, altered vessel permeability, bronchochonstriction, leukotriene release, prostaglandin release, fever, elevated white blood cell count, erythrocyte sedimentation rate, C-reactive protein level, and fibrinosis. Any methods of monitoring, evaluating, or assaying an inflammation related phenotype known in the art may be utilized in the methods of the invention. Methods of monitoring inflammation related phenotype include but are not limited to visual inspection, photometric assays, temperature evaluation, white blood cell concentrations, erythrocyte sedimentation rate assays, C-reactive protein level assays, pain assays, gallium imaging, indium imaging, and gene expression assays such as those described elsewhere herein.

[0063] IL-13/sIL-13Rα2 complex modulating agents include, but are not limited to, isolated IL-13/sIL-13Rα2, modified IL-13/sIL-13Rα2 complexes such as IL-13/sIL-13Rα2 fusion polypeptides, cross-linked IL-13/sIL-13Rα2 polypeptides, sIL-13Rα2-binding antibodies, IL-13 binding antibodies, peptidomimetics, cross-linking agents, complex disrupting agents, an IL-13-like molecule, a sIL-13Rα-like molecule, and a sIL-13Rα2 agonist. By "isolated IL-13/sIL-13Rα2" is intended an IL-13/sIL-13Rα2 complex substantially free of other cellular material or culture medium when produced by recombinant techniques or substantially free of chemical precursors or other chemicals when chemically synthesized. It is recognized that

isolated IL-13/sIL-13Rα2 may be obtained from a biological sample as complex, prepared *in vitro* from one or two chemically synthesized components, or prepared *in vitro* from one or two components obtained from a biological sample. IL-13/sIL-13Rα2 complex modulating agents are not bound by a particular mechanism but may function by impacting various aspects of the IL-13/sIL-13Rα2 complex including but not limited to complex structure, complex solubility, or complex's effect on nucleotide sequence of interest expression levels. An IL-13/sIL-13Rα2 complex modulating agent that impacts the complex's effect on nucleotide sequence of interest expression levels could affect among things, the specificity of the effect on nucleotide sequence of interest expression levels, the degree of effect on a nucleotide sequence of interest expression level, increase the effect on a nucleotide sequence of interest expression, or decrease the effect on a nucleotide sequence of interest expression.

[0064] The term "administer" is used in its broadest sense and includes any method of introducing a compound into a transgenic animal of the present invention. This includes producing polypeptides or polynucleotides in vivo as by transcription or translation in vivo of polynucleotides that have been exogenously introduced into a subject. Thus, polypeptides or nucleic acids produced in the subject from the exogenous compositions are encompassed in the term "administer."

[0065] An IL-13/sIL-13Rα2 complex modulating agent may additionally comprise a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, such media can be used in the compositions of the invention. Supplementary active compounds can also be incorporated into the compositions. A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial

agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[0066] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor ELTM (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, and sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

[0067] Sterile injectable solutions can be prepared by incorporating the active compound (e.g., a carboxypeptidase protein or anti- carboxypeptidase antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of

preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[0068] Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For oral administration, the agent can be contained in enteric forms to survive the stomach or further coated or mixed to be released in a particular region of the GI tract by known methods. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose, saccharin, phenylalanine, or sucralose; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

[0069] For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser, which contains a suitable propellant, e.g., a gas such as carbon dioxide, a nebulizer, or an inhaler. Compounds may also be delivered with supplemental oxygen administered to a subject.

[0070] Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

[0071] In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811.

[0072] It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. "Dosage unit form" as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

[0073] The following examples are offered by way of illustration and not limitation.

EXPERIMENTAL

Example 1. Evaluation of sIL-13Rα2 and sIL-4R levels in serum

[0074] BALB/c wild-type mice were obtained from Taconic. Eight to sixteen week old untreated BALB/c mice (5/gp) were bled. A first and second aliquot were obtained from the biological samples. Levels of IL-4/sIL-4Rα complex and IL-13/sIL13-Rα2 complex in the first aliquots were measured by ELISA. Affinity purified goat anti-IL-13 antibodies were used to capture the IL-13/sIL-13Rα2 complex onto microtiter plate walls followed by biotin-labeled anti-IL13 monoclonal antibody (C531), followed by a horseradish peroxidase streptavidin conjugate (Pierce Chemical Co.) and a luminogenic substrate for horseradish peroxidase. C531 binds IL-13 and IL-13 that is complexed to sIL-13Rα2. To evaluate IL-

4/sIL4Rα levels, microtiter plate wells were coated with goat anti-IL4Rα monoclonal antibody. Captured IL-4/sIL-4Rα complex was detected with biotin anti-IL-4 monoclonal antibody (BVD6-24G2.3). Luminescence was measured with a Fluoroskan Ascent FL microtiter plate luminometer/fluorometer (Labsystems). Total levels of sIL-13Rα2 or sIL-4Rα in the second aliquots were detected similarly. Isolated IL-13 (100 ng/ml) or IL-4 (20 ng/ml), respectively was added to the serum before performing the ELISA on the second aliquots. The percentage of saturation of the soluble receptor (sR) with the cytokine was determined by dividing the concentration of the cytokine/SR complex by the total soluble cytokine receptor concentration. Data obtained from one such experiment are presented in Figure 1.

Example 2. Preparation of Affinity Purified Antibodies

[0075] Mouse IL-13Rα2-human IgGFc fusion protein (Wyeth) was combined with complete Freund's adjuvant (Difco) and injected into a goat. The goat was provided a boost preparation of mouse IL-13Rα2-human IgGFc fusion protein combined with incomplete Freund's adjuvant (Difco). Serum was harvested. Human IgG was coupled to CNBr-activated Sepharose (Pharmacia). The serum was adsorbed with the IgG coupled Sepharose. The non-adsorbing material was collected and incubated with mouse IL-13Rα2-mouse IgGFc fusion protein coupled to CNBr-activated Sepharose. The Sepharose beads were washed. A solution containing 3.5 M $MgCl_2$ was added to the mouse IL-13Rα2-mouse IgGFc fusion protein coupled Sepharose beads. The eluant containing affinity purified goat anti-mouse IL-13Rα2 Ab was collected.

[0076] A goat was immunized with mouse sIL-4Rα (Immunex) in complete Freund's adjuvant. The goat was boosted with the same antigen in incomplete Freund's adjuvant. Antibodies were affinity purified by adsorption to and 3.5 M $MgCl_2$ elution from mouse IL-4Rα coupled to CNBr-activated Sepharose.

Example 3. Determination of the Percent Saturation of the sIL-13Rα2 and sIL-4R in serum

[0077] Serum IL-13/sIL-13Rα2 complexes were measured by ELISA using affinity purified goat anti-IL-13Rα2 Ab adhered onto microtiter plate walls, followed by biotin-labeled C531 anti-IL-13 mAb, streptavidin-horseradish peroxidase, and luminogenic substrate. Serum levels of IL-4/sIL-4Rα complex were detected by an ELISA in which microtiter plates were coated with goat anti-IL-4Rα mAb and captured complex was detected with biotin-anti-IL-4

mAb (BVD6-24G2.3). Total levels of sIL-13Rα2 or sIL-4Rα were detected in the same way, except that recombinant IL-13 (100 ng/ml) or IL-4 (20 ng/ml) respectively was added to the serum prior to performing the assay. Percentage of saturation of the soluble receptor with cytokine was determined by dividing the concentration of cytokine/soluble receptor complex by the total soluble cytokine receptor concentration.

[0078] Data obtained from one such experiment are presented in Figure 1.

