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[Continued on next page]

(54) Title: ANTI-IL-4 ANTIBODIES AND BISPECIFIC ANTIBODIES AND USES THEREOF

(57) Abstract: The invention provides anti-IL-4 antibodies and bispecific antibodies and methods of using the same.

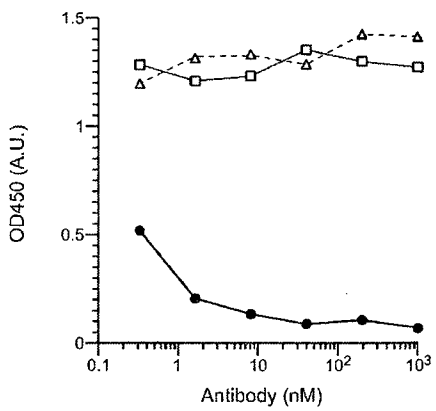

FIG. 1A

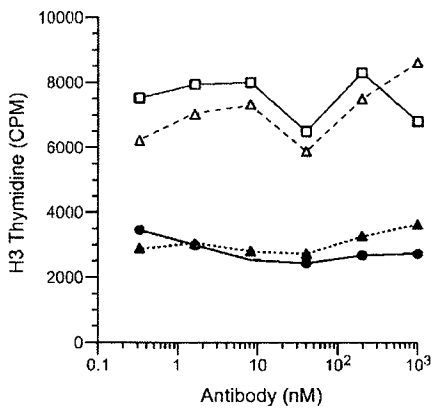

FIG. 1B

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ANTI-IL-4 ANTIBODIES AND BISPECIFIC ANTIBODIES AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of priority of provisional U.S. Application No. 61/808,748 filed April 5, 2013, which is hereby incorporated by reference in its entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on March 12, 2014, is named 2014.MAR.12 P5609R1-WO_SL and is 75,442 bytes in size.

FIELD

[0003] The present invention relates to anti-IL-4 antibodies and bispecific antibodies and methods of using the same.

BACKGROUND

[0004] Asthma is a complex disease with increasing worldwide incidence. Among other events, eosinophilic inflammation has been reported in the airways of asthma patients. The pathophysiology of the disease is characterized by variable airflow obstruction, airway inflammation, mucus hypersecretion, and subepithelial fibrosis. Clinically, patients may present with cough, wheezing, and shortness of breath. While many patients are adequately treated with currently available therapies, some patients with asthma have persistent disease despite the use of current therapies.

[0005] A number of studies have implicated IL-4, IL-13, and their receptors in the pathogenesis of asthma and allergy (*see, e.g.*, Wills-Karp, 2004, *Immunol. Rev.* 202, 175–190; Brightling et al., 2010, *Clin. Exp. Allergy* 40, 42–49; Finkelman et al., 2010, *J Immunol* 184, 1663–1674; Maes et al., 2012, *Am. J. Respir. Cell Mol. Biol.* 47, 261–270; Steinke and Borish, 2001, *Respir. Res.* 2, 66–70). IL-4 binds to two receptors, one a heterodimer of IL-4Rα and the common gamma chain (γc), and the other a heterodimer of IL-4 receptor alpha (IL-4Rα) and IL-13 receptor alpha 1 (IL-13Rα1). The latter receptor IL-4Rα / IL-13Rα1 is a shared receptor with IL-13, which also uniquely binds a single chain receptor consisting of IL-13 receptor alpha 2 (IL-13Rα2). Polymorphisms of the IL-4, IL-13, and IL-4Rα genes are associated with asthma and allergy, including features such as IgE levels, prevalence of atopy, and severity of asthma disease. In addition, expression of IL-4, IL-13, and their receptors are

increased in asthma and other allergic diseases. Moreover, neutralization or deficiency of IL-4, IL-13, and their receptors ameliorates disease in preclinical models of asthma.

[0006] A number of drugs are on the market or in development for treating asthma. One of the numerous targets for asthma therapy is IL-13. IL-13 is a pleiotropic TH2 cytokine produced by activated T cells, NKT cells, basophils, eosinophils, and mast cells, and it has been strongly implicated in the pathogenesis of asthma in preclinical models. IL-13 antagonists, including anti-IL-13 antibodies, have previously been described. *See, e.g.*, Intn'l Patent Application Pub. No. WO 2005/062967. Such antibodies have also been developed as human therapeutics. Recently, several studies have shown clinical activity of monoclonal antibodies against IL-13 in the treatment of asthma (*See, e.g.,* Corren et al., 2011, *N. Engl. J. Med.* 365, 1088-1098; Gauvreau et al., 2011, *Am. J. Respir. Crit. Care Med.* 183, 1007-1014; Ingram and Kraft, 2012, *J. Allergy Clin. Immunol.* 130, 829-42; Webb, 2011, *Nat Biotechnol* 29, 860-863). Of these, lebrikizumab, a humanized IgG4 antibody that neutralizes IL-13 activity, improved lung function in asthmatics who were symptomatic despite treatment with, for the majority, inhaled corticosteroids and a long-acting beta2-adrenergic receptor agonist (Corren et al., 2011, *N. Engl. J. Med.* 365, 1088-1098). In addition, a bispecific antibody that binds IL-13 and IL-4 has been described. *See, e.g.*, U.S. Publication No. 2010/0226923.

[0007] Yet moderate to severe asthmatic patients are still in need of alternative treatment options. Thus, there is a need to identify better therapies for treating asthma and improved methods for understanding how to treat asthma patients.

[0008] Idiopathic pulmonary fibrosis (IPF) is a restrictive lung disease characterized by progressive interstitial fibrosis of lung parenchyma, affecting approximately 100,000 patients in the United States (Raghu et al., *Am J Respir Crit Care Med* 174:810-816 (2006)). This interstitial fibrosis associated with IPF leads to progressive loss of lung function, resulting in death due to respiratory failure in most patients. The median survival from the time of diagnosis is 2-3 years (Raghu et al., *Am J Respir Crit Care Med* 183:788-824 (2011)). The etiology and key molecular and pathophysiological drivers of IPF are unknown. The only treatment shown to prolong survival in IPF patients is lung transplantation (Thabut et al., *Annals of internal medicine* 151:767-774 (2009)). Lung transplantation, however, is associated with considerable morbidity, not all IPF patients are appropriate candidates for it, and there is a relative paucity of suitable donor lungs. Despite numerous attempts, no drug therapies to date have been shown to substantially prolong survival in a randomized, placebo-controlled interventional trial in IPF patients, although some interventions have appeared to

slow the rate of lung function decline in some patients (Raghu et al., *Am J Respir Crit Care Med* 183:788-824 (2011); Richeldi et al., *The New England J. of Med.* 365:1079-1087 (2011)).

[0009] IL-4 and IL-13 signaling can induce fibrogenic responses from a number of cell types *in vitro*. Treatment of fibroblasts with IL-4 or IL-13 has been shown to induce collagen production and differentiation to a myofibroblast phenotype (Borowski et al., *J. British Soc. Allergy Clin. Immunol.*, 38: 619-628 (2008); Hashimoto et al., *J. Allergy Clin. Immunol.*, 107: 1001-1008 (2001); Murray, et al., *Int. J. Biochem. Cell Biol.*, 40: 2174-2182 (2008); Saito et al., *Intl. Archives Allergy Immunol.*, 132: 168-176 (2003)). Alternatively activated macrophages have also been proposed to be major contributors to fibrogenic processes, in part based on their ability to produce growth factors, such as TGFβ and PDGF, that stimulate fibroblasts and myofibroblasts. IL-4 and IL-13 are potent inducers of the alternatively activated macrophage phenotype and may drive fibrogenic responses at least partially through its activity on these cells (Doyle et al., *Eur. J. Immunol.*, 24: 1441-1445 (1994); Song et al., *Cell. Immunol.*, 204: 19-28 (2000); Wynn and Barron, *Seminars Liver Dis.*, 30: 245-257 (2010).

[0010] IL-4 and IL-13 can also drive fibrogenic responses in multiple tissues *in vivo*. Transgenic overexpression of IL-4 or IL-13 in the lungs of mice is sufficient to induce collagen gene expression and profound sub-epithelial fibrosis (Lee et al., *J. Exper. Med.*, 194: 890-821 (2001); Ma et al. *J. Clin. Invest.*, 116: 1274-1283 (2006); Zhu et al., *J. Clin. Invest.* 103: 779-788 (1999)). Additionally, a number of studies have demonstrated a role for IL-4 and IL-13 as drivers of fibrosis in pre-clinical animal models. Mice with targeted disruption of IL-13 or that are treated with blocking antibodies specific for IL-13 show reduced extracellular matrix deposition in Bleomycin- and FITC-induced pulmonary fibrosis models (Belperio et al., *Am. J. Respir. Cell Mol. Biol.*, 27: 419-427 (2002); Kolodsick et al., *J. Immunol.*, 172: 4068-4076 (2004); Liu et al., *J. Immunol.*, 173: 3425-3431 (2004)). Similarly, IL-4 has been shown to be important in sustaining fibrotic responses in the Bleomycin-induced pulmonary fibrosis model (Huaux et al., *J. Immunol.*, 170: 2083-2092 (2003).

[0011] Multiple studies have concluded that expression and activity of IL-4 and/or IL-13 is elevated in IPF patients. The expression of IL-4, IL-13 and IL-4/IL-13 receptor subunits were found to be increased in lung biopsy samples from IPF patients compared to normal controls, both at the level of mRNA and protein (Jakubziak et al., *J. Clin. Pathol.*, 57: 477-

486 (2004)). Notably, in this study IL-13Rα2, a gene that is highly induced by IL-4 or IL-13 signaling (David et al., *Oncogene*, 22: 2286-3394 (2003)), was found to be expressed in fibroblastic foci in IPF biopsies by immunohistochemistry, suggesting active IL-4 or IL-13 signaling in these cells. IL-4 and IL-13 were also found to be elevated in bronchoalveolar lavage fluid of IPF patients compared to normal controls. Notably, the level of IL-13 in these samples negatively correlated with the key measures of lung function, percent predicted FVC and DLCO (Park et al., *J. Korean Med. Sci.*, 24: 614-620 (2009)), suggesting pathogenic functions of IL-13 in IPF patients.

[0012] IPF patients are still in need of alternative treatment options. Thus, there is a need to identify better therapies for treating IPF and improved methods for understanding how to treat IPF patients

[0013] All references cited herein, including patent applications and publications, are incorporated by reference herein in their entirety for any purpose.

SUMMARY

[0014] In some embodiments, a multispecific antibody is provided, wherein the multispecific antibody comprises an antigen-binding domain that comprises a first VH/VL unit that specifically binds IL-4 and a second VH/VL unit that specifically binds IL-13. In some embodiments, the multispecific antibody:

a) inhibits binding of IL-4 to IL-4 receptor alpha (IL-4Rα),

b) inhibits IL-4-induced proliferation of cells *in vitro*, and/or

b) inhibits IL-13-induced proliferation of cells *in vitro*.

[0015] In some embodiments, the first VH/VL unit of the multispecific antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 14, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 17, and HVR-H2 comprising the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 18. In some embodiments, the first VH/VL unit comprises HVR-H1 comprising the amino acid sequence of SEQ ID NO: 12, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 18, and HVR-H3 comprising the amino acid sequence of SEQ ID NO: 14. In some embodiments, the first VH/VL unit comprises HVR-L1 comprising the amino acid sequence of SEQ ID NO: 15, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 16, and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 17. In some embodiments, the first VH/VL unit comprises (a) a VH sequence having at least 95% sequence identity to the amino acid

sequence of SEQ ID NO: 9; (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 10; or (c) a VH sequence as in (a) and a VL sequence as in (b). In some embodiments, the first VH/VL unit comprises a VH sequence selected from SEQ ID NOs: 1 and 3 to 9. In some embodiments, the first VH/VL unit comprises a VL sequence selected from SEQ ID NOs: 2, 10, and 11. In some embodiments, the first VH/VL unit comprises the VH sequence of SEQ ID NO: 9 and the VL sequence of SEQ ID NO: 10.

[0016] In any of the embodiments described herein, the second VH/VL unit of the multispecific antibody may comprise: (a) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26, and HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22; or (b) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 52, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 55, and HVR-H2 comprising the amino acid sequence of SEQ ID NO: 51. In any of the embodiments described herein, the second VH/VL unit of the multispecific antibody may comprise: (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 21 or the amino acid sequence of SEQ ID NO: 60, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22, and HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23; or (b) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 50, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 51, and HVR-H3 comprising the amino acid sequence of SEQ ID NO: 52. In any of the embodiments described herein, the second VH/VL unit of the multispecific antibody may comprise: (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 24, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 25, and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26; or (b) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 53, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 54, and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 55. In any of the embodiments described herein, the second VH/VL unit of the multispecific antibody may comprise: (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 19; (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 20; (c) a VH sequence as in (a) and a VL sequence as in (b); (d) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 49; (e) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 48; or (f) a VH sequence as in (d) and a VL sequence as in (e). In any of the embodiments described herein, the second VH/VL unit of the multispecific antibody may comprise the VH sequence of SEQ ID NO: 19,

56, or 49. In any of the embodiments described herein, the second VH/VL unit of the multispecific antibody may comprise the VL sequence of SEQ ID NO: 20, 57, or 48. In any of the embodiments described herein, the second VH/VL unit of the multispecific antibody may comprise the VH sequence of SEQ ID NO: 19 or 56 and the VL sequence of SEQ ID NO: 20 or 57; or the VH sequence of SEQ ID NO: 49 and the VL sequence of SEQ ID NO: 48.

[0017] In some embodiments, the multispecific antibody competes for binding to IL-4 with an antibody comprising a VH sequence of SEQ ID NO: 9 and a VL sequence of SEQ ID NO: 10. In some embodiments, the multispecific antibody competes for binding to IL-13 with an antibody comprising a VH sequence of SEQ ID NO: 19 and a VL sequence of SEQ ID NO: 20, or with an antibody comprising a VH sequence of SEQ ID NO: 49 and a VL sequence of SEQ ID NO: 48. In some embodiments, the multispecific antibody binds an epitope within amino acids 77 to 89 of SEQ ID NO: 29, or within amino acids 82 to 89 of SEQ ID NO: 29.

[0018] In some embodiments, a multispecific antibody is provided that comprises a first VH/VL unit that specifically binds IL-4 and a second VH/VL unit that specifically binds IL-13, wherein the first VH/VL unit comprises the VH sequence of SEQ ID NO: 9 and the VL sequence of SEQ ID NO: 10, and the second VH/VL unit comprises the VH sequence of SEQ ID NO: 19 and the VL sequence of SEQ ID NO: 20.

