[0006] Moderate asthma is currently treated with a daily inhaled anti-inflammatory-corticosteroid or mast cell inhibitor such as cromolyn sodium or nedocromil plus an inhaled beta2-agonist as needed (3-4 times per day) to relieve breakthrough symptoms or allergen- or exercise-induced asthma. Cromolyn sodium and nedocromil block bronchospasm and inflammation, but are usually effective only for asthma that is associated with allergens or exercise and typically, only for juvenile asthmatics. Inhaled corticosteroids improve inflammation, airways hyperreactivity, and obstruction, and reduce the number of acute exacerbations. However, it takes at least a month before effects are apparent and up to a year for marked improvement to occur. The most frequent side effects are hoarseness and oral fungal infection, i.e., candidiasis. More serious side effects have been reported, *e.g.,* partial adrenal suppression, growth inhibition, and reduced bone formation, but only with the use of higher doses. Beclomethasone, triamcinolone, and flunisolide probably have a similar potency; whereas budesonide and fluticasone are more potent and reportedly have fewer systemic side effects.

[0007] Even patients with mild disease show airway inflammation, including infiltration of the mucosa and epithelium with activated T cells, mast cells, and eosinophils. T cells and mast cells release cytokines that promote eosinophil growth and maturation and the production of IgE antibodies, and these, in turn, increase microvascular permeability, disrupt the epithelium, and stimulate neural reflexes and mucus-secreting glands. The result is airways hyperreactivity, bronchoconstriction, and hypersecretion, manifested by wheezing, coughing, and dyspnea.

[0008] Traditionally, asthma has been treated with oral and inhaled bronchodilators. These agents help the symptoms of asthma, but do nothing for the underlying inflammation. Recognition during the last 10 years of the importance of inflammation in the etiology of asthma has led to the increased use of corticosteroids, but many patients continue to suffer from uncontrolled asthma.

[0009] Because of the importance of treating inflammatory diseases in humans, particularly asthma, new bioactive compounds having fewer side effects are continually being sought. The development of potent and specific inhibitors of IL13, which remain active when administered long term to asthmatic airways, offers a novel approach to the treatment of asthma, as well as in other IL13- and IgE-mediated diseases.

Summary

[0010] The present disclosure relates at least in part to antibodies that bind specifically and with high affinity to both glycosylated and non-glycosylated human IL13; does not bind mouse IL13, and neutralize human IL13 activity at an approximate molar ratio of 1:2 (MAb:IL13). Also included in the present disclosure are antibodies comprising the antigen binding regions derived from the light and/or heavy chain variable regions of said antibodies. The antibodies of the disclosure may be monoclonal, and a monoclonal antibody may be a human antibody, a chimeric antibody, or a humanized antibody.

[0011] Examples of these antibodies are 228B/C-1, 228A-4, 227-26, and 227-43. The hybridomas that produce these antibodies were deposited on November 20, 2003, with the American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209, under Accession Numbers PTA-5657, PTA-5656, PTA-5654, and PTA-5655, respectively.

[0012] The present disclosure includes antibodies which have a VL sequence at least 95% homologous to that set forth in SEQ ID NO: 3, and a VH sequence at least 95% homologous to that set forth in SEQ ID NO: 4; antibodies which have a VL sequence at least 95% homologous to that set forth in SEQ ID NO: 5, and a VH sequence at least 95% homologous to that set forth in SEQ ID NO: 6; and antibodies which have a VL sequence at least 95% homologous to that set forth in SEQ ID NO: 7, and a VH sequence at least 95% homologous to that set forth in SEQ ID NO: 8. The present disclosure also includes a recombinant antibody molecule, or an IL13-binding fragment thereof, comprising at least one antibody heavy chain, or an IL13-binding fragment thereof, comprising non-human CDRs at positions 31-35 (CDR1), 50-65 (CDR2) and 95-102 (CDR3) (Kabat numbering) from a mouse anti-IL13 antibody, wherein positions 27-30 have the amino acid Gly 26, Phe 27, Ser 28, Leu 29, Asn 30, (SEQ ID NO: 18); and at least one antibody light chain, or an IL13-binding fragment thereof, comprising non-human CDRs at positions 24-34 (CDR1), 50-56 (CDR2) and 89-97 (CDR3) from a mouse anti-IL13 antibody, and framework regions from a human monoclonal antibody.