Example 4. Assessment of sIL-13Rα2 and sIL-4Rα Response to Th2 Stimulation

[0079] BALB/c mice were bled to obtain an initial timepoint. Mice were immunized with GaMD (goat anti-mouse IgD antiserum) or inoculated subcutaneously with 500 *N. brasiliensis* third stage infectious larvae. *N. brasiliensis* and GaMD stimulate Th2 cytokine production. Blood samples were obtained at days 1, 3, 5, 7, 10 and 14 post-inoculation or days 2, 6, and 14 post-infection.

[0080] Serum concentrations of sIL-4Rα and sIL-13Rα2 and their percent saturation with their cytokine ligands (IL-4 or IL-13 respectively) were determined as described above herein. Data from one such experiment are presented in Figure 2.

Example 5. Cytokine Requirements for the sIL-13Rα2 Response to *S. mansoni* Infection

[0081] The sIL-13Rα2 response to *Schistosoma mansoni* infection was determined in multiple mouse strains to evaluate the response in the absence of various cytokines. BALB/c wild-type mice, IL-13 deficient mice and IL-4/IL-13 double deficient mice were bled to obtain a zero timepoint. The mice were inoculated percutaneously via the tail with 25-30 cercariae of a Puerto Rican strain of *S. mansoni* that were obtained from infected *Biomphalaria glabrata* snails. Mice were bled at 3, 6, 9, 12, and 16 days post-inoculation.

[0082] Serum concentrations of sIL-13Rα2 and its percent saturation with its cytokine ligand IL-13 were determined as described above herein. Data from one such experiment are presented in Figure 3.

Example 6. Assessment of Effect of Rapid Production of IL-4 and IL-13 on serum sIL-13Rα2 and sIL-4R Concentration and Saturation

[0083] *BALB/c* mice were used to investigate the effect of rapid production of IL-4 and IL-13 on serum sIL-13Rα2 and sIL-4R concentration and saturation. Mice (n=5) were injected with either saline or GaMD. Fourteen days later the GaMD injected mice were challenged with either saline or rat anti-IgE mAb (100 μg). Blood samples were obtained four hours after the challenge. Serum concentrations of sIL-4R and sIL-13Rα2 and their percent saturation with their cytokine ligands was determined as described elsewhere herein.

[0084] Mice were injected with saline or anti-CD3 mAb (10 μg). Blood samples were obtained two hours after the injection. Serum concentrations of sIL-4R and sIL-13Rα2 and their percent saturation with their cytokine ligands were determined as described elsewhere herein. Data from one such experiment are presented in Figure 4.

Example 7. Assessment of Response to Exogenous Administration of IL-4 or IL-13

[0085] BALB/C mice were injected with 1μg of IL-4 or IL-13. Blood samples were obtained at various timepoints post-injection. Serum concentrations of sIL-4R and sIL-13Rα2 and their percent saturation with their cytokine ligands was determined as described elsewhere herein.

[0086] Data from one such experiment are presented in Figure 5.

Example 8. Evaluation of Pathway Involvement in IL-4 and IL-13 Upregulation of serum sIL-13Rα2 concentration

[0087] IL-4C was prepared by mixing IL-4 and anti-IL-4 mAb at a 2:1 molar ratio. BALB/c wild-type, IL-4Rα-deficient and STAT6 deficient mice were injected intraperitoneally with IL-4C that contained 2μm IL-4. Mice were bled on day 0 and day 3 and the serum level of sIL-13Rα2 was determined. BALB/c wild-type, IL-4Rα-deficient STAT6 deficient mice and IL-13Rα2 deficient mice were injected intraperitoneally with 1 μg IL-13. Mice were bled on day 0 and day 1, and the serum levels of sIL-13Rα2 were determined. Data from one such experiment are presented in Figure 6.

Example 9. Analysis of Serum Receptor and Cytokine/Receptor Complex Half-lives

[0088] Normal mouse serum was obtained and concentrated 5 fold. IL-4Rα deficient mice were injected with 0.5 ml of 5X normal sera or 5X normal sera plus IL-4. Mice were bled at

15 minutes, 45 minutes and 6 hours post-administration. Serum levels of IL-4/sIL-4Rα complex and total sIL-4Rα were determined as described elsewhere herein.

[0089] Wild-type BALB-c and IL-13 deficient BALB-c mice were infected with *N. brasiliensis* as described elsewhere herein. Serum was obtained from the infected mice and concentrated 10 fold. The 10X serum (0.5 ml) was injected into IL-13/sIL-13Rα2 double-deficient mice. In one experiment mice were bled at 0.5, 2, 6, and 12 hours post treatment; in another, the mice were bled at 0.5, 2, 6, 12, and 24 hours post treatment. Amounts of free sIL-13Rα2 or IL-13/sIL-13Rα2 were determined as described elsewhere herein.

[0090] Mean values from two independent experiments were calculated. Half-life curves were calculated for both sets of points using exponential equations. Data from one such experiment are presented in Figure 7.

Example 10. Preparation of IL-13 Rich Serum

[0091] IL-13Rα2-deficient mice were inoculated with 200 or 500 infective *N. brasiliensis* larvae on day 0 and day 14. The mice were injected intraperitoneally with 10 μg anti-CD3 mAb on day 21. Mice were bled on day 6, 7, 8, 19, and 2 hours after the anti-CD3 injection. Sera were pooled and concentrated 2-fold.

Example 11. Determination of IL-13 Half-Life

[0092] IL-13 rich serum was prepared as described above herein. IL-13/IL-13Rα2 double deficient mice were generated by breeding IL-13 deficient and IL-13Rα2 deficient mice. Offspring were typed by PCR. Nine IL-13/IL-13Rα2 double deficient mice were injected with 0.5 ml of IL-13 rich serum. Groups of three recipient mice were bled at 20, 40 or 80 minutes post-injection. Urine samples were obtained and pooled from the same sets of mice at 30, 90 or 720 minutes post-injection. IL-13 levels were determined by ELISA.

Example 12. Assessment of IL-4Rα Involvement in Induction of sIL-13Rα2

[0093] IL-4Rα deficient BALB/c mice were injected with 1 μg of IL-13. Total sIL-13Rα2 levels were determined at 0, 4, 8, and 12 hours post injection.

Example 13. Renal Secretion Analysis

[0094] BALB/c mice were injected intravenously with saline, recombinant mouse IL-13, or IL-4C. IL-13Rα2 deficient mice were injected with IL-4C. IL-13 deficient BALB/c mice were injected with saline or IL-4C.

[0095] Blood and urine samples were collected one day post treatment. Total sIL-13Rα2 and IL-13/sIL-13Rα2 complex concentrations were determined.

Example 14. Expression Level Analysis

[0096] Gene expression summary values for the Affymetrix GeneChip data in CEL files were computed using RMAExpress (http://rmaexpress.bmbolsted.com). Data analyses were carried out with GeneSpring version 7.3.1 (Agilent Technologies) software, including filtering, statistical analysis and clustering. Hybridization signals were transformed from log base 2 to linear values and then the relative expression of each sequence on the array was normalized to its expression in mice treated with PBS or other appropriate controls in the same experiment.