[0019] In any of the embodiments described herein, the multispecific antibody may be an IgG antibody. In any of the embodiments described herein, the multispecific antibody may be an IgG1 or IgG4 antibody. In any of the embodiments described herein, the multispecific antibody may be an IgG4 antibody.

[0020] In any of the embodiments described herein, the multispecific antibody may comprise a first heavy chain constant region and a second heavy chain constant region, wherein the first heavy chain constant region comprises a knob mutation and the second heavy chain constant region comprises a hole mutation. In some embodiments, the first heavy chain constant region is fused to the heavy chain variable region portion of a VH/VL unit that binds IL-4. In some embodiments, the second heavy chain constant region is fused to the heavy chain variable region portion of a VH/VL unit that binds IL-13. In some embodiments, the first heavy chain constant region is fused to the heavy chain variable region portion of a VH/VL unit that binds IL-13. In some embodiments, the second heavy chain

constant region is fused to the heavy chain variable region portion of a VH/VL unit that binds IL-4.

[0021] In some embodiments, the multispecific antibody is an IgG1 antibody comprising a knob mutation that comprises a T366W mutation. In some embodiments, the multispecific antibody is an IgG1 antibody comprising a hole mutation that comprises at least one, at least two, or three mutations selected from T366S, L368A, and Y407V. In some embodiments, the multispecific antibody is an IgG4 antibody comprising a knob mutation that comprises a T366W mutation. In some embodiments, the multispecific antibody is an IgG4 antibody comprising a hole mutation that comprises at least one, at least two, or three mutations selected from T366S, L368A, and Y407V. In some embodiments, the multispecific antibody comprises a first heavy chain constant region comprising the sequence of SEQ ID NO: 34 or SEQ ID NO: 36. In some embodiments, the multispecific antibody comprises a second heavy chain constant region comprising the sequence of SEQ ID NO: 35 or SEQ ID NO: 37.

[0022] In some embodiments, a multispecific antibody is provided, wherein the antibody comprises a first heavy chain comprising the sequence of SEQ ID NO: 38, a first light chain comprising the sequence of SEQ ID NO: 39, a second heavy chain comprising the sequence of SEQ ID NO: 40, and a second light chain comprising the sequence of SEQ ID NO: 41.

[0023] In some embodiments, isolated antibodies that bind to IL-4 are provided. In some embodiments, the antibody comprises: (a) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 14, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 17, and HVR-H2 comprising the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 18; or (b) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 12, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 18, and HVR-H3 comprising the amino acid sequence of SEQ ID NO: 14; or (c) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 15, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 16, and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 17; or (d) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 9; or (e) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 10. In some embodiments, the antibody comprises HVR-H1 comprising the amino acid sequence of SEQ ID NO: 12, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 18, HVR-H3 comprising the amino acid sequence of SEQ ID NO: 14, HVR-L1 comprising the amino acid sequence of SEQ ID NO: 15, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 16, and HVR-L3 comprising the amino acid sequence of

SEQ ID NO: 17. In some embodiments, the antibody comprises a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 9 and a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 10. In some embodiments, the antibody comprises a VH sequence selected from SEQ ID NOs: 1 and 3 to 9. In some embodiments, the antibody comprises a VL sequence selected from SEQ ID NOs: 2, 10, and 11.

[0024] In some embodiments, an isolated antibody that binds to IL-4 is provided, wherein the antibody comprises the VH sequence of SEQ ID NO: 9 and the VL sequence of SEQ ID NO: 10.

[0025] In some embodiments, an isolated nucleic acid is provided that encodes any of the bispecific antibodies or isolated antibodies described herein. In some embodiments, an isolated nucleic acid is provided that encodes a first VH/VL unit of any of the multispecific antibodies described herein. In some embodiments, an isolated nucleic acid is provided that encodes a second VH/VL unit of any of the multispecific antibodies described herein. In some embodiments, a host cell is provided that comprises the isolated nucleic acid. In some embodiments, the host cell is an *E. coli* cell or a CHO cell. In some embodiments, a method of producing an antibody is provided comprising culturing the host cell.

[0026] In some embodiments, an immunoconjugate is provided, wherein the immunoconjugate comprises any of the multispecific antibodies or isolated antibodies described herein and a cytotoxic agent.

[0027] In some embodiments, pharmaceutical formulations are provided, comprising any of the multispecific antibodies or isolated antibodies described herein and a pharmaceutically acceptable carrier.

[0028] In some embodiments, the antibodies described herein are provided for use as a medicament. In some embodiments, the antibodies described herein are provided for use in treating an eosinophilic disorder, an IL-13 mediated disorder, an IL-4 mediated disorder, or a respiratory disorder. In some embodiments, use of the antibodies described herein in the manufacture of a medicament for treating an eosinophilic disorder, an IL-13 mediated disorder, an IL-4 mediated disorder, or a respiratory disorder is provided. In some embodiments, methods of treating an eosinophilic disorder, an IL-13 mediated disorder, an IL-4 mediated disorder, or a respiratory disorder in an individual are provided comprising administering to the individual an effective amount of an antibody described herein. In some such embodiments, a method further comprises administering to the individual a TH2

pathway inhibitor. In some embodiments, the TH2 pathway inhibitor inhibits at least one target selected from ITK, BTK , IL-9, IL-5, IL-13, IL-4, OX40L, TSLP, IL-25, IL-33, IgE, IL-9 receptor, IL-5 receptor, IL-4 receptor alpha, IL-13receptoralpha1, IL-13receptoralpha2, OX40, TSLP-R, IL-7Ralpha, IL17RB, ST2, CCR3, CCR4, CRTH2, FcepsilonRI, FcepsilonRII/CD23, Flap, Syk kinase; CCR4, TLR9, CCR3, IL5, IL3, and GM-CSF. In some embodiments, the individual is suffering from moderate to severe asthma. In some embodiments, the individual is suffering from idiopathic pulmonary fibrosis.

[0029] In any of the embodiments described herein, the eosinophilic disorder may be selected from asthma, severe asthma, chronic asthma, atopic asthma, atopic dermatitis, allergy, allergic rhinitis, non-allergic rhinitis, contact dermatitis, erythema multiform, bullous skin disease, psoriasis, eczema, rheumatoid arthritis, juvenile chronic arthritis, chronic eosinophilic pneumonia, allergic bronchopulmonary aspergillosis, coeliac disease, Churg-Strauss syndrome (periarteritis nodosa plus atopy), eosinophilic myalgia syndrome, hypereosinophilic syndrome, oedematous reactions including episodic angioedema, helminth infections, urticaria, onchocercal dermatitis, eosinophil-associated gastrointestinal disorders, eosinophilic esophagitis, eosinophilic gastritis, eosinophilic gastroenteritis, eosinophilic enteritis, eosinophilic colitis, ulcerative colitis, Whipple's disease, nasal micropolyposis, nasal polyposis, aspirin intolerance, obstructive sleep apnea, Crohn's disease, scleroderma, endomyocardial fibrosis, fibrosis, inflammatory bowel disease, idiopathic interstitial pneumonia, eosinophilic pneumonia, hypersensitivity pneumonitis, goblet cell metaplasia, pulmonary fibrosis, idiopathic pulmonary fibrosis (IPF), pulmonary fibrosis secondary to sclerosis, chronic obstructive pulmonary disease (COPD), hepatic fibrosis, uveitis, cancer, glioblastoma, Hodgkin's lymphoma, and non-Hodgkin's lymphoma. In some embodiments, the IL-13 mediated disease is selected from atopic dermatitis, allergic rhinitis, asthma, fibrosis, inflammatory bowel disease, Crohn's disease, lung inflammatory disorders, pulmonary fibrosis, idiopathic pulmonary fibrosis (IPF), chronic obstructive pulmonary disease (COPD), hepatic fibrosis, cancer, glioblastoma, and non-Hodgkin's lymphoma. In any of the embodiments described herein, the IL-4 mediated disease may be selected from atopic dermatitis, allergic rhinitis, asthma, fibrosis, inflammatory bowel disease, Crohn's disease, lung inflammatory disorders, pulmonary fibrosis, idiopathic pulmonary fibrosis (IPF), chronic obstructive pulmonary disease (COPD), hepatic fibrosis, cancer, glioblastoma, and non-Hodgkin's lymphoma. In any of the embodiments described herein, the respiratory disorder may be selected from asthma, allergic asthma, non-allergic asthma, bronchitis,

chronic bronchitis, chronic obstructive pulmonary disease (COPD), emphysema, cigarette-induced emphysema, airway inflammation, cystic fibrosis, pulmonary fibrosis, allergic rhinitis, and bronchiectasis.

BRIEF DESCRIPTION OF THE DRAWINGS

[0030] **Figure 1** shows that antibody 19C11 is a potent antagonist of IL-4 receptor activation, as described in Example 2. (A) 19C11 blocks IL-4 binding to immobilized IL-4Rα. 19C11 (filled circle), control IgG (open square), no IgG (open triangle). (B) 19C11 antibody inhibits IL-4-induced proliferation of TF-1 cells. 19C11 (filled circle), control IgG (open square), no IgG (open triangle), no IL-4 added (filled triangle).

[0031] **Figure 2** shows a Western blot of (A) non-reduced and (B) reduced samples of anti-IL-13.knob and anti-IL-4.hole as IgG1-isotype in *E. coli*, as described in Example 4. Fragment designations are heavy chain (H) and light chain (L) and lane labels are M (molecular weight standard) C (control, no antibody expression plasmid). Figure 2 also shows an immunoblot comparing the different isotypes and mutations for anti-IL-13.knob (C) and anti-IL-4.hole (D), as described in Example 5. The upper panels show non-reduced conditions, representing the assembled half-antibody (HL), while the lower panels show reducing conditions, demonstrating that similar amounts of heavy and light chain are synthesized for all variants.

[0032] **Figure 3** shows analytical characterization of the bispecific antibody, as described in Example 6. (A) Size-exclusion chromatography of the assembled bispecific antibody. The insert shows a zoomed in view of the same graph on the high-molecular weight area. (B) Non-reduced CE-SDS PAGE of the assembled bispecific antibody confirmed formation of the hinge-disulfides and the integrity of inter-chain disulfides. The main peak area corresponds to an intact antibody with formed interchain disulfides. The few minor peak species are reflective of intact antibody lacking complete interchain disulfide to stabilize the hetero-dimer. (C) Reduced CE-SDS confirmed the presence of the expected distribution of light and heavy chains and demonstrated the purity of the material. Aside the main peaks for intact light and heavy chain only trace peaks are detected.

[0033] **Figure 4** shows ESI-TOF mass spectrometry analysis of the intact (A) IgG1-, (B) IgG4- and (C) $IgG4_{R409K}$-isotype based bispecific antibodies, as described in Example 6.

[0034] **Figure 5** shows dose-depending inhibition of human IL-4- (A), human IL-13- (B), or human IL-4/IL-13- (C) induced proliferation by anti-IL-4/IL-13 IgG1-isotype and anti-IL-

4/IL-13 IgG4-isotype bispecific antibodies, as described in Example 8. Anti-IL-4/IL-13 IgG1-isotype (filled circles), anti-IL-4/IL-13 IgG4-isotype (open triangles), no antibody added (open square), no cytokine and antibody added (filled square).

[0035] **Figure 6** shows dose-depending inhibition of cynomolgus monkey IL-4- (A) and cynomolgus monkey IL-13- (B) induced proliferation by anti-IL-4/IL-13 IgG1-isotype and anti-IL-4/IL-13 IgG4-isotype bispecific antibodies, as described in Example 8. Anti-IL-4/IL-13 IgG1-isotype (filled circles), anti-IL-4/IL-13 IgG4-isotype (filled circles in (A), open triangles in (B)), no antibody added (open square), no cytokine and antibody added (filled square).

[0036] **Figure 7** shows mean (±SD) serum anti-IL-4/IL-13 IgG4 (A) and IgG1 (B) bispecific antibody concentrations following administration of a single intravenous or subcutaneous dose in cynomolgus monkeys, as described in Example 9. The limit of quantitation (LOQ) for the ELISA was 0.078 μg/mL. All data above LOQ were used and all data below LOQ were excluded. SD was not calculated when $n \leq 2$.

[0037] **Figure 8** shows bronchoalveolar lavage (BAL) fluid concentrations and epithelial lining fluid (ELF) concentrations of anti-IL-4/IL-13 IgG4 and anti-IL-4/IL-13 IgG1 antibodies following intravenous administration to cynomolgus monkeys challenged with *A. suum* extract to elicit allergic inflammatory responses that mimic those of asthmatics exposed to allergens, as described in Example 10. The limit of quantitation (LOQ) for the ELISA for anti-IL-4/IL-13 was 0.078 μg/mL. All data above LOQ were used and all data below LOQ were excluded. SD was not calculated when $n \leq 2$.

[0038] **Figure 9** shows (A) the study design for treatment of an allergic airway inflammation and asthma mouse model, as described in Example 11. Figure 9 also shows (B) lung eosinophil numbers, (C) bronchoalveolar lavage eosinophil numbers, (D) levels of antigen-specific IgE, and (E) serum TARC levels, in the allergic airway inflammation and asthma mouse model animals following various treatments, as described in Example 11. For each bar graph, the first four bars are, from left to right: control treatment, anti-IL-4 antibody treatment, anti-IL-13 antibody treatment, and anti-IL-4/IL-13 bispecific antibody treatment. The fifth and sixth bars, where present, are naïve mice.

[0039] **Figure 10** shows the amino acid sequences for the human κ1 light chain variable region consensus sequence (SEQ ID NO: 61), the mu19C11 antibody light chain variable region (SEQ ID NO: 2), and the 19C11-κ1 graft light chain variable region (SEQ ID NO: 10),

as described in Example 3. Positions are numbered according to Kabat and hypervariable regions grafted from mu19C11 to the variable light Kappa I consensus framework are boxed.

[0040] **Figure 11** shows the amino acid sequences for the human κ3 light chain variable region consensus sequence (SEQ ID NO: 62), the mu19C11 antibody light chain variable region (SEQ ID NO: 2), and the 19C11-κ3 graft light chain variable region (SEQ ID NO: 11), as described in Example 3. Positions are numbered according to Kabat and hypervariable regions grafted from mu19C11 to the variable light Kappa I consensus framework are boxed.