[0013] The present disclosure includes human antigen-binding antibody fragments of the antibodies of the present disclosure including, but are not limited to, Fab, Fab' and $F(ab')_2$, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv). The disclosure also includes single-domain antibodies comprising either a VL or VH domain. On example of an scFv is depicted in Figure 21, having the sequence of SEQ ID NO 152.

[0014] The present disclosure includes humanized sequences of monoclonal antibody 228B/C-1. These humanized recombinant antibody molecules comprise a variable light chain region comprising an amino acid sequence having the formula: FRL1-CDRL1-FRL2-CDRL2-FRL3-CDRL3-FRL4, wherein FRL1 consists of any one of SEQ ID Nos: 20-25; CDRL1 consists of any one of SEQ ID NOs: 99-103; FRL2 consists of SEQ ID NO: 29; CDRL2 consists of any one of SEQ ID NOs: 104-114; FRL3 consists of any one of SEQ ID NOs: 30-56; CDRL3 consists of any of SEQ ID NOs: 115-116; and FRL4 consists of SEQ ID NO: 57-59; and comprising a variable heavy chain region comprising an amino acid sequence having the formula: FRH1-CDRH1-FRH2-CDRH2-FRH3-CDRH3-FRH4, wherein FRH1 consists of any one of SEQ ID NOs: 60-66; CDRH1 consists of any one of SEQ ID NOs: 117-122 ; FRH2 consists of any one of SEQ ID NOs: 67-75 ; CDRH2 consists of any one of SEQ ID NOs: 123-134; FRH3 consists of any one of SEQ ID NOs: 76-90; CDRH3 consists of any of SEQ ID NOs: 135-141; and FRH4 consists of SEQ ID NO: 91-92. The variable heavy chain region may further comprise at least the CH1 domain of a constant region or the CH1, CH2 and CH3 domains of a constant region. The heavy chain constant region may comprise an IgG antibody. wherein the IgG antibody is an IgG1 antibody, an IgG2 antibody, an IgG3 antibody, or an IgG4 antibody.

[0015] The present disclosure also includes recombinant antibody molecules wherein the variable light chain is chosen from any one of SEQ ID Nos: 3, 5, 7, 93, 95, 97, 142, 144, and 150, and a variable heavy chain chosen from any one of SEQ ID Nos: 4, 6, 8, 94, 96, 98, 143, 145, 146, 147, 148, and 149. One particular antibody comprises the variable light chain having the sequence set forth in SEQ ID NO:142, and a variable heavy chain having the sequence set forth in SEQ ID NO:143.

[0016] The present disclosure includes the hybridoma cell lines that produce the monoclonal antibodies 228B/C-1, 228A-4, 227-26, and 227-43. The present disclosure includes nucleic acids encoding the monoclonal antibodies 228B/C-1, 228A-4, 227-26, and 227-43, cell lines comprising a nucleic acid encoding these antibodies or chains thereof, and vectors comprising the nucleic acid encoding these antibodies or chains thereof.

[0017] The present disclosure also includes antibodies that bind the same epitope as 228B/C-1. Exemplary polypeptides comprise all or a portion of SEQ ID NO. 1 or variants thereof, or SEQ ID NO. 2, wherein amino acid 13 is changed from glutamic acid to lysine. The disclosure also relates to the epitope recognized by the antibodies of the present disclosure. Epitope peptides include a peptide comprising essentially or consisting of ESLINVSG (SEQ ID NO: 18) or YCAALESLINVS (SEQ ID NO:19).

[0018] The present disclosure includes a composition comprising the antibodies according to the claimed disclosure in combination with a pharmaceutically acceptable carrier, diluent, excipient, or stabilizer.