[0097] Relative expression levels of particular genes of interest were determined by real-time PCR. Total RNA was extracted from frozen lungs using Trizol Reagent® (Invitrogen) per the manufacturer's protocols, followed by purification with RNeasy mini kit and DNAse digestion (Qiagen). RNA purity was confirmed with a Nanodrop® Spectrophotometer (Nanodrop) and RNA integrity was confirmed using a Bioanalyzer (model 2100, Agilent Technologies). Purified total lung RNA was reverse transcribed into single-stranded cDNA using random hexamers and Superscript II (Invitrogen). Real-time RT-PCR was performed on the iCycler (Roche Diagnostics) using a total volume of 20 µl, containing 100 µM of iCycler-DNA Master SYBR Green (Roche Diagnostics), ddH_2O, and 4 µl cDNA, which corresponds to approximately 33 ng of total RNA. The cDNA was added as template and 5 µl (3 mM) of the primer of interest was added to the PCR reaction. The amount of mRNA transcripts encoding β-actin, ApoA1, Vnn1, Pira1, IL-13Rα2 and 18S RNA was determined using the following formula: relative Gene Expression = $(1.8^{(a-b)} \times 100{,}000$ where a= crossing point of β-actin and b=crossing point of gene of interest. Data were analyzed by ANOVA and Fisher's protected least significant difference for statistical significance, using Statview. Values of $p<0.05$ were considered statistically significant.

Example 15. Assessment of IL-4 Effect on Steady-State IL-13Rα2 Expression Levels

[0098] BALB/c and IL-13 deficient BALB/c mice were injected intravenously with vehicle or IL-4C (2 μg IL-4/10 μg BVD4-11D11 in 200 μl saline). Mice were sacrificed three days later.

[0099] Levels of sIL-13Rα2 mRNA relative to mRNA levels for a housekeeping gene (18S RNA) were determined as described elsewhere herein. Results obtained from one such experiment are presented in Figure 10, panel A.

Example 16. Assessment of IL-4Rα Involvement in Steady-State IL-13Rα2 Expression Levels

[0100] BALB/c and IL-4Rα deficient BALB/c mice were injected with vehicle or 2 μg IL-13. IL-4Rα deficient BALB/c mice were injected with vehicle, 3 μg IL-13, or 3 μg IL-13 + 9 μg sIL-13Rα2-Fc. Mice were sacrificed one day post-injection.

[0101] Levels of sIL-13Rα2 mRNA relative to mRNA levels for a housekeeping gene (18S RNA) were determined as described elsewhere herein. Results obtained from one such experiment are presented in Figure 10, panels B and C.

Example 17. Assessment of IL-13/sIL-13Rα2 Complexes on Pulmonary Gene Expression

[0102] Wild-type and IL-13/sIL-13Rα2 double deficient mice were infected with *N. brasiliensis*. Sera were harvested from the mice. The sera was saturated with recombinant mouse IL-13 then absorbed to remove any free IL-13.

[0103] In some experiments, aliquots of the sera were either mock absorbed with Ab-agarose or absorbed with anti-IL-13Rα2 Ab-agarose prior to inoculation into mice.

[0104] IL-13/sIL-13Rα2 double-deficient mice were inoculated intratracheally daily on 3 consecutive days with 50 μl PBS, serum from *N. brasiliensis* infected wild-type or serum from *N. brasiliensis* infected IL-13/sIL-13Rα2 double-deficient mice. Mice were sacrificed 16 hours after the third dose of PBS or serum.

[0105] The lungs were harvested. RNA was purified from the lungs and used for a gene scan or real-time PCR. Gene scans were analyzed as described elsewhere herein. Real-time PCR was performed as described elsewhere herein.

[0106] Real-time PCR was performed on 4 individual mice/group in each of 2 separate experiments to determine *Pira1* (SEQ ID NO:3), *Vnn-1* (SEQ ID NO:4), and *ApoA1* (SEQ ID NO:5) gene expression levels relative to levels of the β-actin gene. Results from two identical experiments were pooled and are presented in Figure 12.

Example 18. Assessment of IL-13/sIL-13Rα2 Complex Stability

[0107] Recombinant sIL-13Rα2-IgGFc fusion protein (150 µl) was bound to agarose and saturated with 4.5 ng IL-13. The agarose was washed extensively with PBS. The agarose was step-wise eluted with buffers at pH 7, 6.5, 6, 5.5, 5, 4.5 and 4.1. Lastly, 3.5 M $MgCl_2$ was incubated with the agarose complex. Eluants were collected and assayed for IL-13 by ELISA. Results from one such experiment are presented in Figure 11.

[0108] All publications, patents, and patent applications mentioned in the specification are indicative of the level of those skilled in the art to which this invention pertains. All publications, patents, and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually incorporated by reference.

[0109] Having described the invention with reference to the exemplary embodiments, it is to be understood that it is not intended that any limitations or elements describing the exemplary embodiment set forth herein are to be incorporated into the meanings of the patent claims unless such limitations or elements are explicitly listed in the claims. Likewise, it is to be understood that it is not necessary to meet any or all of the identified advantages or objects of the invention disclosed herein in order to fall within the scope of any claims, since the invention is defined by the claims and since inherent and/or unforeseen advantages of the present invention may exist even though they may not be explicitly discussed herein.

[0110] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

[0111] THAT WHICH IS CLAIMED:

1. A method of determining the ratio of sIL13Rα2 and IL-13/sIL13Rα2 complex in a biological sample comprising the steps of:

(a) obtaining a biological sample;

(b) incubating said biological sample with a first IL-13/sIL-13Rα2 complex detecting reagent; and

(c) assaying said first complex detecting reagent.

2. The method of claim 1, wherein said first IL-13/sIL-13Rα2 complex detecting reagent comprises an antibody selected from the group consisting of an antibody that binds an IL-13 polypeptide and an antibody that binds a sIL-13Rα2 polypeptide.

3. The method of claim 1 further comprising the steps of:

(a) incubating said biological sample and first IL-13/sIL-13Rα2 with a second IL-13/sIL-13Rα2 complex detecting reagent; and

(b) assaying said second IL-13/sIL-13Rα2 complex detecting reagent.

4. The method of claim 3, wherein said second IL-13/sIL-13Rα2 complex detecting reagent comprises an antibody selected from the group consisting of an antibody that binds an IL-13 polypeptide and an antibody that binds a sIL-13Rα2 polypeptide.

5. The method of claim 1, comprising the step of incubating a biological sample with a polypeptide selected from the group consisting of:

(a) a polypeptide having the amino acid sequence set forth in SEQ ID NO:6;

(b) a polypeptide comprising at least 30 consecutive amino acids of the amino acid sequence set forth in SEQ ID NO:6, wherein said polypeptide is capable of binding sIL-13Rα2;

(c) a polypeptide having at least a 95% identity to the amino acid sequence set forth in SEQ ID NO:6, wherein said polypeptide is capable of binding sIL-13Rα2; and

(d) a polypeptide consisting of at least 30 consecutive amino acids of the amino acid sequence set forth in SEQ ID NO:6, wherein said polypeptide is capable of binding to sIL-13Rα2.

6. The method of claim 1, comprising the step of comparing the sIL-13Rα2 level and the IL-13/sIL-13Rα2 level.

7. A method of determining the ratio of sIL13Rα2 and IL-13/sIL13Rα2 complex in a biological sample comprising the steps of:

(a) obtaining a biological sample from a subject;

(b) providing a first aliquot of said biological sample and a second aliquot of said biological sample;

(c) incubating said first aliquot with isolated IL-13;

(d) incubating said first aliquot with a first IL-13/sIL-13Rα2 complex detecting reagent; and

(e) incubating said second aliquot with first IL-13/sIL-13Rα2 complex detecting reagent.

8. The method of claim 7, further comprising the step of incubating said first aliquot, said IL-13 and said first IL-13/sIL-13Rα2 complex detecting reagent with a second IL-13/sIL-13Rα2 complex detecting reagent and incubating said second aliquot and said first IL-13/sIL-13Rα2 complex detecting reagent with a second IL-13/sIL-13Rα2 complex detecting reagent.