[0041] **Figure 12** shows the amino acid sequences for the human VH1 heavy chain variable region consensus sequence (SEQ ID NO: 63), the mu19C11 antibody heavy chain variable region (SEQ ID NO: 1), and the 19C11-VH1 graft (SEQ ID NO: 3), the 19C11-VH1.L (SEQ ID NO: 4), and 19C11-VH1.FFL (SEQ ID NO: 5) heavy chain variable regions, as described in Example 3. Positions are numbered according to Kabat and hypervariable regions and vernier positions taken from mu19C11 to the variable heavy subgroup I consensus framework are boxed.

[0042] **Figure 13** shows the amino acid sequences for the human VH3 heavy chain variable region consensus sequence (SEQ ID NO: 64), the mu19C11 antibody heavy chain variable region (SEQ ID NO: 1), and the 19C11-VH3 graft (SEQ ID NO: 6), the 19C11-VH3.FLA (SEQ ID NO: 7), 19C11-VH3.LA (SEQ ID NO: 8), and 19C11-VH3.LA.SV (SEQ ID NO: 9) heavy chain variable regions, as described in Example 3. Positions are numbered according to Kabat and hypervariable regions and vernier positions taken from mu19C11 to the variable heavy subgroup I consensus framework are boxed.

[0043] **Figure 14** shows a table of surface plasmon resonance (SPR) affinity measurements of the humanized antibodies for IL-4, as described in Example 3.

[0044] **Figure 15** shows a plot of inhibition of biotinylated human IL-4 binding to human IL-4R by increasing concentrations of anti-IL-4/IL-13 bispecific antibody, as described in Example 7.

[0045] **Figure 16** shows a plot of inhibition of biotinylated human IL-13 binding to human IL-13Rα1 by increasing concentrations of anti-IL-4/IL-13 bispecific antibody, as described in Example 7.

[0046] **Figure 17** shows a plot of inhibition of biotinylated human IL-13 binding to human IL-13Rα2 by increasing concentrations of anti-IL-4/IL-13 bispecific antibody, as described in Example 7.

[0047] **Figure 18** shows SPR sensograms for binding of IL-13Rα2 to IL-13 in the presence of anti-IL-4/IL-13 bispecific antibody, as described in Example 7. The lines shown represent a two-fold concentration series of the receptor ranging from 12.5 nM to 200 nM.

DETAILED DESCRIPTION

[0048] Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Singleton et al., Dictionary of Microbiology and Molecular Biology 2nd ed., J. Wiley & Sons (New York, N.Y. 1994), and March, Advanced Organic Chemistry Reactions, Mechanisms and Structure 4th ed., John Wiley & Sons (New York, N.Y. 1992), provide one skilled in the art with a general guide to many of the terms used in the present application.

CERTAIN DEFINITIONS

[0049] For purposes of interpreting this specification, the following definitions will apply and whenever appropriate, terms used in the singular will also include the plural and vice versa. In the event that any definition set forth below conflicts with any document incorporated herein by reference, the definition set forth below shall control.

[0050] As used in this specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a protein" or an "antibody" includes a plurality of proteins or antibodies, respectively; reference to "a cell" includes mixtures of cells, and the like.

[0051] The term "biological sample" as used herein includes, but is not limited to, blood, serum, plasma, sputum, bronchoalveolar lavage, tissue biopsies (e.g., lung samples), and nasal samples including nasal swabs or nasal polyps.

[0052] FE_{NO} assay refers to an assay that measures FE_{NO} (fractional exhaled nitric oxide) levels. Such levels can be evaluated using, e.g., a hand-held portable device, NIOX MINO™ (Aerocrine, Solna, Sweden), in accordance with guidelines published by the American Thoracic Society (ATS) in 2005. FE_{NO} may be noted in other similar ways, e.g., FeNO or FENO, and it should be understood that all such similar variations have the same meaning.

[0053] Asthma is a complex disorder characterized by variable and recurring symptoms, reversible airflow obstruction (e.g., by bronchodilator) and bronchial hyperresponsiveness which may or may not be associated with underlying inflammation. Examples of asthma include aspirin sensitive/exacerbated asthma, atopic asthma, severe asthma, mild asthma, moderate to severe asthma, corticosteroid naïve asthma, chronic asthma, corticosteroid

resistant asthma, corticosteroid refractory asthma, newly diagnosed and untreated asthma, asthma due to smoking, asthma uncontrolled on corticosteroids and other asthmas as mentioned in *J Allergy Clin Immunol* (2010) 126(5):926-938.

[0054] "Eosinophilic Disorder" means a disorder associated with excess eosinophil numbers in which atypical symptoms may manifest due to the levels or activity of eosinophils locally or systemically in the body. Disorders associated with excess eosinophil numbers or activity include, but are not limited to, asthma (including aspirin sensitive asthma, chronic asthma, and severe asthma), atopic asthma, atopic dermatitis, allergy, allergic rhinitis (including seasonal allergic rhinitis), non-allergic rhinitis, contact dermatitis, erythema multiform, bullous skin diseases, psoriasis, eczema, rheumatoid arthritis, juvenile chronic arthritis, chronic eosinophilic pneumonia, allergic bronchopulmonary aspergillosis, coeliac disease, Churg-Strauss syndrome (periarteritis nodosa plus atopy), eosinophilic myalgia syndrome, hypereosinophilic syndrome, oedematous reactions including episodic angiodema, helminth infections, urticaria, onchocercal dermatitis, Eosinophil- Associated Gastrointestinal Disorders (EGID) (including but not limited to, eosinophilic esophagitis, eosinophilic gastritis, eosinophilic gastroenteritis, eosinophilic enteritis, and eosinophilic colitis), ulcerative colitis, Whipple's disease, nasal micropolyposis and polyposis, aspirin intolerance, obstructive sleep apnea, Crohn's disease, scleroderma, endomyocardial fibrosis, cancer (e.g., glioblastoma (such as glioblastoma multiforme), non-Hodgkin's lymphoma (NHL), Hodgkin's lymphoma), fibrosis, inflammatory bowel disease, idiopathic interstitial pneumonia, eosinophilic pneumonia, hypersensitivity pneumonitis, goblet cell metaplasia, pulmonary fibrosis (including idiopathic pulmonary fibrosis (IPF) and pulmonary fibrosis secondary to sclerosis), chronic obstructive pulmonary disease (COPD), hepatic fibrosis, and uveitis. Eosinophil-derived secretory products have also been associated with the promotion of angiogenesis and connective tissue formation in tumors and the fibrotic responses seen in conditions such as chronic asthma, Crohn's disease, scleroderma, and endomyocardial fibrosis (Munitz A, Levi-Schaffer F. *Allergy* 2004; 59: 268-75, Adamko et al. *Allergy* 2005; 60: 13-22, Oldhoff, et al. *Allergy* 2005; 60: 693-6).

[0055] IL-13 mediated disorder means a disorder associated with excess IL-13 levels or activity in which atypical symptoms may manifest due to the levels or activity of IL-13 locally and/or systemically in the body. Examples of IL-13 mediated disorders include: cancers (e.g., non-Hodgkin's lymphoma, glioblastoma), atopic dermatitis, allergic rhinitis, asthma, fibrosis,

inflammatory bowel disease, Crohn's disease, lung inflammatory disorders (including pulmonary fibrosis such as IPF), COPD, and hepatic fibrosis.

[0056] IL-4 mediated disorder means: a disorder associated with excess IL-4 levels or activity in which atypical symptoms may manifest due to the levels or activity of IL-4 locally and/or systemically in the body. Examples of IL-4 mediated disorders include: cancers (e.g., non-Hodgkin's lymphoma, glioblastoma), atopic dermatitis, allergic rhinitis, asthma, fibrosis, inflammatory bowel disease, Crohn's disease, lung inflammatory disorders (including pulmonary fibrosis such as IPF), COPD, and hepatic fibrosis.

[0057] Asthma-Like Symptom includes a symptom selected from the group consisting of shortness of breath, cough (changes in sputum production and/or sputum quality and/or cough frequency), wheezing, chest tightness, bronchioconstriction and nocturnal awakenings ascribed to one of the symptoms above or a combination of these symptoms (Juniper et al (2000) *Am. J. Respir. Crit. Care Med.*, 162(4), 1330−1334.).

[0058] The term "respiratory disorder" includes, but is not limited to, asthma (e.g., allergic and non-allergic asthma (e.g., due to infection, e.g., with respiratory syncytial virus (RSV), e.g., in younger children)); bronchitis (e.g., chronic bronchitis); chronic obstructive pulmonary disease (COPD) (e.g., emphysema (e.g., cigarette-induced emphysema); conditions involving airway inflammation, eosinophilia, fibrosis and excess mucus production, e.g., cystic fibrosis, pulmonary fibrosis, and allergic rhinitis. Examples of diseases that can be characterized by airway inflammation, excessive airway secretion, and airway obstruction include asthma, chronic bronchitis, bronchiectasis, and cystic fibrosis.

[0059] Exacerbations (commonly referred to as asthma attacks or acute asthma) are episodes of new or progressive increase in shortness of breath, cough (changes in sputum production and/or sputum quality and/or cough frequency), wheezing, chest tightness, nocturnal awakenings ascribed to one of the symptoms above or a combination of these symptoms. Exacerbations are often characterized by decreases in expiratory airflow (PEF or FEV1). However, PEF variability does not usually increase during an exacerbation, although it may do so leading up to or during the recovery from an exacerbation. The severity of exacerbations ranges from mild to life-threatening and can be evaluated based on both symptoms and lung function. Severe asthma exacerbations as described herein include exacerbations that result in any one or combination of the following hospitalization for asthma treatment, high corticosteroid use (e.g., quadrupling the total daily corticosteroid dose

or a total daily dose of greater or equal to 500 micrograms of FP or equivalent for three consecutive days or more), or oral/parenteral corticosteroid use.

[0060] A "TH2 pathway inhibitor" or "TH2 inhibitor" is an agent that inhibits the TH2 pathway. Examples of a TH2 pathway inhibitor include inhibitors of the activity of any one of the targets selected from the group consisting of: ITK, BTK , IL-9 (e.g., MEDI-528), IL-5 (e.g., Mepolizumab, CAS No. 196078-29-2; resilizumab), IL-13 (e.g., IMA-026, IMA-638 (also referred to as, anrukinzumab, INN No. 910649-32-0; QAX-576; IL-4/IL-13 trap), tralokinumab (also referred to as CAT-354, CAS No. 1044515-88-9); AER-001, ABT-308 (also referred to as humanized 13C5.5 antibody), IL-4 (e.g., AER-001, IL-4/IL-13 trap), OX40L, TSLP, IL-25, IL-33 and IgE (e.g., XOLAIR, QGE-031; MEDI-4212); and receptors such as: IL-9 receptor, IL-5 receptor (e.g., MEDI-563 (benralizumab, CAS No. 1044511-01-4), IL-4receptor alpha (e.g., AMG-317, AIR-645), IL-13receptoralpha1 (e.g., R-1671) and IL-13receptoralpha2, OX40, TSLP-R, IL-7Ralpha (a co-receptor for TSLP), IL17RB (receptor for IL-25), ST2 (receptor for IL-33), CCR3, CCR4, CRTH2 (e.g., AMG-853, AP768, AP-761, MLN6095, ACT129968), FcepsilonRI, FcepsilonRII/CD23 (receptors for IgE), Flap (e.g., GSK2190915), Syk kinase (R-343, PF3526299); CCR4 (AMG-761), TLR9 (QAX-935) and multi-cytokine inhibitor of CCR3, IL5, IL3, GM-CSF (e.g., TPI ASM8). Examples of inhibitors of the aforementioned targets are disclosed in, for example, WO2008/086395; WO2006/085938; US 7,615,213; US 7,501,121; WO2006/085938; WO 2007/080174; US 7,807,788; WO2005007699; WO2007036745; WO2009/009775; WO2007/082068; WO2010/073119; WO2007/045477; WO2008/134724; US2009/0047277; and WO2008/127,271).

[0061] The term "small molecule" refers to an organic molecule having a molecular weight between 50 Daltons to 2500 Daltons.

[0062] The term "antibody" is used in the broadest sense and specifically covers, for example, monoclonal antibodies, polyclonal antibodies, antibodies with polyepitopic specificity, single chain antibodies, multi-specific antibodies and fragments of antibodies. Such antibodies can be chimeric, humanized, human and synthetic. Such antibodies and methods of generating them are described in more detail below.

[0063] The term "multispecific antibody" is used in the broadest sense and specifically covers an antibody comprising an antigen-binding domain that has polyepitopic specificity (i.e., is capable of specifically binding to two, or more, different epitopes on one biological molecule or is capable of specifically binding to epitopes on two, or more, different biological

molecules). In some embodiments, an antigen-binding domain of a multispecific antibody (such as a bispecific antibody) comprises two VH/VL units, wherein a first VH/VL unit specifically binds to a first epitope and a second VH/VL unit specifically binds to a second epitope, wherein each VH/VL unit comprises a heavy chain variable domain (VH) and a light chain variable domain (VL). Such multispecific antibodies include, but are not limited to, full length antibodies, antibodies having two or more VL and VH domains, antibody fragments such as Fab, Fv, dsFv, scFv, diabodies, bispecific diabodies and triabodies, antibody fragments that have been linked covalently or non-covalently. A VH/VL unit that further comprises at least a portion of a heavy chain constant region and/or at least a portion of a light chain constant region may also be referred to as a "hemimer" or "half antibody." According to some embodiments, the multispecific antibody is an IgG antibody that binds to each epitope with an affinity of 5 μM to 0.001 pM, 3 μM to 0.001 pM, 1 μM to 0.001 pM, 0.5 μM to 0.001 pM, or 0.1 μM to 0.001 pM. In some embodiments, a hemimer comprises a sufficient portion of a heavy chain variable region to allow intramolecular disulfide bonds to be formed with a second hemimer. In some embodiments, a hemimer comprises a knob mutation or a hole mutation, for example, to allow heterodimerization with a second hemimer or half antibody that comprises a complementary hole mutation or knob mutation. Knob mutations and hole mutations are discussed further below.

[0064] A "bispecific antibody" is a multispecific antibody comprising an antigen-binding domain that is capable of specifically binding to two different epitopes on one biological molecule or is capable of specifically binding to epitopes on two different biological molecules. A bispecific antibody may also be referred to herein as having "dual specificity" or as being "dual specific."