[0018a] In one aspect, the present disclosure refers to a pharmaceutical composition comprising an antagonistic anti-human IL13 antibody or antigen-binding fragment thereof that binds specifically to human IL13 and a physiologically acceptable carrier, wherein said anti-human IL13 antibody comprises a variable heavy chain region comprising the complementarity determining regions CDRH1, CDRH2 and CDRH3 having the amino acid sequences of SEQ ID NO: 117, SEQ ID NO: 123, and SEQ ID NO: 135, respectively, and a variable light chain region comprising the complementarity determining regions CDRL1, CDRL2 and CDRL3 having the amino acid sequences of SEQ ID NO: 99, SEQ ID NO: 104, and SEQ ID NO: 115, respectively, and wherein said anti-human IL13 antibody or functional fragment thereof is formulated to be administered intraperitoneally, intramuscularly, subcutaneously or orally.

[0019] The present disclosure includes a method of treating a subject suffering from asthmatic symptoms comprising administering to a subject, e.g. a subject in need thereof, an amount of an antibody according to the claimed disclosure effective to reduce the asthmatic symptoms, wherein the antibody may down-regulate the activity of IL13 in the patient, reduce bronchial hyperresponsiveness in the patient, and/or reduce eosinophilia in the lungs of the subject. The present disclosure also includes a method of inhibiting the infection of respiratory syncytial virus (RSV) comprising administering to a subject, e.g. a subject in need thereof, an inhibiting amount of the antibody of the claimed disclosure.

[0020] The antibody of the present disclosure may be administered by one or more of the routes including intravenous, intraperitoneal, inhalation, intramuscular, subcutaneous and oral routes. The present disclosure includes an inhalation device that delivers to a patient a therapeutically effective amount of an antibody according to the claimed disclosure.

[0021] The present disclosure includes a method for detecting interleukin-13 protein in a subject, e.g., a patient suffering from an allergic disease, comprising, e.g., the steps of allowing the antibody of the claimed disclosure to contact a sample; and detecting the interleukin-13 through the occurrence of immunoreaction. Also described are and methods for diagnosing overexpression of IL13 in a subject, comprising the steps of (a) obtaining a sample from the subject; (b) combining the sample with an antibody according to the claimed disclosure under conditions which would allow immunoreaction with IL13; and (c) determining whether or not IL13 is overexpressed relative to a normal level of expression of IL13.

[0022] The present disclosure includes a method for producing the antibodies of the claimed disclosure, comprising the steps of: a) producing an immunogenic compound comprising a glycosylated IL13 moiety and an immunogenic moiety; b) preparing an injectable solution comprising said immunogenic compound in phosphate buffered saline (PBS) and an adjuvant; c) immunizing a mouse with said injectable solution by a combination of intravenous and intraperitoneal injections, d)

producing a hybridoma by fusing a spleen cell from said immunized mouse with a myeloma cell; e) selecting a hybridoma producing an antibody having the characteristics of the antibody of the claimed disclosure; and f) isolating said antibody.

[0023] The present disclosure includes a method for inhibiting IgE antibody production in a patient, which comprises administrating to the patient an effective amount of an IgE antibody production inhibiting effective amount of an antibody according to the claimed disclosure. The inhibition of IgE antibody production may prevent bronchial asthma, allergic rhinitis, allergic dermatitis, and anaphylaxis, and also treat bronchial asthma, allergic rhinitis, uticaria, and atopic dermatitis.

[0023a] In one aspect, the present disclosure refers to the pharmaceutical composition disclosed herein, for use in the prevention of bronchial asthma, allergic rhinitis, allergic dermatitis or anaphylaxis.

[0023b] In one aspect, the present disclosure refers to the pharmaceutical composition disclosed herein, for use in the treatment of bronchial asthma, allergic asthma, nonallergic asthma, allergic rhinitis, atopic dermatitis, allergic dermatitis, allergic conjunctivitis, eczema, urticaria, food allergies, chronic obstructive pulmonary disease, ulcerative colitis, RSV infection, uveitis, scleroderma, or osteoporosis.

[0023c] In one aspect, the present disclosure refers to the pharmaceutical composition disclosed herein, for use in the treatment of idiopathic pulmonary fibrosis or inflammatory and fibrotic lung disease.