9. A method of identifying an allergic response in a subject comprising the steps of:

(a) obtaining a biological sample from a subject;

(b) incubating said biological sample with a first IL-13/sIL-13Rα2 complex detecting reagent;

(c) determining the IL-13/sIL-13Rα2 complex level in the biological sample; and

(d) comparing the IL-13/sIL-13Rα2 complex level in said sample with a standard IL-13/sIL-13Rα2 complex level.

10. The method of claim 9, wherein said first IL-13/sIL-13Rα2 complex detecting reagent comprises an antibody.

11. The method of claim 10, wherein the antibody is selected from the group of antibodies consisting of an antibody that binds an IL-13 polypeptide and an antibody that binds a sIL-13Rα2 polypeptide.

12. The method of claim 9, comprising the step of incubating said biological sample and said first IL-13/sIL-13Rα2 complex detecting agent with a second IL-13/sIL-13Rα2 complex detecting reagent.

13. The method of claim 12, wherein said second IL-13/sIL-13Rα2 complex detecting reagent comprises an antibody selected from the group of antibodies consisting of an antibody that binds an IL-13 polypeptide and an antibody that binds a sIL-13Rα2 polypeptide.

14. The method of claim 9, wherein said biological sample is selected from the group consisting of blood and serum.

15. A kit for identifying an allergic response in a subject comprising a first IL-13/sIL-13Rα2 complex detecting reagent and a second IL-13/sIL-13Rα2 complex detecting reagent.

16. The kit of claim 15, wherein said first or second IL-13/sIL-13Rα2 complex detecting reagent comprises an antibody that binds an IL-13 polypeptide and said first or second IL-13/sIL-13Rα2 complex detecting reagent comprises an antibody that binds a sIL-13Rα2 polypeptide.

17. A method of identifying an inflammation related disorder in a subject comprising the steps of:

(a) obtaining a biological sample from a subject;

(b) incubating said biological sample with a first IL-13/sIL-13Rα2 complex detecting reagent;

(c) determining the IL-13/sIL-13Rα2 complex level in the biological sample; and

(d) comparing the IL-13/sIL-13Rα2 complex level in said sample with a standard IL-13/sIL-13Rα2 complex level.

18. A method of identifying an altered IL-13/sIL-13Rα2 complex level in a subject, said method comprising the steps of:

(a) obtaining a biological sample from a subject;

(b) assaying the expression level of at least one nucleotide sequence of interest in said biological sample; and

(c) comparing the expression level of said at least one nucleotide sequence of interest with a standard expression level for said nucleotide sequence of interest.

19. The method of claim 18, wherein said subject exhibits an inflammation related disorder.

20. The method of claim 18, comprising assaying the expression level of at least two nucleotide sequences of interest in said biological sample.

21. The method of claim 18, comprising assaying the expression level of three nucleotide sequences of interest in said biological sample.

22. The method of claim 18, wherein said nucleotide sequence of interest is selected from the group of nucleotide sequences having a nucleotide sequence set forth in SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and a nucleotide sequence having at least 95% identity to a nucleotide sequence set forth in SEQ ID NO:3, SEQ ID NO:4, or SEQ ID NO:5.

23. The method of claim 22 wherein an increased expression level of a nucleotide sequence of interest set forth in SEQ ID NO:3 or SEQ ID NO:4 or a nucleotide sequence having at least 95% identity to a nucleotide sequence set forth in SEQ ID NO:3 or SEQ ID NO:4 indicates an increased IL-13/sIL-13Rα2 complex level in said subject.

24. The method of claim 22 wherein a decreased expression level of a nucleotide sequence of interest set forth in SEQ ID NO:5 or a nucleotide sequence having at least 95% identity to the nucleotide sequence set forth in SEQ ID NO:5 indicates an increased IL-13/sIL-13Rα2 complex level in said subject.

25. A method of identifying a subject suitable for inclusion in an IL-13/sIL-13Rα2 complex related study comprising the steps of:

(a) obtaining a biological sample from a subject;

(b) assaying the expression level of at least one nucleotide sequence of interest in said biological sample;

(c) comparing the expression level of said at least one nucleotide sequence of interest with a standard expression level for said nucleotide sequence of interest;

(d) identifying the subject as a subject with either an altered IL-13/sIL-13Rα2 complex level or a normal IL-13/sIL-13Rα2 complex level;

(e) identifying a subject with an attribute of an altered or normal IL-13/sIL-13Rα2 complex level; and

(f) identifying said subject as a subject having a preferred attribute for said IL-13/sIL-13Rα2 complex related study.

26. The method of claim 25 further comprising characterizing a subject with an increased expression level of a nucleotide sequence of interest set forth in SEQ ID NO:3 or SEQ ID NO:4 or a nucleotide sequence having at least 95% identity to a nucleotide sequence set forth in SEQ ID NO:3 or SEQ ID NO:4 or a decreased expression level of a nucleotide sequence of interest set forth in SEQ ID NO:5 or a nucleotide sequence having at least 95% identity to the nucleotide sequence set forth in SEQ ID NO:5 as a subject with an elevated IL-13/sIL-13Rα2 complex level.

27. A method of identifying a subject suitable for inclusion in an inflammatory disorder related study comprising the steps of:

(a) obtaining a biological sample from a subject;

(b) assaying the expression level of at least one nucleotide sequence of interest in said biological sample;

(c) comparing the expression level of said at least one nucleotide sequence of interest with a predetermined standard expression level; and

(d) identifying the subject as a subject with either an altered IL-13/sIL-13Rα2 complex level or a normal IL-13/sIL-13Rα2 complex level.

28. A method of modulating inflammation in a subject comprising the steps of:

(a) providing a subject exhibiting an IL-13/sIL-13Rα2 related disorder;

(b) administering an IL-13/sIL-13Rα2 complex modulating agent to said subject; and

(c) monitoring an inflammation related phenotype in said subject.

29. The method of claim 28 wherein said IL-13/sIL-13Rα2 complex modulating agent comprises isolated IL-13/sIL-13Rα2 complex.

30. The method of claim 28, wherein said IL-13/sIL-13Rα2 complex modulating agent comprises an agent selected from the group consisting of an IL-13-like molecule, a sIL-13Rα2-like molecule, a sIL-13Rα2 agonist, and a sIL-13Rα2 binding antibody.

31. The method of claim 28, wherein an inflammation related phenotype increases.

32. The method of claim 28, wherein an inflammation related phenotype decreases.

33. The method of claim 28, wherein said inflammation related phenotype is expression of a nucleotide sequence of interest selected from the group of nucleotide sequences of interest having the nucleotide sequence set forth in SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:5 and said expression is altered.

34. The method of claim 28, wherein said IL-13/sIL-13Rα2 related disorder is selected from the group consisting of: asthma, allergic responses, endofibrotic disorders, and inflammatory disorders.

35. A method of modulating expression of a nucleotide sequence of interest selected from the group consisting of nucleotide sequences of interest having the nucleotide sequence set forth in SEQ ID NO:3 (Pira1), SEQ ID NO:4 (Vannin1), and SEQ ID NO:5 (ApoA1) comprising the steps of:

(a) providing a subject exhibiting a nucleotide sequence of interest-related disorder; and

(b) administering an IL-13/sIL-13Rα2 complex modulating agent to said subject.