[0065] The term "knob-into-hole" or "KnH" technology as used herein refers to the technology directing the pairing of two polypeptides together *in vitro* or *in vivo* by introducing a protuberance (knob) into one polypeptide and a cavity (hole) into the other polypeptide at an interface in which they interact. For example, KnHs have been introduced in the Fc:Fc binding interfaces, C_L:C_{H1} interfaces or V_H/V_L interfaces of antibodies (*see, e.g.*, US 2011/0287009, US2007/0178552, WO 96/027011, WO 98/050431, and Zhu et al., 1997, *Protein Science* 6:781-788). In some embodiments, KnHs drive the pairing of two different heavy chains together during the manufacture of multispecific antibodies. For example, multispecific antibodies having KnH in their Fc regions can further comprise single variable domains linked to each Fc region, or further comprise different heavy chain variable domains

that pair with similar or different light chain variable domains. KnH technology can be also be used to pair two different receptor extracellular domains together or any other polypeptide sequences that comprises different target recognition sequences (e.g., including affibodies, peptibodies and other Fc fusions).

[0066] The term "knob mutation" as used herein refers to a mutation that introduces a protuberance (knob) into a polypeptide at an interface in which the polypeptide interacts with another polypeptide. In some embodiments, the other polypeptide has a hole mutation.

[0067] The term "hole mutation" as used herein refers to a mutation that introduces a cavity (hole) into a polypeptide at an interface in which the polypeptide interacts with another polypeptide. In some embodiments, the other polypeptide has a knob mutation.

[0068] The term "therapeutic agent" refers to any agent that is used to treat a disease. A therapeutic agent may be, for example, a polypeptide(s) (e.g., an antibody, an immunoadhesin or a peptibody), an aptamer or a small molecule that can bind to a protein or a nucleic acid molecule that can bind to a nucleic acid molecule encoding a target (i.e., siRNA), etc.

[0069] The term "controller" or "preventor" refers to any therapeutic agent that is used to control asthma inflammation. Examples of controllers include corticosteroids, leukotriene receptor antagonists (e.g., inhibit the synthesis or activity of leukotrienes such as montelukast, zileuton, pranlukast, zafirlukast), LABAs, corticosteroid/LABA combination compositions , theophylline (including aminophylline), cromolyn sodium, nedocromil sodium, omalizumab, LAMAs, MABA (e.g, bifunctional muscarinic antagonist-beta2 Agonist), 5-Lipoxygenase Activating Protein (FLAP) inhibitors, and enzyme PDE-4 inhibitor (e.g., roflumilast). A "second controller" typically refers to a controller that is not the same as the first controller.

[0070] The term "corticosteroid sparing" or "CS" means the decrease in frequency and/or amount, or the elimination of, corticosteroid used to treat a disease in a patient taking corticosteroids for the treatment of the disease due to the administration of another therapeutic agent. A "CS agent" refers to a therapeutic agent that can cause CS in a patient taking a corticosteroid.

[0071] The term "corticosteroid" includes, but is not limited to fluticasone (including fluticasone propionate (FP)), beclometasone, budesonide, ciclesonide, mometasone, flunisolide, betamethasone and triamcinolone. "Inhalable corticosteroid" means a corticosteroid that is suitable for delivery by inhalation. Exemplary inhalable corticosteroids are fluticasone, beclomethasone dipropionate, budenoside, mometasone furoate, ciclesonide, flunisolide, triamcinolone acetonide and any other corticosteroid currently available or

becoming available in the future. Examples of corticosteroids that can be inhaled and are combined with a long-acting beta2-agonist include, but are not limited to: budesonide/formoterol and fluticasone/salmeterol.

[0072] Examples of corticosteroid/LABA combination drugs include fluticasone furoate/vilanterol trifenatate and indacaterol/mometasone.

[0073] The term "LABA" means long-acting beta-2 agonist, which agonist includes, for example, salmeterol, formoterol, bambuterol, albuterol, indacaterol, arformoterol and clenbuterol.

[0074] The term "LAMA" means long-acting muscarinic antagonist, which agonists include: tiotropium.

[0075] Examples of LABA/LAMA combinations include, but are not limited to: olodaterol tiotropium (Boehringer Ingelheim's) and indacaterol glycopyrronium (Novartis)

[0076] The term "SABA" means short-acting beta-2 agonists, which agonists include, but are not limited to, salbutamol, levosalbutamol, fenoterol, terbutaline, pirbuterol, procaterol, bitolterol, rimiterol, carbuterol, tulobuterol and reproterol

[0077] Leukotriene receptor antagonists (sometimes referred to as a leukast) (LTRA) are drugs that inhibit leukotrienes. Examples of leukotriene inhibitors include montelukast, zileuton, pranlukast, and zafirlukast.

[0078] The term "FEV1" refers to the volume of air exhaled in the first second of a forced expiration. It is a measure of airway obstruction. Provocative concentration of methacholine required to induce a 20% decline in FEV1 (PC20) is a measure of airway hyper-responsiveness. FEV1 may be noted in other similar ways, e.g., FEV_1, and it should be understood that all such similar variations have the same meaning.

[0079] The term "relative change in FEV1" = (FEV1 at week 12 of treatment – FEV1 prior to start of treatment) divided by FEV1.

[0080] As used herein, "FVC" refers to "Forced Vital Capacity" which refers to a standard test that measures the change in lung air volume between a full inspiration and maximal expiration to residual volume (as opposed to the volume of air expelled in one second as in FEV1). It is a measure of the functional lung capacity. In patients with restrictive lung diseases such as interstitial lung disease including IPF, hypersensitivity pneumonitis, sarcoidosis, and systemic sclerosis, the FVC is reduced typically due to scarring of the lung parenchyma.

[0081] The term "mild asthma" refers to a patient generally experiencing symptoms or exacerbations less than two times a week, nocturnal symptoms less than two times a month, and is asymptomatic between exacerbations. Mild, intermittent asthma is often treated as needed with the following: inhaled bronchodilators (short-acting inhaled beta2- agonists); avoidance of known triggers; annual influenza vaccination; pneumococcal vaccination every 6 to 10 years, and in some cases, an inhaled beta2-agonist, cromolyn, or nedocromil prior to exposure to identified triggers. If the patient has an increasing need for short-acting beta2-agonist (e.g., uses short-acting beta2-agonist more than three to four times in 1 day for an acute exacerbation or uses more than one canister a month for symptoms), the patient may require a stepup in therapy.

[0082] The term "moderate asthma" generally refers to asthma in which the patient experiences exacerbations more than two times a week and the exacerbations affect sleep and activity; the patient has nighttime awakenings due to asthma more than two times a month; the patient has chronic asthma symptoms that require short-acting inhaled beta2-agonist daily or every other day; and the patient's pretreatment baseline PEF or FEV1 is 60 to 80 percent predicted and PEF variability is 20 to 30 percent.

[0083] The term "severe asthma" generally refers to asthma in which the patient has almost continuous symptoms, frequent exacerbations, frequent nighttime awakenings due to the asthma, limited activities, PEF or FEV1 baseline less than 60 percent predicted, and PEF variability of 20 to 30 percent.

[0084] Examples of rescue medications include albuterol, ventolin and others.

[0085] "Resistant" refers to a disease that demonstrates little or no clinically significant improvement after treatment with a therapeutic agent. For example, asthma which requires treatment with high dose ICS (e.g., quadrupling the total daily corticosteroid dose or a total daily dose of greater or equal to 500 micrograms of FP (or equivalent) for at least three consecutive days or more, or systemic corticosteroid for a two week trial to establish if asthma remains uncontrolled or FEV1 does not improve is often considered severe refractory asthma.

[0086] A therapeutic agent as provided herein can be administered by any suitable means, including parenteral, subcutaneous, intraperitoneal, intrapulmonary, and intranasal. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. In some embodiments, the therapeutic agent is inhaled. According to some embodiments, the dosing is given by injections, e.g., intravenous or subcutaneous injections.

In some embodiments, the therapeutic agent is administered using a syringe (e.g., prefilled or not) or an autoinjector.

[0087] For the prevention or treatment of disease, the appropriate dosage of a therapeutic agent may depend on the type of disease to be treated, the severity and course of the disease, whether the therapeutic agent is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the therapeutic agent, and the discretion of the attending physician. The therapeutic agent is suitably administered to the patient at one time or over a series of treatments. The therapeutic agent composition will be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners.

[0088] "Patient response" or "response" (and grammatical variations thereof) can be assessed using any endpoint indicating a benefit to the patient, including, without limitation, (1) inhibition, to some extent, of disease progression, including slowing down and complete arrest; (2) reduction in the number of disease episodes and/or symptoms; (3) reduction in lesional size; (4) inhibition (i.e., reduction, slowing down or complete stopping) of disease cell infiltration into adjacent peripheral organs and/or tissues; (5) inhibition (i.e. reduction, slowing down or complete stopping) of disease spread; (6) decrease of auto-immune response, which may, but does not have to, result in the regression or ablation of the disease lesion; (7) relief, to some extent, of one or more symptoms associated with the disorder; (8) increase in the length of disease-free presentation following treatment; and/or (9) decreased mortality at a given point of time following treatment.

[0089] "Affinity" refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (Kd). Affinity can be measured by common methods known in the art, including those described herein. Specific illustrative and exemplary embodiments for measuring binding affinity are described herein.

[0090] An "affinity matured" antibody refers to an antibody with one or more alterations in one or more hypervariable regions (HVRs), compared to a parent antibody which does not possess such alterations, such alterations resulting in an improvement in the affinity of the antibody for antigen.

[0091] The terms "anti-IL-4 antibody" and "an antibody that binds to IL-4" refer to an antibody that is capable of binding IL-4 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting IL-4. In some embodiments, the extent of binding of an anti-IL-4 antibody to an unrelated, non-IL-4 protein is less than about 10% of the binding of the antibody to IL-4 as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to IL-4 has a dissociation constant (Kd) of $\leq$ 1μM, $\leq$ 100 nM, $\leq$ 10 nM, $\leq$ 1 nM, $\leq$ 0.1 nM, $\leq$ 0.01 nM, or $\leq$ 0.001 nM (e.g. 10-8 M or less, e.g. from 10-8 M to 10-13 M, e.g., from 10-9 M to 10-13 M). In certain embodiments, an anti-IL-4 antibody binds to an epitope of IL-4 that is conserved among IL-4 from different species. In some embodiments, an anti-IL-4 antibody is a multispecific antibody, such as a bispecific antibody.

[0092] The terms "anti-IL-13 antibody" and "an antibody that binds to IL-13" refer to an antibody that is capable of binding IL-13 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting IL-13. In some embodiments, the extent of binding of an anti-IL-13 antibody to an unrelated, non-IL-13 protein is less than about 10% of the binding of the antibody to IL-13 as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to IL-13 has a dissociation constant (Kd) of $\leq$ 1μM, $\leq$ 100 nM, $\leq$ 10 nM, $\leq$ 1 nM, $\leq$ 0.1 nM, $\leq$ 0.01 nM, or $\leq$ 0.001 nM (e.g. 10-8 M or less, e.g. from 10-8 M to 10-13 M, e.g., from 10-9 M to 10-13 M). In certain embodiments, an anti-IL-13 antibody binds to an epitope of IL-13 that is conserved among IL-13 from different species. In some embodiments, an anti-IL-13 antibody is a multispecific antibody, such as a bispecific antibody.

[0093] The term "antibody" herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity.

[0094] An "antibody fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH,

$F(ab')_2$; diabodies; linear antibodies; single-chain antibody molecules (e.g. scFv); and multispecific antibodies formed from antibody fragments.

[0095] An "antibody that binds to the same epitope" as a reference antibody refers to an antibody that blocks binding of the reference antibody to its antigen in a competition assay by 50% or more, and conversely, the reference antibody blocks binding of the antibody to its antigen in a competition assay by 50% or more. An exemplary competition assay is provided herein.

[0096] An "acceptor human framework" for the purposes herein is a framework comprising the amino acid sequence of a light chain variable domain (VL) framework or a heavy chain variable domain (VH) framework derived from a human immunoglobulin framework or a human consensus framework, as defined below. An acceptor human framework "derived from" a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain amino acid sequence changes. In some embodiments, the number of amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. In some embodiments, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.

[0097] The term "chimeric" antibody refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species.

[0098] The "class" of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α, δ, ε, γ, and μ, respectively.

[0099] The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents a cellular function and/or causes cell death or destruction. Cytotoxic agents include, but are not limited to, radioactive isotopes (e.g., At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu); chemotherapeutic agents or drugs (e.g., methotrexate, adriamycin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents); growth inhibitory agents; enzymes and fragments thereof such as nucleolytic enzymes; antibiotics; toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant

or animal origin, including fragments and/or variants thereof; and the various antitumor or anticancer agents disclosed below.

[00100] "Effector functions" refer to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g. B cell receptor); and B cell activation.

[00101] An "effective amount" of an agent, e.g., a pharmaceutical formulation, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result.

[0100] The term "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. In some embodiments, a human IgG heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991.

[0101] "Framework" or "FR" refers to variable domain residues other than hypervariable region (HVR) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in the following sequence in VH (or VL): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.

[0102] The terms "full length antibody," "intact antibody," and "whole antibody" are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure or having heavy chains that contain an Fc region as defined herein.

[0103] The terms "host cell," "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same

function or biological activity as screened or selected for in the originally transformed cell are included herein.

[0104] A "human antibody" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

[0105] A "human consensus framework" is a framework which represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat et al., Sequences of Proteins of Immunological Interest, Fifth Edition, NIH Publication 91-3242, Bethesda MD (1991), vols. 1-3. In some embodiments, for the VL, the subgroup is subgroup kappa I as in Kabat et al., supra. In some embodiments, for the VH, the subgroup is subgroup III as in Kabat et al., supra.

[0106] A "humanized" antibody refers to a chimeric antibody comprising amino acid residues from non-human HVRs and amino acid residues from human FRs. In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (e.g., CDRs) correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A "humanized form" of an antibody, e.g., a non-human antibody, refers to an antibody that has undergone humanization.

[0107] The term "hypervariable region" or "HVR" as used herein refers to each of the regions of an antibody variable domain which are hypervariable in sequence ("complementarity determining regions" or "CDRs") and/or form structurally defined loops ("hypervariable loops") and/or contain the antigen-contacting residues ("antigen contacts"). Generally, antibodies comprise six HVRs: three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). Exemplary HVRs herein include:

(a) hypervariable loops occurring at amino acid residues 26-32 (L1), 50-52 (L2), 91-96 (L3), 26-32 (H1), 53-55 (H2), and 96-101 (H3) (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987));

(b) CDRs occurring at amino acid residues 24-34 (L1), 50-56 (L2), 89-97 (L3), 31-35b (H1), 50-65 (H2), and 95-102 (H3) (Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991));

(c) antigen contacts occurring at amino acid residues 27c-36 (L1), 46-55 (L2), 89-96 (L3), 30-35b (H1), 47-58 (H2), and 93-101 (H3) (MacCallum et al. *J. Mol. Biol.* 262: 732-745 (1996)); and

(d) combinations of (a), (b), and/or (c), including HVR amino acid residues 46-56 (L2), 47-56 (L2), 48-56 (L2), 49-56 (L2), 26-35 (H1), 26-35b (H1), 49-65 (H2), 93-102 (H3), and 94-102 (H3).