[0023d] In one aspect, the present disclosure refers to the use of the pharmaceutical composition disclosed herein, in the manufacture of a medicament for the prevention of bronchial asthma, allergic rhinitis, allergic dermatitis or anaphylaxis.

[0023e] In one aspect, the present disclosure refers to the use of the pharmaceutical composition disclosed herein, in the manufacture of a medicament for the treatment of bronchial asthma, allergic asthma, nonallergic asthma, allergic rhinitis, atopic dermatitis, allergic dermatitis, allergic conjunctivitis, eczema, urticaria, food allergies, chronic obstructive pulmonary disease, ulcerative colitis, RSV infection, uveitis, scleroderma, or osteoporosis.

[0023f] In one aspect, the present disclosure refers to the use of the pharmaceutical composition disclosed herein, in the manufacture of a medicament for the treatment of idiopathic pulmonary fibrosis or inflammatory and fibrotic lung disease.

[0024] The present disclosure includes a method of treating an IL13-mediated disorder in a patient, comprising administering to the patient an effective amount of an antibody or antigen-binding fragment thereof according to the claimed disclosure, wherein said antibody or antigen-binding fragment thereof inhibits binding of IL13 to its receptor and inhibits one or more functions associated with binding of the interleukin to said receptor.

[0025] The present disclosure includes a method of treating an IgE-mediated disorder in a patient, comprising administering to the patient an effective amount of an antibody or antigen-binding fragment thereof according to the claimed disclosure, wherein said antibody or antigen-binding fragment thereof inhibits binding of IL13 to its receptor and inhibits one or more functions associated with binding of the interleukin to said receptor.

[0026] The present disclosure includes a method for reducing the severity of asthma in a mammal comprising administering to the mammal a therapeutically effective amount of an anti-IL13 monoclonal antibody having at least one of the following characteristics: the ability to bind human IL13 with a K_D between about $1X10^{10}$ to about $1X\ 10^{12}$ M; the ability to inhibit one or more functions associated with binding of the interleukin IL13 to the IL13 receptor; and the inability of the antibody does to bind to mouse IL13.

[0027] Diseases and/or conditions mediated by IL13 that are contemplated by the disclosure include, but are not limited to, allergic asthma, non-allergic (intrinsic) asthma, allergic rhinitis, atopic dermatitis, allergic conjunctivitis, eczema, urticaria, food allergies, chronic obstructive pulmonary disease, ulcerative colitis, RSV infection, uveitis, scleroderma, and osteoporosis .

Brief Description of the Drawings

[0028] FIGURE 1 depicts the binding of anti-IL13 monoclonal antibodies to human IL13.

[0029] FIGURE 2 depicts the binding of anti-IL13 monoclonal antibodies mutant IL13-Fc.

[0030] FIGURE 3 illustrates that there is no inhibition of MAb 228B/C-1 binding to human IL13 by MAb JES10-5A2 (Pharmingen).

[0031] FIGURE 4 illustrates the effect of anti-IL13 monoclonal antibodies on the proliferation of Hodgkin Lymphoma L-1236 cells.

[0032] FIGURE 5 illustrates the effect of anti-IL13 monoclonal antibodies on IL13-induced suppression of CD14 expression in human monocytes.

[0033] FIGURE 6 illustrates the effect of anti-IL13 monoclonal antibodies on IL13-induced up-regulation of CD23 expression in human monocytes.

Claims

1. A pharmaceutical composition comprising an antagonistic anti-human IL13 antibody or antigen-binding fragment thereof that binds specifically to human IL13 and a physiologically acceptable carrier, wherein said anti-human IL13 antibody comprises a variable heavy chain region comprising the complementarity determining regions CDRH1, CDRH2 and CDRH3 having the amino acid sequences of SEQ ID NO: 117, SEQ ID NO: 123, and SEQ ID NO: 135, respectively, and a variable light chain region comprising the complementarity determining regions CDRL1, CDRL2 and CDRL3 having the amino acid sequences of SEQ ID NO: 99, SEQ ID NO: 104, and SEQ ID NO: 115, respectively, and wherein said anti-human IL13 antibody or functional fragment thereof is formulated to be administered intraperitoneally, intramuscularly, subcutaneously or orally.