36. The method of claim 35, wherein SEQ ID NO:3 expression levels increase.

37. The method of claim 35, wherein SEQ ID NO:4 expression levels increase.

38. The method of claim 35, wherein SEQ ID NO:5 expression levels decrease.

39. The method of claim 35, wherein expression levels of at least 2 nucleotide sequences of interest are altered.

40. The method of claim 35, wherein expression levels of at least 3 nucleotide sequences of interest are altered.

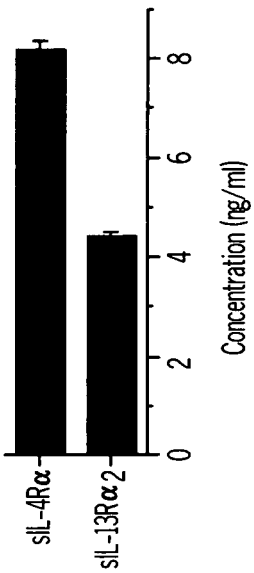
sIL-4Rα
sIL-13Rα2
0
2
4
6
8
Concentration (ng/ml)

FIG. 1A

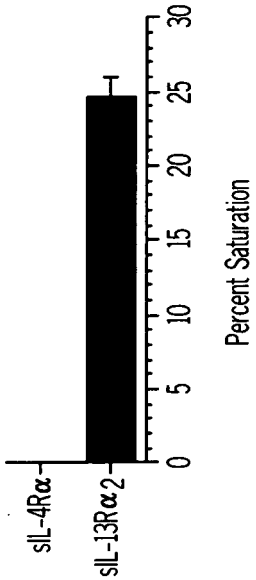
sIL-4Rα
sIL-13Rα2
0
5
10
15
20
25
30
Percent Saturation

FIG. 1B

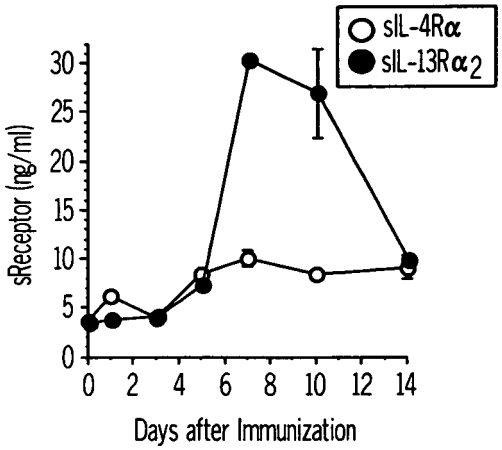

FIG. 2A

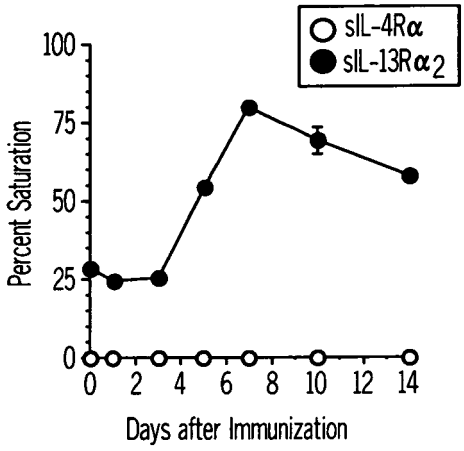

FIG. 2B

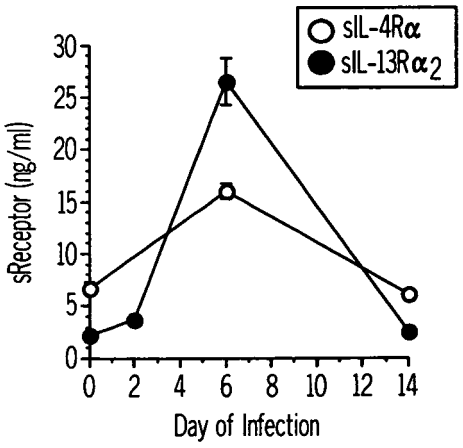

FIG. 2C

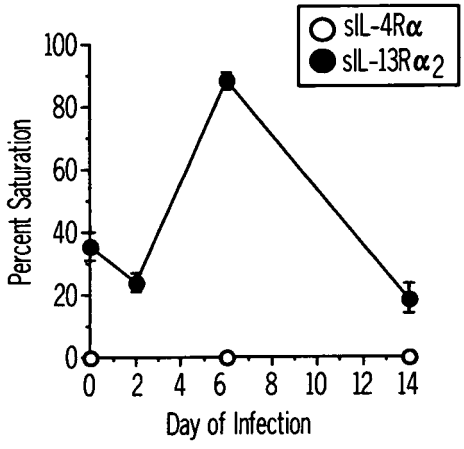

FIG. 2D

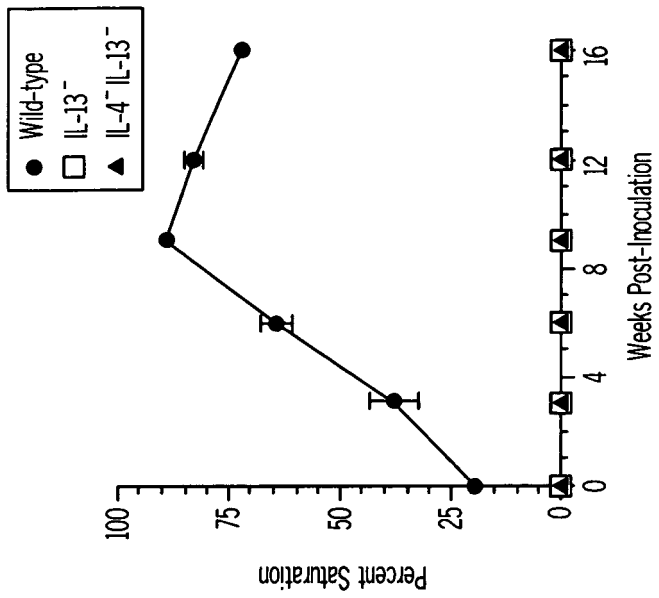
Wild-type
IL-13⁻
IL-4⁻ IL-13⁻
Percent Saturation
100
75
50
25
0
0
4
8
12
16
Weeks Post-Inoculation

FIG. 3B

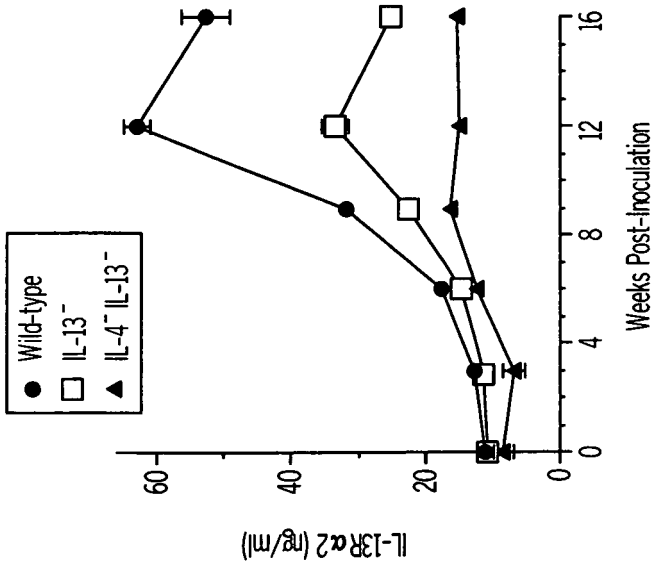
Wild-type
IL-13⁻
IL-4⁻ IL-13⁻
IL-13Rα2 (ng/ml)
60
40
20
0
0
4
8
12
16
Weeks Post-Inoculation