[0108] In some embodiments, HVR residues comprise those identified in Figures 10 to 13 or elsewhere in the specification.

[0109] Unless otherwise indicated, HVR residues and other residues in the variable domain (e.g., FR residues) are numbered herein according to Kabat et al., *supra*.

[0110] An "immunoconjugate" is an antibody conjugated to one or more heterologous molecule(s), including but not limited to a cytotoxic agent.

[0111] An "individual" or "subject" is a mammal. Mammals include, but are not limited to, domesticated animals (e.g., cows, sheep, cats, dogs, and horses), primates (e.g., humans and non-human primates such as monkeys), rabbits, and rodents (e.g., mice and rats). In certain embodiments, the individual or subject is a human.

[0112] An "isolated" antibody is one which has been separated from a component of its natural environment. In some embodiments, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (e.g., ion exchange or reverse phase HPLC). For review of methods for assessment of antibody purity, *see, e.g.*, Flatman et al., *J. Chromatogr.* B 848:79-87 (2007).

[0113] An "isolated" nucleic acid refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

[0114] "Isolated nucleic acid encoding an anti-IL-4 antibody" refers to one or more nucleic acid molecules encoding antibody heavy and light chains (or fragments thereof),

including such nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.

[0115] "Isolated nucleic acid encoding an anti-IL3 antibody" refers to one or more nucleic acid molecules encoding antibody heavy and light chains (or fragments thereof), including such nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.

[0116] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies, e.g., containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, the modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, monoclonal antibodies may be made by a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described herein. In some embodiments, a monoclonal antibody is a multispecific (such as bispecific) antibody.

[0117] A "naked antibody" refers to an antibody that is not conjugated to a heterologous moiety (e.g., a cytotoxic moiety) or radiolabel. The naked antibody may be present in a pharmaceutical formulation.

[0118] "Native antibodies" refer to naturally occurring immunoglobulin molecules with varying structures. For example, native IgG antibodies are heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light chains and two identical heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (CH1, CH2, and CH3). Similarly, from N- to C-terminus, each light chain has a variable region (VL), also called a variable light domain or a light chain variable domain, followed by a constant light (CL) domain. The light chain of an antibody may be

assigned to one of two types, called kappa (κ) and lambda (λ), based on the amino acid sequence of its constant domain.

[0119] The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products. The term "package insert" is also used to refer to instructions customarily included in commercial packages of diagnostic products that contain information about the intended use, test principle, preparation and handling of reagents, specimen collection and preparation, calibration of the assay and the assay procedure, performance and precision data such as sensitivity and specificity of the assay.

[0120] "Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, California, or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

[0121] In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid

sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

100 times the fraction X/Y

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

[0122] The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

[0123] A "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

[0124] The term "IL-4," as used herein, refers to any native IL-4 from any vertebrate source, including mammals such as primates (e.g. humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses "full-length," unprocessed IL-4 as well as any form of IL-4 that results from processing in the cell. The term also encompasses naturally occurring variants of IL-4, e.g., splice variants or allelic variants. The amino acid sequences of exemplary human IL-4 are shown in SEQ ID NOs: 27 and 28, and in Swiss-Prot Accession No. P05112.2. The amino acid sequence of an exemplary cynomolgus monkey IL-4 is shown in SEQ ID NO: 33.

[0125] The term "IL-13," as used herein, refers to any native IL-13 from any vertebrate source, including mammals such as primates (e.g. humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses "full-length," unprocessed IL-13 as well as any form of IL-13 that results from processing in the cell. The term also encompasses naturally occurring variants of IL-13, e.g., splice variants or allelic variants. The amino acid sequences of exemplary human IL-13 are shown in SEQ ID NOs: 29 and 30, and in Swiss-

Prot Accession No. P35225.2. The amino acid sequence of an exemplary cynomolgus monkey IL-13 is shown in SEQ ID NO: 32.

[0126] As used herein, "treatment" (and grammatical variations thereof such as "treat" or "treating") refers to clinical intervention in an attempt to alter the natural course of the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, antibodies are used to delay development of a disease or to slow the progression of a disease.

[0127] The term "variable region" or "variable domain" refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). (*See, e.g.*, Kindt et al. *Kuby Immunology*, 6th ed., W.H. Freeman and Co., page 91 (2007).) A single VH or VL domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a VH or VL domain from an antibody that binds the antigen to screen a library of complementary VL or VH domains, respectively. *See, e.g.*, Portolano et al., *J. Immunol.* 150:880-887 (1993); Clarkson et al., *Nature* 352:624-628 (1991).

[0128] The term "vector," as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors."

COMPOSITIONS AND METHODS

[0129] In certain embodiments, antibodies that bind to IL-4 are provided. In certain embodiments, bispecific antibodies that bind to IL-4 and IL-13 are provided. The antibodies are useful, e.g., for the diagnosis or treatment of eosinophilic disorders, including respiratory disorders (such as asthma and IPF), IL-4 mediated disorders, and IL-13 mediated disorders.

Exemplary Anti-IL-4 Antibodies

[0130] In some embodiments, isolated antibodies that bind IL-4 are provided. In some embodiments, an anti-IL-4 antibody comprises at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 12; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 18; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 14; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 15; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 16; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 17.

[0131] In some embodiments, an antibody is provided that comprises at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 12; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 18; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 14. In some embodiments, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 14. In some embodiments, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 14 and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 17. In some embodiments, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 14, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 17, and HVR-H2 comprising the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 18. In some embodiments, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 12; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 18; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 14.

[0132] In some embodiments, an antibody is provided that comprises at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 15; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 16; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 17. In some embodiments, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 15; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 16; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 17.

[0133] In some embodiments, an antibody comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 12, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 18, and (iii) HVR-H3 comprising an amino acid sequence

selected from SEQ ID NO: 14; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 15, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 16, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 17.

[0134] In some embodiments, an antibody is provided that comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 12; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 18; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 14; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 15; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 16; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO: 17. In some embodiments, an antibody is provided that comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 12; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 13; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 14; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 15; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 16; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO: 17.

[0135] In any of the above embodiments, an anti-IL-4 antibody is humanized. In some embodiments, an anti-IL-4 antibody comprises HVRs as in any of the above embodiments, and further comprises an acceptor human framework, e.g. a human immunoglobulin framework or a human consensus framework. In some embodiments, an anti-IL-4 antibody comprises HVRs as in any of the above embodiments, and further comprises a VH comprising FR1, FR2, FR3, and FR4 of any one of SEQ ID NOs: 3 to 9. In some embodiments, an anti-IL-4 antibody comprises HVRs as in any of the above embodiments, and further comprises a VH comprising FR1, FR2, FR3, and FR4 of SEQ ID NO: 9. In some embodiments, an anti-IL-4 antibody comprises HVRs as in any of the above embodiments, and further comprises a VL comprising FR1, FR2, FR3, and FR4 of any one of SEQ ID NOs: 10 and 11. In some embodiments, an anti-IL-4 antibody comprises HVRs as in any of the above embodiments, and further comprises a VL comprising FR1, FR2, FR3, and FR4 of SEQ ID NO: 10.

[0136] In some embodiments, an anti-IL-4 antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 1 and 3 to 9. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%,

94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-IL-4 antibody comprising that sequence retains the ability to bind to IL-4. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 9. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-IL-4 antibody comprises the VH sequence in SEQ ID NO: 9, including post-translational modifications of that sequence. In a particular embodiment, the VH comprises one, two or three HVRs selected from: (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 12, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 18, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 14.

[0137] In some embodiments, an anti-IL-4 antibody is provided, wherein the antibody comprises a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 2, 10, and 11. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-IL-4 antibody comprising that sequence retains the ability to bind to IL-4. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 10. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-IL-4 antibody comprises the VL sequence in SEQ ID NO: 10, including post-translational modifications of that sequence. In a particular embodiment, the VL comprises one, two or three HVRs selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 15; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 16; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 17.

[0138] In some embodiments, an anti-IL-4 antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above. In some embodiments, the antibody comprises the VH and VL sequences in SEQ ID NO: 9 and SEQ ID NO: 10, respectively, including post-translational modifications of those sequences.

[0139] In some embodiments, an antibody is provided that competes for binding to IL-4 with an anti-IL-4 antibody comprising a VH sequence of SEQ ID NO: 9 and a VL sequence

of SEQ ID NO: 10. In some embodiments, an antibody is provided that binds to the same epitope as an anti-IL-4 antibody provided herein. For example, in certain embodiments, an antibody is provided that binds to the same epitope as an anti-IL-4 antibody comprising a VH sequence of SEQ ID NO: 9 and a VL sequence of SEQ ID NO: 10.

[0140] In some embodiments, an anti-IL-4 antibody according to any of the above embodiments is a monoclonal antibody, including a chimeric, humanized or human antibody. In some embodiments, an anti-IL-4 antibody is an antibody fragment, e.g., a Fv, Fab, Fab', scFv, diabody, or $F(ab')_2$ fragment. In some embodiments, the antibody is a full length antibody, e.g., an intact IgG1 or IgG4 antibody or other antibody class or isotype as defined herein.

[0141] In some embodiments, an anti-IL-4 antibody according to any of the above embodiments may incorporate any of the features, singly or in combination, as described in Sections 1-7 below.

Exemplary Anti-IL-13 Antibodies

[0142] In some embodiments, isolated antibodies that bind IL-13 are provided. In some embodiments, an anti-IL-13 antibody comprises at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 21 or SEQ ID NO: 60; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 24; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 25; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26.

[0143] In some embodiments, an antibody is provided that comprises at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 21 or SEQ ID NO: 60; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23. In some embodiments, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23. In some embodiments, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23 and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26, and HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22. In some embodiments, the antibody comprises (a) HVR-H1 comprising the amino

acid sequence of SEQ ID NO: 21 or SEQ ID NO: 60; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23.

[0144] In some embodiments, an antibody is provided that comprises at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 24; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 25; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 24; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 25; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26.

[0145] In some embodiments, an antibody comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 21 or SEQ ID NO: 60, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO: 23; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 24, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 25, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26.

[0146] In some embodiments, an antibody is provided that comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 21 or SEQ ID NO: 60; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 24; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 25; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO: 26.

[0147] In any of the above embodiments, an anti-IL-13 antibody is humanized. In some embodiments, an anti-IL-13 antibody comprises HVRs as in any of the above embodiments, and further comprises an acceptor human framework, e.g. a human immunoglobulin framework or a human consensus framework. In some embodiments, an anti-IL-13antibody comprises HVRs as in any of the above embodiments, and further comprises a VH comprising FR1, FR2, FR3, and/or FR4 sequences of SEQ ID NO: 19. In some embodiments, an anti-IL-13antibody comprises HVRs as in any of the above embodiments, and further comprises a VL comprising FR1, FR2, FR3, and/or FR4 sequences of SEQ ID NO: 20. In some embodiments, an anti-IL-13antibody comprises HVRs as in any of the

above embodiments, and further comprises a VH comprising FR1, FR2, FR3, and/or FR4 sequences of SEQ ID NO: 56. In some embodiments, an anti-IL-13antibody comprises HVRs as in any of the above embodiments, and further comprises a VL comprising FR1, FR2, FR3, and/or FR4 sequences of SEQ ID NO: 57.

[0148] In some embodiments, an anti-IL-13 antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 19. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-IL-13 antibody comprising that sequence retains the ability to bind to IL-13. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 19. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). In some embodiments, the anti-IL-13 antibody comprises the VH sequence in SEQ ID NO: 19, including post-translational modifications of that sequence. In some embodiments, the anti-IL-13 antibody comprises the VH sequence in SEQ ID NO: 56, including post-translational modifications of that sequence. In some embodiments, the VH comprises one, two or three HVRs selected from: (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 21 or SEQ ID NO: 60, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23.

[0149] In some embodiments, an anti-IL-13 antibody is provided, wherein the antibody comprises a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 20. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-IL-13 antibody comprising that sequence retains the ability to bind to IL-13. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 20. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). In some embodiments, the anti-IL-13 antibody comprises the VL sequence in SEQ ID NO: 20, including post-translational modifications of that sequence. In some embodiments, the anti-IL-13 antibody comprises the VL sequence in

SEQ ID NO: 57, including post-translational modifications of that sequence. In some embodiments, the VL comprises one, two or three HVRs selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 24; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 25; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26.

[0150] In some embodiments, an anti-IL-13 antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above. In some embodiments, the antibody comprises the VH sequence in SEQ ID NO: 19 or SEQ ID NO: 56 and the VL sequence in SEQ ID NO: 20 or SEQ ID NO: 57, including post-translational modifications of those sequences.

[0151] In some embodiments, an antibody is provided that competes for binding to IL-13 with an anti-IL-13 antibody comprising a VH sequence of SEQ ID NO: 19 and a VL sequence of SEQ ID NO: 20. In some embodiments, an antibody is provided that binds to the same epitope as an anti-IL-13 antibody provided herein. *See, e.g.*, Ultsch, M. et al., Structural Basis of Signaling Blockade by Anti-IL-13 Antibody Lebrikizumab, *J. Mol. Biol.* (2013), dx.doi.org/10.1016/j.jmb.2013.01.024. In some embodiments, an antibody is provided that binds to the same epitope as an anti-IL-13 antibody provided herein. For example, in certain embodiments, an antibody is provided that binds to the same epitope as an anti-IL-13 antibody comprising a VH sequence of SEQ ID NO: 19 and a VL sequence of SEQ ID NO: 20. In certain embodiments, an antibody is provided that binds to an epitope within amino acids 63 to 74 of human precursor IL-13 (SEQ ID NO: 29) or amino acids 45 to 56 of the mature form of human IL-13 (SEQ ID NO: 30), which are YCAALESLINVS (SEQ ID NO: 43). In certain embodiments, an antibody is provided that binds to an epitope within amino acids 68 to 75 of human precursor IL-13 (SEQ ID NO: 29) or amino acids 50-57 of the mature form of human IL-13 (SEQ ID NO: 30), which are ESLINVSG (SEQ ID NO: 42).