2. The pharmaceutical composition of claim 1, wherein said anti-human IL13 antibody is a humanized antibody.

3. The pharmaceutical composition of claim 1 or 2, wherein said anti-human IL13 antibody comprises a variable heavy chain region comprising an amino acid sequence having the formula: FRH1-CDRH1-FRH2-CDRH2-FRH3-CDRH3-FRH4, wherein FRH1 consists of SEQ ID NO:65; CDRH1 consists of SEQ ID NO:117; FRH2 consists of SEQ ID NO:68; CDRH2 consists of SEQ ID NO:123; FRH3 consists of SEQ ID NO:80; CDRH3 consists of SEQ ID NO:135; and FRH4 consists of SEQ ID NO:92 and a variable light chain region comprising an amino acid sequence having the formula: FRL1-CDRL1-FRL2-CDRL2-FRL3-CDRL3-FRL4, wherein FRL1 consists of SEQ ID NO:21; CDRL1 consists of SEQ ID NO:99; FRL2 consists of SEQ ID NO:29; CDRL2 consists of SEQ ID NO:104; FRL3 consists of SEQ ID NO:31; CDRL3 consists of SEQ ID NO:115; and FRL4 consists of SEQ ID NO:58.

4. The pharmaceutical composition of any one of claims 1 to 3, wherein said anti-human IL13 antibody comprises a variable light chain region comprising the amino acid sequence of SEQ ID NO:142 and a variable heavy chain region comprising the amino acid sequence of SEQ ID NO:143.

5. The pharmaceutical composition of any one of claims 1 to 4, wherein said anti-human IL13 antibody or antigen binging fragment thereof binds specifically and with high affinity to both glycosylated and non-glycosylated human IL13, does not bind mouse IL13, and neutralizes human IL13 activity at an approximate molar ratio of 1:2 (MAb:IL13).

6. The pharmaceutical composition of any one of claims 1 to 5, wherein said anti-human IL13 antibody or antigen binding fragment thereof binds to the same epitope as the antibody produced by hybridoma 228B/C-1 (PTA-5657).

7. The pharmaceutical composition of any one of claims 1 to 6, wherein said anti-human IL13 antibody is a monoclonal antibody.

8. The pharmaceutical composition of any one of claims 1 to 7, wherein said anti-IL13 antibody is a monovalent antibody, a multispecific antibody, a single chain antibody, a Fab fragment, or a F(ab') fragment.

9. The pharmaceutical composition of any one of claims 1 to 8, wherein said anti-human-IL13 antibody is a multispecific antibody which is a bispecific antibody.

10. The pharmaceutical composition of any one of claims 1 to 9, for use in the prevention of bronchial asthma, allergic rhinitis, allergic dermatitis or anaphylaxis.

11. The pharmaceutical composition of any one of claims 1 to 9, for use in the treatment of bronchial asthma, allergic asthma, nonallergic asthma, allergic rhinitis, atopic dermatitis, allergic dermatitis, allergic conjunctivitis, eczema, urticaria, food allergies, chronic obstructive pulmonary disease, ulcerative colitis, RSV infection, uveitis, scleroderma, or osteoporosis.

12. The pharmaceutical composition of any one of claims 1 to 9, for use in the treatment of idiopathic pulmonary fibrosis or inflammatory and fibrotic lung disease.

13. Use of the pharmaceutical composition of any one of claims 1 to 9, in the manufacture of a medicament for the prevention of bronchial asthma, allergic rhinitis, allergic dermatitis or anaphylaxis.

14. Use of the pharmaceutical composition of any one of claims 1 to 9, in the manufacture of a medicament for the treatment of bronchial asthma, allergic asthma, nonallergic asthma, allergic rhinitis, atopic dermatitis, allergic dermatitis, allergic conjunctivitis, eczema, urticaria, food allergies, chronic obstructive pulmonary disease, ulcerative colitis, RSV infection, uveitis, scleroderma, or osteoporosis.

15. Use of the pharmaceutical composition of any one of claims 1 to 9, in the manufacture of a medicament for the treatment of idiopathic pulmonary fibrosis or inflammatory and fibrotic lung disease.