FIG. 3A

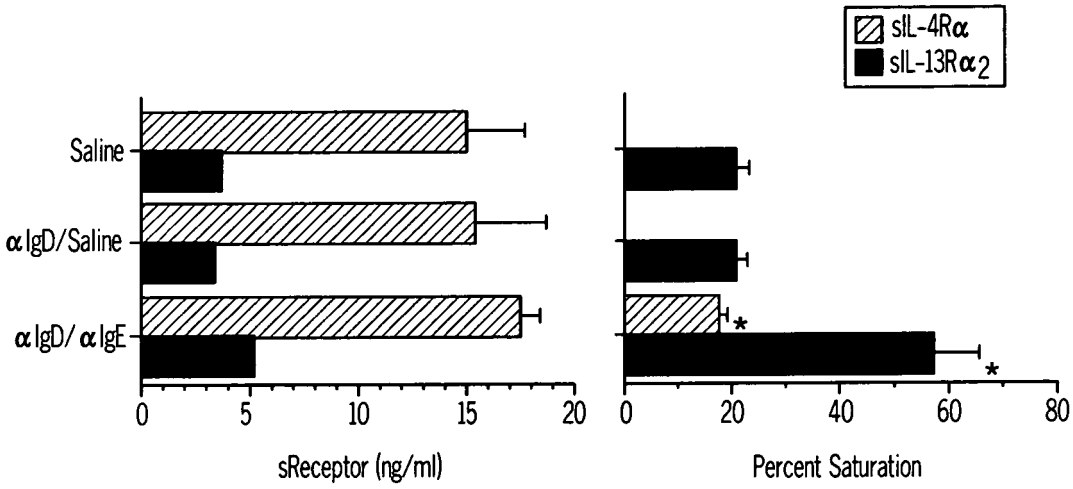
sIL-4Rα
sIL-13Rα2
Saline
αIgD/Saline
αIgD/αIgE
0
5
10
15
20
sReceptor (ng/ml)
0
20
40
60
80
Percent Saturation
*
*

FIG. 4A

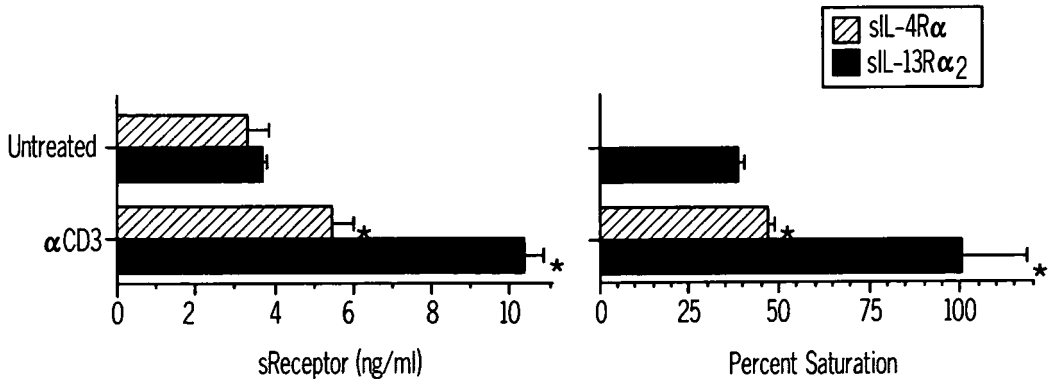
sIL-4Rα
sIL-13Rα2
Untreated
αCD3
0
2
4
6
8
10
sReceptor (ng/ml)
0
25
50
75
100
Percent Saturation
*
*
*
*