[0152] Another exemplary anti-IL-13 antibody is 11H4 and humanized versions thereof, including hu11H4v6. Mu11H4 comprises heavy chain and light chain variable regions comprising the amino acid sequences of SEQ ID NOs: 45 and 44, respectively. Humanized hu11H4v6 comprises a heavy chain variable region and a light chain variable region comprising the amino acid sequence of SEQ ID NOs: 49 and 48, respectively. Humanized hu11H4v6 comprises a heavy chain and a light chain comprising the amino acid sequence of SEQ ID NOs: 47 and 46, respectively.

[0153] In some embodiments, an anti-IL-13 antibody comprises at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 50; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 51; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 52; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 53; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 54; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 55.

[0154] In some embodiments, an antibody is provided that comprises at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 50; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 51; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 52. In some embodiments, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 52. In some embodiments, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 52 and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 55. In some embodiments, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 52, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 55, and HVR-H2 comprising the amino acid sequence of SEQ ID NO: 51. In some embodiments, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 50; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 51; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 52.

[0155] In some embodiments, an antibody is provided that comprises at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 53; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 54; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 55. In some embodiments, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 53; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 54; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 55.

[0156] In some embodiments, an antibody comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 50, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 51, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO: 52; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 53,

(ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 54, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 55.

[0157] In some embodiments, an antibody is provided that comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 50; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 51; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 52; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 53; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 54; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO: 55.

[0158] In any of the above embodiments, an anti-IL-13 antibody is humanized. In some embodiments, an anti-IL-13 antibody comprises HVRs as in any of the above embodiments, and further comprises an acceptor human framework, e.g. a human immunoglobulin framework or a human consensus framework. In some embodiments, an anti-IL-13antibody comprises HVRs as in any of the above embodiments, and further comprises a VH comprising FR1, FR2, FR3, and/or FR4 sequences of SEQ ID NO: 49. In some embodiments, an anti-IL-13antibody comprises HVRs as in any of the above embodiments, and further comprises a VL comprising FR1, FR2, FR3, and/or FR4 sequences of SEQ ID NO: 48.

[0159] In some embodiments, an anti-IL-13 antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 49. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-IL-13 antibody comprising that sequence retains the ability to bind to IL-13. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 49. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-IL-13 antibody comprises the VH sequence in SEQ ID NO: 49, including post-translational modifications of that sequence. In a particular embodiment, the VH comprises one, two or three HVRs selected from: (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 50, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 51, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 52.

[0160] In some embodiments, an anti-IL-13 antibody is provided, wherein the antibody comprises a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%,

95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 48. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-IL-13 antibody comprising that sequence retains the ability to bind to IL-13. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 48. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-IL-13 antibody comprises the VL sequence in SEQ ID NO: 48, including post-translational modifications of that sequence. In a particular embodiment, the VL comprises one, two or three HVRs selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 53; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 54; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 55.

[0161] In some embodiments, an anti-IL-13 antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above. In some embodiments, the antibody comprises the VH and VL sequences in SEQ ID NO: 49 and SEQ ID NO: 48, respectively, including post-translational modifications of those sequences.

[0162] In some embodiments, an antibody is provided that competes for binding to IL-13 with an anti-IL-13 antibody comprising a VH sequence of SEQ ID NO: 49 and a VL sequence of SEQ ID NO: 48. In some embodiments, an antibody is provided that binds to the same epitope as an anti-IL-13 antibody provided herein. *See, e.g.*, Ultsch, M. et al., Structural Basis of Signaling Blockade by Anti-IL-13 Antibody Lebrikizumab, *J. Mol. Biol.* (2013), dx.doi.org/10.1016/j.jmb.2013.01.053. In some embodiments, an antibody is provided that binds to the same epitope as an anti-IL-13 antibody provided herein. For example, in certain embodiments, an antibody is provided that binds to the same epitope as an anti-IL-13 antibody comprising a VH sequence of SEQ ID NO: 49 and a VL sequence of SEQ ID NO: 48.

[0163] In some embodiments, an anti-IL-13 antibody according to any of the above embodiments is a monoclonal antibody, including a chimeric, humanized or human antibody. In some embodiments, an anti-IL-13 antibody is an antibody fragment, e.g., a Fv, Fab, Fab', scFv, diabody, or $F(ab')_2$ fragment. In some embodiments, the antibody is a full length

antibody, e.g., an intact IgG1 or IgG4 antibody or other antibody class or isotype as defined herein.

[0164] In some embodiments, an anti-IL-13 antibody according to any of the above embodiments may incorporate any of the features, singly or in combination, as described in Sections 1-7 below.

Exemplary Anti-IL-4/IL-13 Bispecific Antibodies

[0165] In some embodiments, a multispecific antibody (such as a bispecific antibody) comprising an antigen-binding domain that specifically binds to IL-4 and IL-13 is provided. In some embodiments, the antigen-binding domain does not specifically bind to other targets. The multispecific antibody that binds IL-4 and IL-13 may comprise a first set of variable regions (VH and VL; also referred to as a VH/VL unit) according to any of the embodiments described herein for anti-IL-4 antibodies, and a second set of variable regions (VH and VL; also referred to as a VH/VL unit) according to any of the embodiments described herein for anti-IL-13 antibodies.

[0166] In some embodiments, the multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where the antibody comprises a first VH/VL unit comprising a VH (heavy chain variable domain) comprising the amino acid sequence of SEQ ID NO: 9. In some embodiments, the multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where the antibody comprises a first VH/VL unit comprising a VL (light chain variable domain) comprising the amino acid sequence of SEQ ID NO: 10. In some embodiments, the multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where the antibody comprises a first VH/VL unit comprising a VH comprising the amino acid sequence of SEQ ID NO: 9 and a VL comprising the amino acid sequence of SEQ ID NO: 10. In some embodiments, the multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where the antibody comprises a first VH/VL unit that competes for binding to IL-4 with an antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 9 and a VL comprising the amino acid sequence of SEQ ID NO: 10.

[0167] In some embodiments, the multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where the antibody comprises a second VH/VL unit comprising a VH (heavy chain variable domain) comprising the amino acid sequence of SEQ ID NO: 19 or SEQ ID NO: 56. In some embodiments, the multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where

the antibody comprises a second VH/VL unit comprising a VL (light chain variable domain) comprising the amino acid sequence of SEQ ID NO: 20 or SEQ ID NO: 57. In some embodiments, the multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where the antibody comprises a second VH/VL unit comprising a VH comprising the amino acid sequence of SEQ ID NO: 19 or SEQ ID NO: 56 and a VL comprising the amino acid sequence of SEQ ID NO: 20 or SEQ ID NO: 57. In some embodiments, the multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where the antibody comprises a second VH/VL unit that competes for binding to IL-13 with an antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 19 and a VL comprising the amino acid sequence of SEQ ID NO: 20. In some embodiments, the multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where the antibody comprises a second VH/VL unit that binds an epitope of IL-13 consisting of amino acids 82 to 89 of SEQ ID NO: 29. In some embodiments, the multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where the antibody comprises a second VH/VL unit that binds an epitope of IL-13 consisting of amino acids 77 to 89 of SEQ ID NO: 29.

[0168] In some embodiments, the multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where the antibody comprises a second VH/VL unit comprising a VH (heavy chain variable domain) comprising the amino acid sequence of SEQ ID NO: 49. In some embodiments, the multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where the antibody comprises a second VH/VL unit comprising a VL (light chain variable domain) comprising the amino acid sequence of SEQ ID NO: 48. In some embodiments, the multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where the antibody comprises a second VH/VL unit comprising a VH comprising the amino acid sequence of SEQ ID NO: 49 and a VL comprising the amino acid sequence of SEQ ID NO: 48. In some embodiments, the multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where the antibody comprises a second VH/VL unit that competes for binding to IL-13 with an antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 49 and a VL comprising the amino acid sequence of SEQ ID NO: 48.

[0169] In some embodiments, the multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where the antibody comprises a first VH/VL

unit comprising a first VH comprising the amino acid sequence of SEQ ID NO: 9 and a first VL comprising the amino acid sequence of SEQ ID NO: 10; and comprises a second VH/VL unit comprising a second VH comprising the amino acid sequence of SEQ ID NO: 19 or SEQ ID NO: 56 and a second VL comprising the amino acid sequence of SEQ ID NO: 20 or SEQ ID NO: 57.

[0170] In some embodiments, the multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where the antibody comprises a first VH/VL unit comprising a first VH comprising the amino acid sequence of SEQ ID NO: 9 and a first VL comprising the amino acid sequence of SEQ ID NO: 10; and comprises a second VH/VL unit comprising a second VH comprising the amino acid sequence of SEQ ID NO: 49 and a second VL comprising the amino acid sequence of SEQ ID NO: 48.

[0171] In some embodiments, the multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 wherein the antibody comprises a first VH/VL unit comprising a VH having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 9 and a VL having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 10. In some embodiments, the multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where the antibody comprises a second VH/VL unit comprising a VH having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 19 and a VL having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 20. In some embodiments, the multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where the antibody comprises a second VH/VL unit comprising a VH having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 49 and a VL having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 48. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in the sequences above. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs).

[0172] In some embodiments, the multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 wherein the antibody comprises a first

VH/VL unit comprising a first VH having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 9 and a first VL having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 10; and a second VH/VL unit comprising a second VH having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 19 and a second VL having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 20. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in the sequences above. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs).

[0173] In some embodiments, the multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 wherein the antibody comprises a first VH/VL unit comprising a first VH having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 9 and a first VL having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 10; and a second VH/VL unit comprising a second VH having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 49 and a second VL having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 48. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in the sequences above. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs).

[0174] In some embodiments, a multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where the antibody comprises a first VH/VL unit comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 12; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 18; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 14; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 15; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 16; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 17. In some embodiments, a multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4

and IL-13 where the antibody comprises a second VH/VL unit comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 21 or SEQ ID NO: 60; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 24; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 25; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, a multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where the antibody comprises a second VH/VL unit comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 50; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 51; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 52; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 53; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 54; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 55.

[0175] In some embodiments, a multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where the antibody comprises a first VH/VL unit comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 12; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 18; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 14; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 15; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 16; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 17; and a second VH/VL unit comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 21 or SEQ ID NO: 60; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 24; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 25; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26.

[0176] In some embodiments, a multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where the antibody comprises a first VH/VL unit comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 12; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 18; (c) HVR-H3 comprising the amino acid

sequence of SEQ ID NO: 14; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 15; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 16; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 17; and a second VH/VL unit comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 50; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 51; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 52; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 53; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 54; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 55.

[0177] In some embodiments, a multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where the antibody comprises a first VH/VL unit comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 12; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 18; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 14. In some embodiments, a multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where the antibody comprises a second VH/VL unit comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 21 or SEQ ID NO: 60; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23. In some embodiments, a multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where the antibody comprises a second VH/VL unit comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 50; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 51; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 52.

[0178] In some embodiments, a multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where the antibody comprises a first VH/VL unit comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 12; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 18; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 14; and a second VH/VL unit comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino

acid sequence of SEQ ID NO: 21 or SEQ ID NO: 60; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23. In some embodiments, a multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where the antibody comprises a first VH/VL unit comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 12; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 18; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 14; and a second VH/VL unit comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 50; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 51; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 52.

[0179] In some embodiments, a multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where the antibody comprises a first VH/VL unit comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 15; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 16; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 17. In some embodiments, a multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where the antibody comprises a second VH/VL unit comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 24; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 25; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, a multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where the antibody comprises a second VH/VL unit comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 53; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 54; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 55.

[0180] In some embodiments, a multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where the antibody comprises a first VH/VL unit comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 15; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 16; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 17; and a second VH/VL unit comprising at least one, at least two,

or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 24; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 25; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, a multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where the antibody comprises a first VH/VL unit comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 15; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 16; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 17; and a second VH/VL unit comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 53; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 54; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 55.

[0181] In some embodiments, a multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where the antibody comprises a first VH/VL unit comprising three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 12; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 18; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 14; and three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 15; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 16; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 17. In some embodiments, a multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where the antibody comprises a first VH/VL unit comprising three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 12; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 13; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 14; and three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 15; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 16; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 17.

[0182] In some embodiments, a multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where the antibody comprises a second VH/VL unit comprising three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 21 or SEQ ID NO: 60; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22; (c) HVR-H3 comprising the amino acid sequence of

SEQ ID NO: 23; and three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 24; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 25; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26.

[0183] In some embodiments, a multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where the antibody comprises a second VH/VL unit comprising three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 50; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 51; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 52; and three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 53; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 54; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 55.

[0184] In some embodiments, a multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where the antibody comprises a first VH/VL unit comprising three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 12; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 18; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 14; and three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 15; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 16; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 17; and a second VH/VL unit comprising three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 21 or SEQ ID NO: 60; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23; and three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 24; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 25; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26.

[0185] In some embodiments, a multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where the antibody comprises a first VH/VL unit comprising three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 12; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 18; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 14; and three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 15; (b) HVR-L2 comprising the amino acid sequence of SEQ ID

NO: 16; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 17; and a second VH/VL unit comprising three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 50; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 51; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 52; and three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 53; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 54; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 55.

[0186] In some embodiments, a multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where the antibody comprises a first VH/VL unit comprising three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 12; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 13; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 14; and three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 15; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 16; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 17; and a second VH/VL unit comprising three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 21 or SEQ ID NO: 60; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23; and three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 24; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 25; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26.

[0187] In some embodiments, a multispecific antibody comprises an antigen-binding domain that specifically binds to IL-4 and IL-13 where the antibody comprises a first VH/VL unit comprising three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 12; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 13; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 14; and three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 15; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 16; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 17; and a second VH/VL unit comprising three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 50; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 51; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 52; and three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID

NO: 53; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 54; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 55.

[0188] In various embodiments, a multispecific antibody comprises a first hemimer comprising a first VH/VL unit that binds IL-4, wherein the first hemimer comprises a knob mutation in the heavy chain constant region, and a second hemimer comprising a second VH/VL unit that binds IL-13, wherein the second hemimer comprises a hole mutation in the heavy chain constant region. In various embodiments, a multispecific antibody comprises a first hemimer comprising a first VH/VL unit that binds IL-4, wherein the first hemimer comprises a hole mutation in the heavy chain constant region, and a second hemimer comprising a second VH/VL unit that binds IL-13, wherein the second hemimer comprises a knob mutation in the heavy chain constant region. In some embodiments, a heavy chain constant region comprising a hole mutation has the sequence shown in SEQ ID NO: 35 (IgG1) or SEQ ID NO: 37 (IgG4). In some embodiments, a heavy chain constant region comprising a knob mutation has the sequence shown in SEQ ID NO: 34 (IgG1) or SEQ ID NO: 36 (IgG4). In some embodiments, a multispecific antibody comprises a first hemimer comprising a first heavy chain having the sequence of SEQ ID NO: 38 and a first light chain having the sequence of SEQ ID NO: 39, and a second hemimer comprising a second heavy chain having the sequence of SEQ ID NO: 40 or 58 and a second light chain having the sequence of SEQ ID NO: 41 or 59. In some embodiments, a multispecific antibody comprises a first hemimer comprising a first heay chain having the sequence of SEQ ID NO: 38 and a first light chain having the sequence of SEQ ID NO: 39, and a second hemimer comprising a second heavy chain having the sequence of SEQ ID NO: 40 and a second light chain having the sequence of SEQ ID NO: 41.

[0189] In some embodiments, an anti-IL-4/IL-13 multispecific antibody according to any of the above embodiments is a monoclonal antibody, including a chimeric, humanized or human antibody. In some embodiments, an anti-IL-4/IL-13 multispecific antibody is an antibody fragment, e.g., a Fv, Fab, Fab', scFv, diabody, or $F(ab')_2$ fragment. In some embodiments, the antibody is a full length antibody, e.g., an intact IgG1 or IgG4 antibody or other antibody class or isotype as defined herein.

[0190] In some embodiments, an anti-IL-4/IL-13 multispecific antibody according to any of the above embodiments may incorporate any of the features, singly or in combination, as described in Sections 1-7 below.

1. Antibody Affinity

[0191] In certain embodiments, an antibody provided herein has a dissociation constant (Kd) for an antigen of ≤ 1μM, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM (e.g. 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M, e.g., from 10^{-9} M to 10^{-13} M).

[0192] In some embodiments, Kd is measured by a radiolabeled antigen binding assay (RIA). In some embodiments, an RIA is performed with the Fab version of an antibody of interest and its antigen. For example, solution binding affinity of Fabs for antigen is measured by equilibrating Fab with a minimal concentration of (^{125}I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (*see, e.g.*, Chen et al., *J. Mol. Biol.* 293:865-881(1999)). To establish conditions for the assay, MICROTITER® multi-well plates (Thermo Scientific) are coated overnight with 5 μg/ml of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23°C). In a non-adsorbent plate (Nunc #269620), 100 pM or 26 pM [^{125}I]-antigen are mixed with serial dilutions of a Fab of interest (e.g., consistent with assessment of the anti-VEGF antibody, Fab-12, in Presta et al., *Cancer Res.* 57:4593-4599 (1997)). The Fab of interest is then incubated overnight; however, the incubation may continue for a longer period (e.g., about 65 hours) to ensure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature (e.g., for one hour). The solution is then removed and the plate washed eight times with 0.1% polysorbate 20 (TWEEN-20®) in PBS. When the plates have dried, 150 μl/well of scintillant (MICROSCINT-20 ™; Packard) is added, and the plates are counted on a TOPCOUNT ™ gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

[0193] According to some embodiments, Kd is measured using a BIACORE® surface plasmon resonance assay. For example, an assay using a BIACORE®-2000 or a BIACORE®-3000 (BIAcore, Inc., Piscataway, NJ) is performed at 25°C with immobilized antigen CM5 chips at ~10 response units (RU). In some embodiments, carboxymethylated dextran biosensor chips (CM5, BIACORE, Inc.) are activated with *N*-ethyl-*N'*- (3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5 μg/ml (~0.2 μM) before injection at a flow rate of 5 μl/minute to achieve

approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% polysorbate 20 (TWEEN-20™) surfactant (PBST) at 25°C at a flow rate of approximately 25 μl/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIACORE® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensograms. The equilibrium dissociation constant (Kd) is calculated as the ratio k_{off}/k_{on}. *See, e.g.*, Chen et al., *J. Mol. Biol.* 293:865-881 (1999). If the on-rate exceeds 10^6 M^{-1} s^{-1} by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm band-pass) at 25°C of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophometer (Aviv Instruments) or a 8000-series SLM-AMINCO™ spectrophotometer (ThermoSpectronic) with a stirred cuvette.

2. Antibody Fragments

[0194] In certain embodiments, an antibody provided herein is an antibody fragment. Antibody fragments include, but are not limited to, Fab, Fab', Fab'-SH, $F(ab')_2$, Fv, and scFv fragments, and other fragments described below. For a review of certain antibody fragments, see Hudson et al. *Nat. Med.* 9:129-134 (2003). For a review of scFv fragments, *see, e.g.*, Pluckthün, in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., (Springer-Verlag, New York), pp. 269-315 (1994); see also WO 93/16185; and U.S. Patent Nos. 5,571,894 and 5,587,458. For discussion of Fab and $F(ab')_2$ fragments comprising salvage receptor binding epitope residues and having increased *in vivo* half-life, see U.S. Patent No. 5,869,046.

[0195] Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific. See, for example, EP 404,097; WO 1993/01161; Hudson et al., *Nat. Med.* 9:129-134 (2003); and Hollinger et al., *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al., *Nat. Med.* 9:129-134 (2003).

[0196] Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an

antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; *see, e.g.*, U.S. Patent No. 6,248,516 B1).

[0197] Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (e.g. *E. coli* or phage), as described herein.

3. Chimeric and Humanized Antibodies

[0198] In certain embodiments, an antibody provided herein is a chimeric antibody. Certain chimeric antibodies are described, e.g., in U.S. Patent No. 4,816,567; and Morrison et al., *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). In one example, a chimeric antibody comprises a non-human variable region (e.g., a variable region derived from a mouse, rat, hamster, rabbit, or non-human primate, such as a monkey) and a human constant region. In a further example, a chimeric antibody is a "class switched" antibody in which the class or subclass has been changed from that of the parent antibody. Chimeric antibodies include antigen-binding fragments thereof.

[0199] In certain embodiments, a chimeric antibody is a humanized antibody. Typically, a non-human antibody is humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which HVRs, e.g., CDRs, (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (e.g., the antibody from which the HVR residues are derived), e.g., to restore or improve antibody specificity or affinity.

[0200] Humanized antibodies and methods of making them are reviewed, e.g., in Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008), and are further described, e.g., in Riechmann et al., *Nature* 332:323-329 (1988); Queen et al., *Proc. Nat'l Acad. Sci. USA* 86:10029-10033 (1989); US Patent Nos. 5, 821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri *et al.*, *Methods* 36:25-34 (2005) (describing specificity determining region (SDR) grafting); Padlan, *Mol. Immunol.* 28:489-498 (1991) (describing "resurfacing"); Dall'Acqua et al., *Methods* 36:43-60 (2005) (describing "FR shuffling"); and Osbourn et al., *Methods* 36:61-68 (2005) and Klimka et al., *Br. J. Cancer*, 83:252-260 (2000) (describing the "guided selection" approach to FR shuffling).

[0201] Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the "best-fit" method (*see, e.g.*, Sims et al. *J. Immunol.* 151 :2296 (1993)); framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (*see, e.g.*, Carter et al. *Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); and Presta et al. *J. Immunol.*, 151:2623 (1993)); human mature (somatically mutated) framework regions or human germline framework regions (*see, e.g.*, Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008)); and framework regions derived from screening FR libraries (*see, e.g.*, Baca et al., *J. Biol. Chem.* 272:10678-10684 (1997) and Rosok et al., *J. Biol. Chem.* 271:22611-22618 (1996)).

4. Human Antibodies

[0202] In certain embodiments, an antibody provided herein is a human antibody. Human antibodies can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel, *Curr. Opin. Pharmacol.* 5: 368-74 (2001) and Lonberg, *Curr. Opin. Immunol.* 20:450-459 (2008).

[0203] Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic mice, the endogenous immunoglobulin loci have generally been inactivated. For review of methods for obtaining human antibodies from transgenic animals, see Lonberg, *Nat. Biotech.* 23:1117-1125 (2005). See also, e.g., U.S. Patent Nos. 6,075,181 and 6,150,584 describing XENOMOUSE™ technology; U.S. Patent No. 5,770,429 describing HUMAB® technology; U.S. Patent No. 7,041,870 describing K-M MOUSE® technology, and U.S. Patent Application Publication No. US 2007/0061900, describing VELOCIMOUSE® technology). Human variable regions from intact antibodies generated by such animals may be further modified, e.g., by combining with a different human constant region.

[0204] Human antibodies can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described. (*See, e.g.*, Kozbor *J. Immunol.*, 133: 3001 (1984); Brodeur et al., *Monoclonal Antibody Production Techniques and Applications*, pp.

51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner et al., *J. Immunol.*, 147: 86 (1991).) Human antibodies generated via human B-cell hybridoma technology are also described in Li et al., *Proc. Natl. Acad. Sci. USA*, 103:3557-3562 (2006). Additional methods include those described, for example, in U.S. Patent No. 7,189,826 (describing production of monoclonal human IgM antibodies from hybridoma cell lines) and Ni, *Xiandai Mianyixue*, 26(4):265-268 (2006) (describing human-human hybridomas). Human hybridoma technology (Trioma technology) is also described in Vollmers and Brandlein, *Histology and Histopathology*, 20(3):927-937 (2005) and Vollmers and Brandlein, *Methods and Findings in Experimental and Clinical Pharmacology*, 27(3):185-91 (2005).

[0205] Human antibodies may also be generated by isolating Fv clone variable domain sequences selected from human-derived phage display libraries. Such variable domain sequences may then be combined with a desired human constant domain. Techniques for selecting human antibodies from antibody libraries are described below.

5. Library-Derived Antibodies

[0206] Antibodies described herein may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, e.g., in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, 2001) and further described, e.g., in the McCafferty et al., *Nature* 348:552-554; Clackson et al., *Nature* 352: 624-628 (1991); Marks et al., *J. Mol. Biol.* 222: 581-597 (1992); Marks and Bradbury, in *Methods in Molecular Biology* 248:161-175 (Lo, ed., Human Press, Totowa, NJ, 2003); Sidhu et al., *J. Mol. Biol.* 338(2): 299-310 (2004); Lee et al., *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee et al., *J. Immunol. Methods* 284(1-2): 119-132(2004).

[0207] In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter et al., *Ann. Rev. Immunol.*, 12: 433-455 (1994). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (e.g., from human) to provide a single source of antibodies to a wide range of non-self and also self antigens without any

immunization as described by Griffiths et al., *EMBO J,* 12: 725-734 (1993). Finally, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement *in vitro*, as described by Hoogenboom and Winter, *J. Mol. Biol.*, 227: 381-388 (1992). Patent publications describing human antibody phage libraries include, for example: US Patent No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

[0208] Antibodies or antibody fragments isolated from human antibody libraries are considered human antibodies or human antibody fragments herein.

6. Multispecific Antibodies

[0209] In certain embodiments, an antibody provided herein is a multispecific antibody, e.g. a bispecific antibody. Multispecific antibodies are monoclonal antibodies that have binding specificities for at least two different sites. In certain embodiments, one of the binding specificities is for IL-4 and the other is for any other antigen. In certain embodiments, one of the binding specificities is for IL-4 and the other is IL-13. In certain embodiments, bispecific antibodies may bind to two different epitopes of IL-4. Bispecific antibodies may also be used to localize cytotoxic agents to cells. Bispecific antibodies can be prepared as full length antibodies or antibody fragments.

[0210] Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (see Milstein and Cuello, *Nature* 305: 537 (1983)), WO 93/08829, and Traunecker et al., *EMBO J.* 10: 3655 (1991)), and "knob-in-hole" engineering (*see, e.g.*, U.S. Patent No. 5,731,168; U.S. Publication No. 2011/0287009). Multi-specific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004A1); cross-linking two or more antibodies or fragments (*see, e.g.*, US Patent No. 4,676,980, and Brennan et al., *Science*, 229: 81 (1985)); using leucine zippers to produce bispecific antibodies (*see, e.g.*, Kostelny et al., *J. Immunol.*, 148(5):1547-1553 (1992)); using a furin cleavable tether between a C_L domain and a V_H domain in a single VH/VL unit (*see, e.g.*, International Patent App. No. PCT/US2012/059810); using "diabody" technology for making bispecific antibody fragments (*see, e.g.*, Hollinger et al., *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993)); and using

single-chain Fv (sFv) dimers (*see, e.g.* Gruber et al., *J. Immunol.*, 152:5368 (1994)); and preparing trispecific antibodies as described, e.g., in Tutt et al. *J. Immunol.* 147: 60 (1991).

[0211] Engineered antibodies with three or more functional antigen binding sites, including "Octopus antibodies," are also included herein (see, e.g. US 2006/0025576A1).

[0212] The antibody or fragment herein also includes a "Dual Acting FAb" or "DAF" comprising an antigen binding site that binds to IL-4 as well as another, different antigen, such as IL-13 (see, US 2008/0069820, for example).

Knobs into Holes

[0213] The use of knobs into holes as a method of producing multispecific antibodies is described, e.g., in U.S. Pat. No. 5,731,168, WO2009/089004, US2009/0182127, US2011/0287009, Marvin and Zhu, *Acta Pharmacol. Sin.* (2005) 26(6):649-658, and Kontermann (2005) *Acta Pharmacol. Sin.*, 26:1-9. A brief nonlimiting discussion is provided below.

[0214] A "protuberance" refers to at least one amino acid side chain which projects from the interface of a first polypeptide and is therefore positionable in a compensatory cavity in the adjacent interface (i.e. the interface of a second polypeptide) so as to stabilize the heteromultimer, and thereby favor heteromultimer formation over homomultimer formation, for example. The protuberance may exist in the original interface or may be introduced synthetically (e.g. by altering nucleic acid encoding the interface). In some embodiments, nucleic acid encoding the interface of the first polypeptide is altered to encode the protuberance. To achieve this, the nucleic acid encoding at least one "original" amino acid residue in the interface of the first polypeptide is replaced with nucleic acid encoding at least one "import" amino acid residue which has a larger side chain volume than the original amino acid residue. It will be appreciated that there can be more than one original and corresponding import residue. The side chain volumes of the various amino residues are shown, for example, in Table 1 of US2011/0287009.

[0215] In some embodiments, import residues for the formation of a protuberance are naturally occurring amino acid residues selected from arginine (R), phenylalanine (F), tyrosine (Y) and tryptophan (W). In some embodiments, an import residue is tryptophan or tyrosine. In some embodiment, the original residue for the formation of the protuberance has a small side chain volume, such as alanine, asparagine, aspartic acid, glycine, serine, threonine or valine.