FIG. 4B

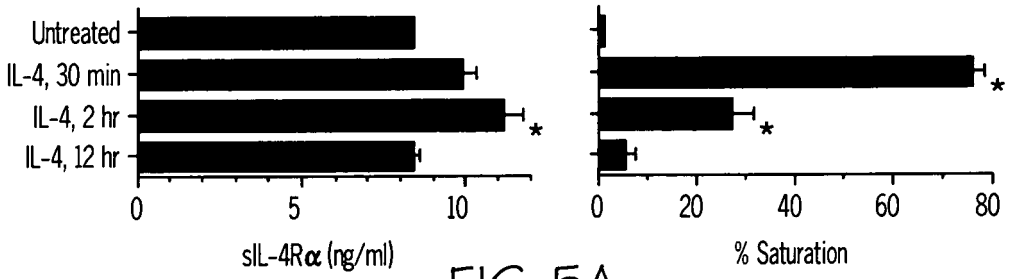

FIG. 5A

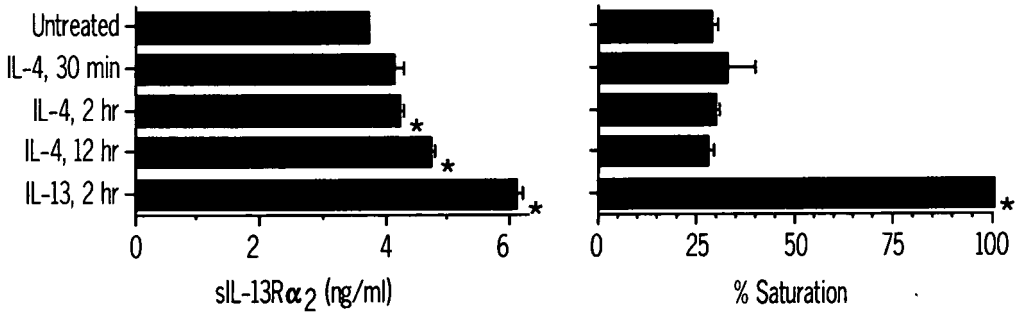

FIG. 5B

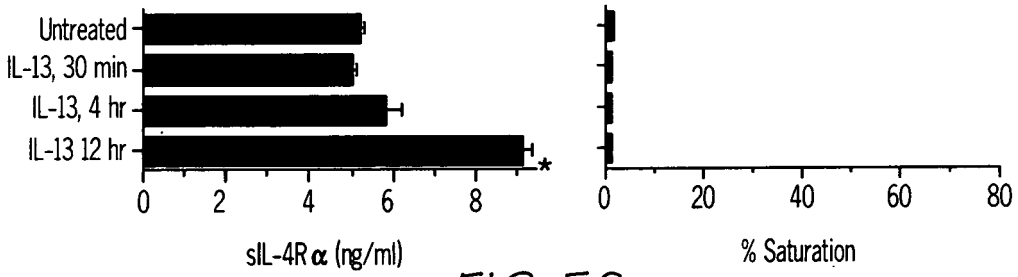

FIG. 5C

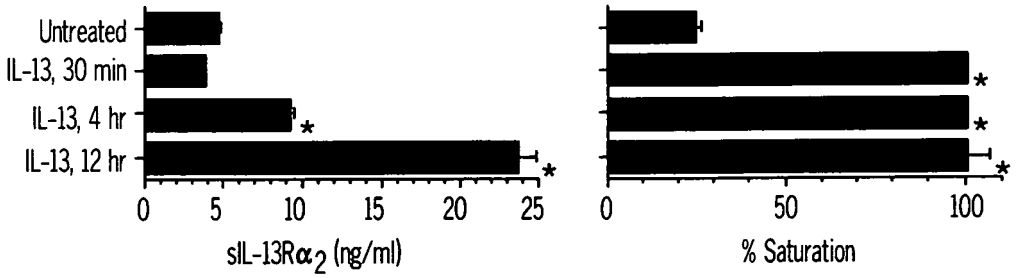

FIG. 5D

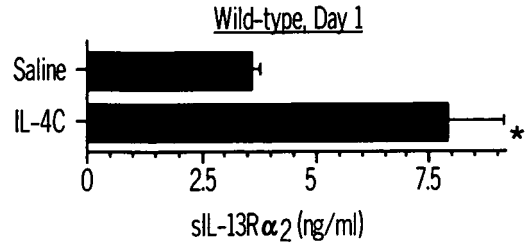

FIG. 6A

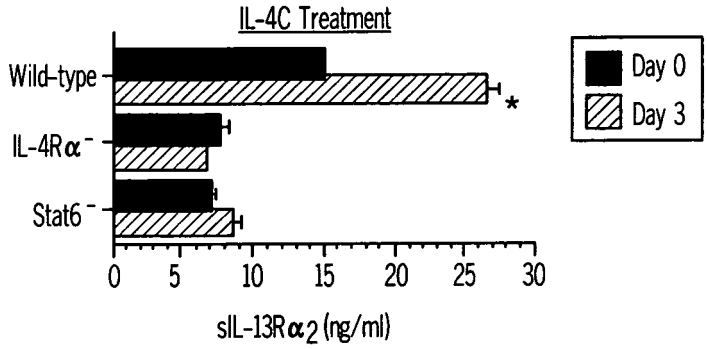

FIG. 6B

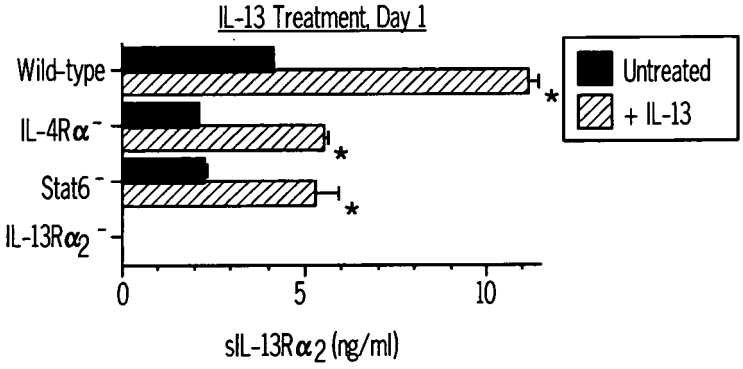

FIG. 6C

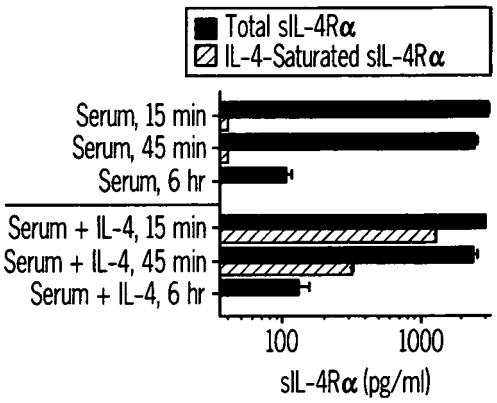

FIG. 7A

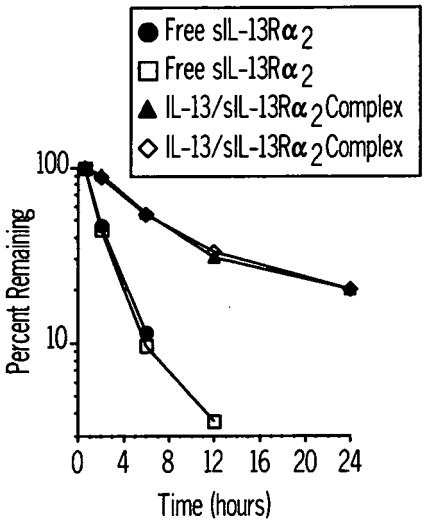

FIG. 7B

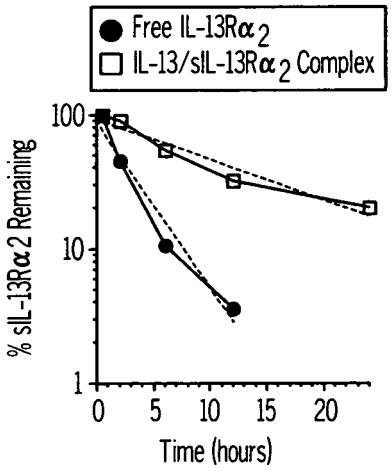

FIG. 7C

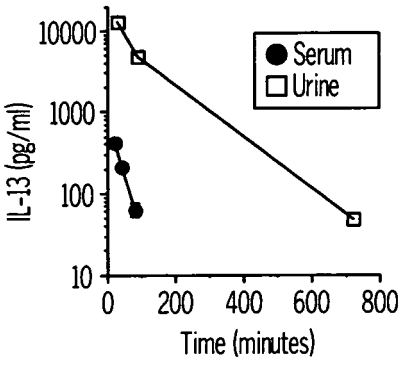

FIG. 7D

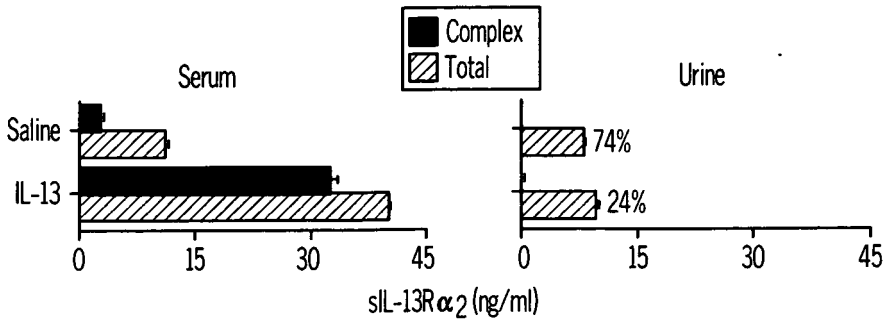

FIG. 8A

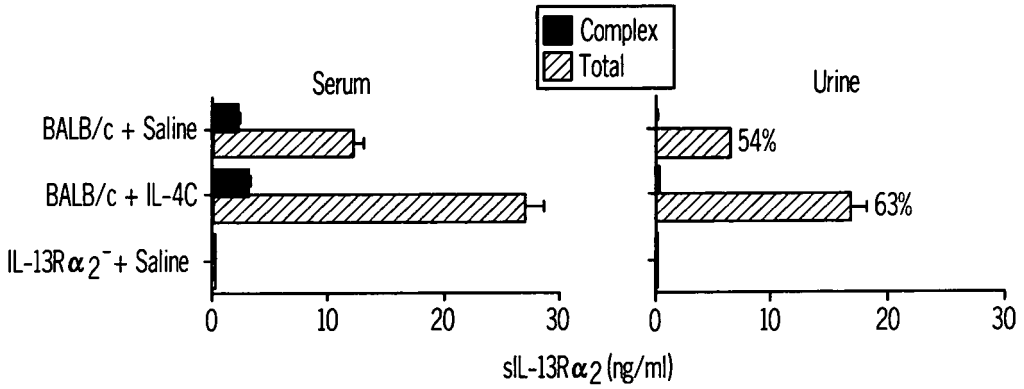

FIG. 8B

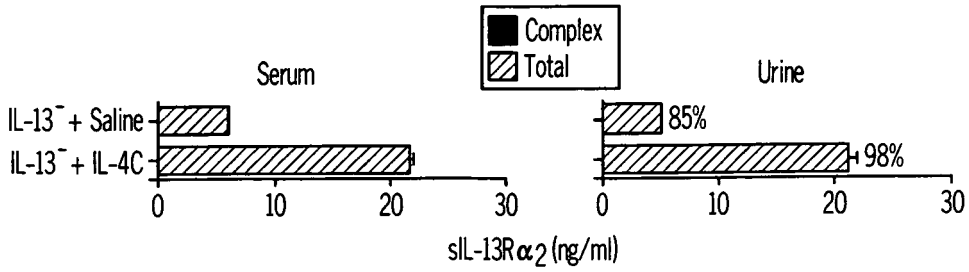

FIG. 8C

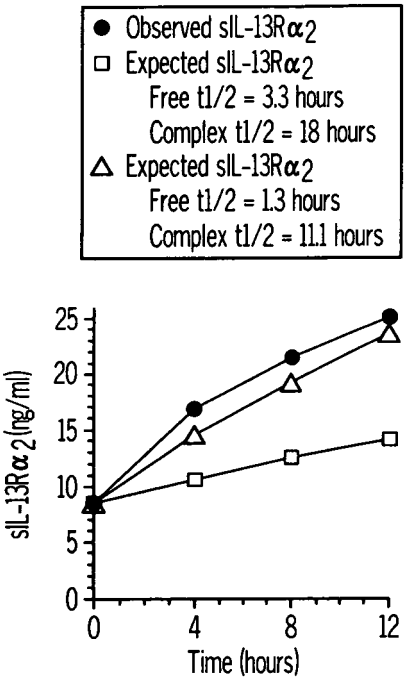
Observed sIL-13Rα2
Expected sIL-13Rα2
Free t1/2 = 3.3 hours
Complex t1/2 = 18 hours
Expected sIL-13Rα2
Free t1/2 = 1.3 hours
Complex t1/2 = 11.1 hours
sIL-13Rα2 (ng/ml)
25
20
15
10
5
0
0
4
8
12
Time (hours)

FIG. 9

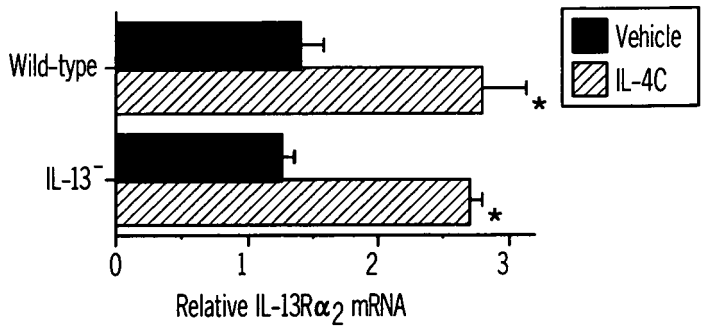

FIG. 10A

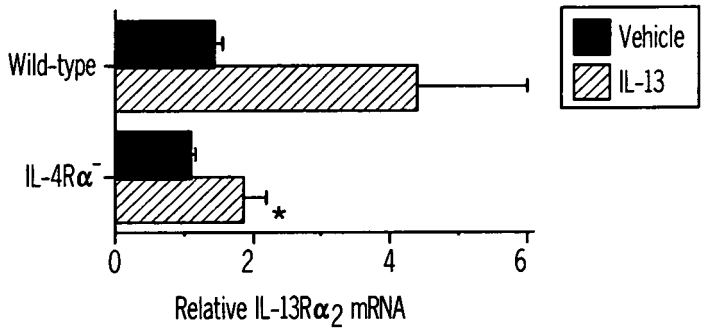

FIG. 10B

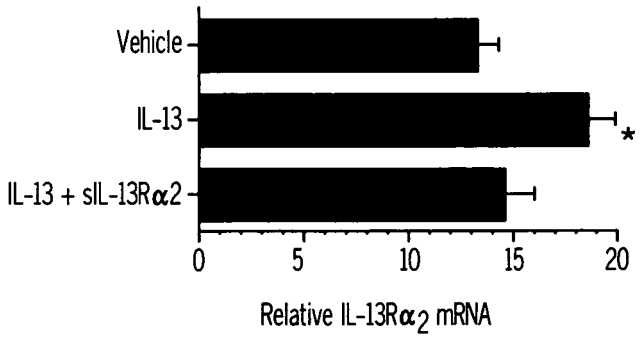

FIG. 10C

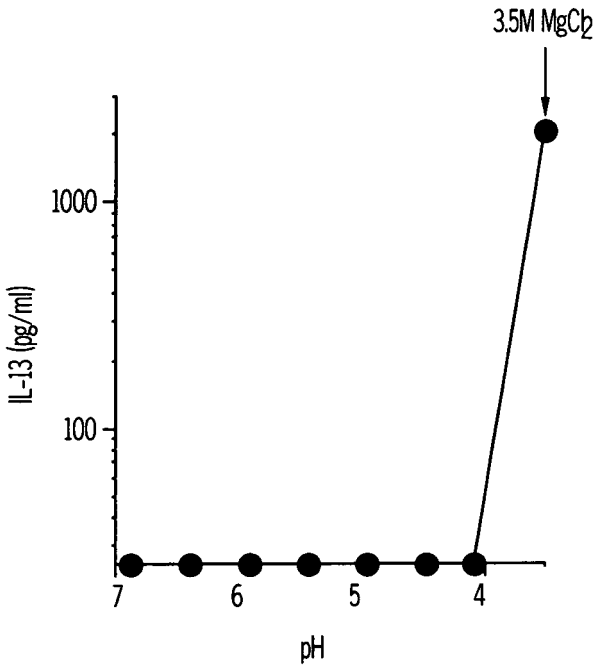

FIG. 11

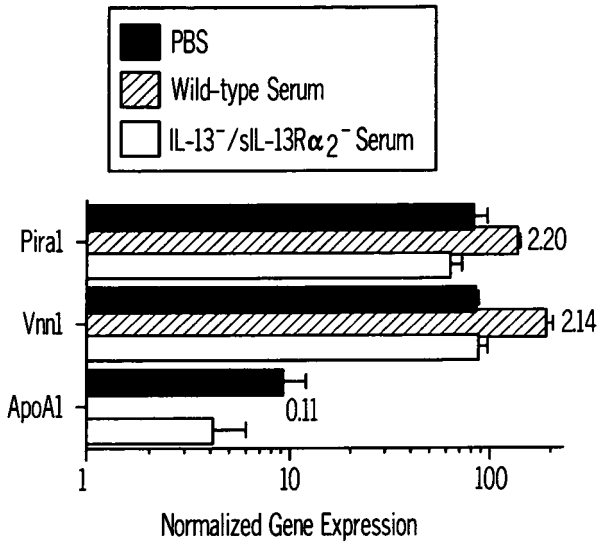

FIG. 12A

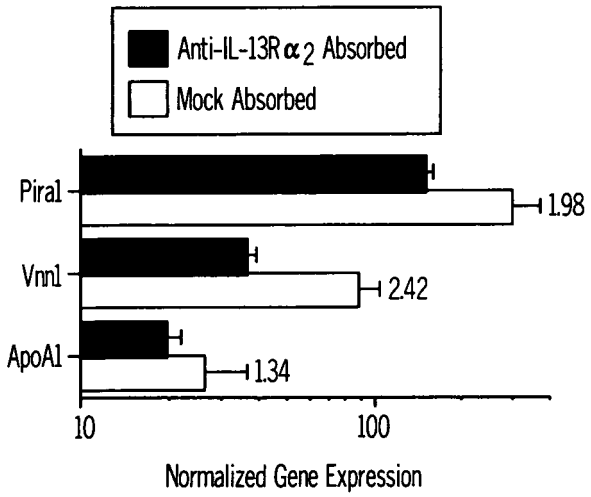

FIG. 12B

专利名称(译)	因此调节炎症和组合物的方法		
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摘要(译)

本发明提供了用于检测或治疗哮喘或其它过敏性或炎性疾病的组合物和方法。在一个方面，本发明的方法包括调节IL-13 / sIL-13Ra2复合物和调节各种目的核苷酸序列（包括Pira1，Vannin1和ApoA1）表达的方法。在一方面，本发明的方法允许检测受试者中改变的IL-13 / sIL-13Ra2复合物水平。本发明的其它方面允许鉴定适合包括在sIL-13Ra2相关研究或炎症相关病症研究中的受试者。