[0216] A "cavity" refers to at least one amino acid side chain which is recessed from the interface of a second polypeptide and therefore accommodates a corresponding protuberance on the adjacent interface of a first polypeptide. The cavity may exist in the original interface or may be introduced synthetically (e.g. by altering nucleic acid encoding the interface). In some embodiments, nucleic acid encoding the interface of the second polypeptide is altered to encode the cavity. To achieve this, the nucleic acid encoding at least one "original" amino acid residue in the interface of the second polypeptide is replaced with DNA encoding at least one "import" amino acid residue which has a smaller side chain volume than the original amino acid residue. It will be appreciated that there can be more than one original and corresponding import residue. In some embodiments, import residues for the formation of a cavity are naturally occurring amino acid residues selected from alanine (A), serine (S), threonine (T) and valine (V). In some embodiments, an import residue is serine, alanine or threonine. In some embodiments, the original residue for the formation of the cavity has a large side chain volume, such as tyrosine, arginine, phenylalanine or tryptophan.

[0217] The protuberance is "positionable" in the cavity which means that the spatial location of the protuberance and cavity on the interface of a first polypeptide and second polypeptide respectively and the sizes of the protuberance and cavity are such that the protuberance can be located in the cavity without significantly perturbing the normal association of the first and second polypeptides at the interface. Since protuberances such as Tyr, Phe and Trp do not typically extend perpendicularly from the axis of the interface and have preferred conformations, the alignment of a protuberance with a corresponding cavity may, in some instances, rely on modeling the protuberance/cavity pair based upon a three-dimensional structure such as that obtained by X-ray crystallography or nuclear magnetic resonance (NMR). This can be achieved using widely accepted techniques in the art.

[0218] In some embodiments, a knob mutation in an IgG1 constant region is T366W. In some embodiments, a hole mutation in an IgG1 constant region comprises one or more mutations selected from T366S, L368A and Y407V. In some embodiments, a hole mutation in an IgG1 constant region comprises T366S, L368A and Y407V. SEQ ID NO: 34 shows an exemplary IgG1 constant region with a knob mutation and SEQ ID NO: 35 shows an exemplary IgG1 constant region with a hole mutation.

[0219] In some embodiments, a knob mutation in an IgG4 constant region is T366W. In some embodiments, a hole mutation in an IgG4 constant region comprises one or more mutations selected from T366S, L368A, and Y407V. In some embodiments, a hole mutation

in an IgG4 constant region comprises T366S, L368A, and Y407V. SEQ ID NO: 36 shows an exemplary IgG4 constant region with a knob mutation and SEQ ID NO: 37 shows an exemplary IgG4 constant region with a hole mutation.

7. Antibody Variants

[0220] In certain embodiments, amino acid sequence variants of the antibodies provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, e.g., antigen-binding.

Substitution, Insertion, and Deletion Variants

[0221] In certain embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the HVRs and FRs. Conservative substitutions are shown in Table 1 under the heading of "conservative substitutions." More substantial changes are provided in Table 1 under the heading of "exemplary substitutions," and as further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, e.g., retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

TABLE 1

Original Residue	Exemplary Substitutions	Conservative Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala

Original Residue	Exemplary Substitutions	Conservative Substitutions
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

[0222] Amino acids may be grouped according to common side-chain properties:

(1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

(2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

(3) acidic: Asp, Glu;

(4) basic: His, Lys, Arg;

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

(6) aromatic: Trp, Tyr, Phe.

[0223] Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

[0224] One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (e.g. a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (e.g., improvements) in certain biological properties (e.g., increased affinity, reduced immunogenicity) relative to the parent antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, e.g., using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more HVR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (e.g. binding affinity).

[0225] Alterations (e.g., substitutions) may be made in HVRs, e.g., to improve antibody affinity. Such alterations may be made in HVR "hotspots," i.e., residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (*see, e.g.*, Chowdhury, *Methods Mol. Biol.* 207:179-196 (2008)), and/or SDRs (a-CDRs), with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, e.g., in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, (2001).) In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (e.g., error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues (e.g., 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, e.g., using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

[0226] In certain embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody to bind antigen. For example, conservative alterations (e.g., conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. Such alterations may be outside of HVR "hotspots" or SDRs. In certain embodiments of the variant VH and VL sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

[0227] A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) Science, 244:1081-1085. In this method, a residue or group of target residues (e.g., charged residues such as arg, asp, his, lys, and glu) are identified and replaced by a neutral or negatively charged amino acid (e.g., alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

[0228] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (e.g. for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

Glycosylation variants

[0229] In certain embodiments, an antibody provided herein is altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

[0230] Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. *See, e.g.*, Wright et al. *TIBTECH* 15:26-32 (1997). The oligosaccharide may include various carbohydrates, e.g., mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an antibody provided herein may be made in order to create antibody variants with certain improved properties.

[0231] In some embodiments, antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (e. g. complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (Eu numbering of Fc region residues); however, Asn297 may also be located about ± 3 amino acids upstream or downstream of position 297, i.e., between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. *See, e.g.,* US Patent Publication Nos. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to "defucosylated" or "fucose-deficient" antibody

variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki et al. *J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. *Arch. Biochem. Biophys.* 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams et al., especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, FUT8, knockout CHO cells (*see, e.g.*, Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004); Kanda, Y. et al., *Biotechnol. Bioeng.*, 94(4):680-688 (2006); and WO2003/085107).

[0232] Antibodies variants are further provided with bisected oligosaccharides, e.g., in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, e.g., in WO 2003/011878 (Jean-Mairet et al.); US Patent No. 6,602,684 (Umana et al.); and US 2005/0123546 (Umana et al.). Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, e.g., in WO 1997/30087 (Patel et al.); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

Fc region variants

[0233] In certain embodiments, one or more amino acid modifications may be introduced into the Fc region of an antibody provided herein, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (e.g., a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (e.g. a substitution) at one or more amino acid positions.

[0234] In some embodiments, and antibody constant region, such as a heavy chain constant region, comprises a knob mutation and/or a hole mutation to facilitate formation of a multispecific antibody. Nonlimiting exemplary knob mutations and hole mutations, and knob-into-hole technology generally, are described, for example, in U.S. Pat. No. 5,731,168, WO2009/089004, US2009/0182127, US2011/0287009, Marvin and Zhu, *Acta Pharmacol. Sin.* (2005) 26(6):649-658, and Kontermann (2005) *Acta Pharmacol. Sin.*, 26:1-9. Certain nonlimiting exemplary knob mutations and hole mutations are discussed herein.

[0235] In certain embodiments, an antibody variant that possesses some but not all effector functions is provided, which make it a desirable candidate for applications in which the half-life of the antibody *in vivo* is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (1991). Non-limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest is described in U.S. Patent No. 5,500,362 (*see, e.g.* Hellstrom, I. et al. *Proc. Nat'l Acad. Sci. USA* 83:7059-7063 (1986)) and Hellstrom, I et al., *Proc. Nat'l Acad. Sci. USA* 82:1499-1502 (1985); 5,821,337 (see Bruggemann, M. et al., *J. Exp. Med.* 166:1351-1361 (1987)). Alternatively, non-radioactive assays methods may be employed (see, for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA; and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, WI). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, e.g., in an animal model such as that disclosed in Clynes et al. *Proc. Nat'l Acad. Sci. USA* 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. *See, e.g.*, C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (*see, e.g.*, Gazzano-Santoro et al., *J. Immunol. Methods* 202:163 (1996); Cragg, M.S. et al., *Blood* 101:1045-1052 (2003); and Cragg, M.S. and M.J. Glennie, *Blood* 103:2738-2743 (2004)). FcRn binding and *in vivo* clearance/half-life determinations can also be performed using methods known in the art (*see, e.g.*, Petkova, S.B. et al., *Int'l. Immunol.* 18(12):1759-1769 (2006)).

[0236] Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581).

[0237] Certain antibody variants with improved or diminished binding to FcRs are described. (*See, e.g.,* U.S. Patent No. 6,737,056; WO 2004/056312, and Shields et al., *J. Biol. Chem.* 9(2): 6591-6604 (2001).)

[0238] In certain embodiments, an antibody variant comprises an Fc region with one or more amino acid substitutions which improve ADCC, e.g., substitutions at positions 298, 333, and/or 334 of the Fc region (EU numbering of residues).

[0239] In some embodiments, alterations are made in the Fc region that result in altered (i.e., either improved or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), e.g., as described in US Patent No. 6,194,551, WO 99/51642, and Idusogie et al. *J. Immunol.* 164: 4178-4184 (2000).

[0240] Antibodies with increased half-lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., *J. Immunol.* 117:587 (1976) and Kim et al., *J. Immunol.* 24:249 (1994)), are described in US2005/0014934A1 (Hinton et al.). Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, e.g., substitution of Fc region residue 434 (US Patent No. 7,371,826).

[0241] *See also* Duncan & Winter, *Nature* 322:738-40 (1988); U.S. Patent No. 5,648,260; U.S. Patent No. 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

[0242] In some embodiments, an antibody constant region comprises more than one of the mutations discussed herein (for example, a knob and/or hole mutation and/or a mutation that increases stability and/or a mutation that decreases ADCC, etc.).

Cysteine engineered antibody variants

[0243] In certain embodiments, it may be desirable to create cysteine engineered antibodies, e.g., "thioMAbs," in which one or more residues of an antibody are substituted with cysteine residues. In particular embodiments, the substituted residues occur at accessible sites of the antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate, as described further herein. In certain embodiments, any one or more of the following residues may be substituted with cysteine: V205 (Kabat numbering) of the light chain; A118 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy chain Fc region.

Cysteine engineered antibodies may be generated as described, e.g., in U.S. Patent No. 7,521,541.

Antibody Derivatives

[0244] In certain embodiments, an antibody provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers, prolypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer is attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc.

[0245] In some embodiments, conjugates of an antibody and nonproteinaceous moiety that may be selectively heated by exposure to radiation are provided. In some embodiments, the nonproteinaceous moiety is a carbon nanotube (Kam et al., *Proc. Natl. Acad. Sci.* USA 102: 11600-11605 (2005)). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the nonproteinaceous moiety to a temperature at which cells proximal to the antibody-nonproteinaceous moiety are killed.

Recombinant Methods and Compositions

[0246] Antibodies may be produced using recombinant methods and compositions, e.g., as described in U.S. Patent No. 4,816,567. In some embodiments, isolated nucleic acid encoding an anti-IL-4 antibody described herein is provided. In some embodiments, isolated nucleic acid encoding an anti-IL-13 antibody described herein is provided. In some embodiments, isolated nucleic acid encoding an anti-IL-4/IL-13 bispecific/ antibody described herein is provided. Such nucleic acids may encode an amino acid sequence

comprising the VL and/or an amino acid sequence comprising the VH of the antibody (e.g., the light and/or heavy chains of the antibody). In some embodiments, one or more vectors (e.g., expression vectors) comprising such nucleic acid are provided. In some embodiments, a host cell comprising such nucleic acid is provided. In one such embodiment, a host cell comprises (e.g., has been transformed with): (1) a vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and an amino acid sequence comprising the VH of the antibody, or (2) a first vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and a second vector comprising a nucleic acid that encodes an amino acid sequence comprising the VH of the antibody.

[0247] In some embodiments, the host cell is eukaryotic, e.g. a Chinese Hamster Ovary (CHO) cell or lymphoid cell (e.g., Y0, NS0, Sp20 cell). In some embodiments, a method of making an antibody is provided, wherein the method comprises culturing a host cell comprising nucleic acid encoding the antibody, as provided above, under conditions suitable for expression of the antibody, and optionally recovering the antibody from the host cell (or host cell culture medium).

[0248] In some embodiments, a method of making a multispecific antibody is provided, wherein the method comprises culturing in a host cell comprising nucleic acid encoding the multispecific antibody under conditions suitable for expression of the antibody, and optionally recovering the multispecific antibody from the host cell (or host cell culture medium). In some embodiments, a method of making a multispecific antibody is provided, wherein the method comprises culturing a first host cell comprising nucleic acid encoding a first VH/VL unit of the multispecific antibody (including constant region, if any, sometimes referred to as a "hemimer" or "half-antibody") under conditions suitable for expression of the first VH/VL unit, and optionally recovering the first VH/VL unit from the host cell (or host cell culture medium), and culturing a second host cell comprising nucleic acid encoding a second VH/VL unit of the multispecific antibody (including constant region, if any) under conditions suitable for expression of the second VH/VL unit, and optionally recovering the second VH/VL unit from the host cell (or host cell culture medium). In some embodiments, the method further comprises assembling the multispecific antibody from an isolated first VH/VL unit and an isolated second VH/VL unit. Such assembly may comprise, in some embodiments, a redox step to form intramolecular disulfides between the two VH/VL units (or hemimers). Nonlimiting exemplary methods of producing multispecific antibodies are

described, e.g., in US 2011/0287009, US 2007/0196363, US2007/0178552, U.S. Patent No. 5,731,168, WO 96/027011, WO 98/050431, and Zhu et al., 1997, *Protein Science* 6:781-788. A nonlimiting exemplary method is also described in the examples below.

[0249] For recombinant production of an anti-IL-4 antibody or anti-IL-4/IL-13 bispecific antibody, nucleic acid encoding an antibody, e.g., as described above, is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acid may be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody).

[0250] Suitable host cells for cloning or expression of antibody-encoding vectors include prokaryotic or eukaryotic cells described herein. For example, antibodies may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For expression of antibody fragments and polypeptides in bacteria, *see, e.g.,* U.S. Patent Nos. 5,648,237, 5,789,199, and 5,840,523. (See also Charlton, *Methods in Molecular Biology, Vol. 248* (B.K.C. Lo, ed., Humana Press, Totowa, NJ, 2003), pp. 245-254, describing expression of antibody fragments in *E. coli*.) After expression, the antibody may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.

[0251] In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been "humanized," resulting in the production of an antibody with a partially or fully human glycosylation pattern. See Gerngross, *Nat. Biotech.* 22:1409-1414 (2004), and Li et al., *Nat. Biotech.* 24:210-215 (2006).

[0252] Suitable host cells for the expression of glycosylated antibody are also derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of *Spodoptera frugiperda* cells.

[0253] Plant cell cultures can also be utilized as hosts. *See,* e.g., US Patent Nos. 5,959,177; 6,040,498; 6,420,548; 7,125,978; and 6,417,429 (describing PLANTIBODIES™ technology for producing antibodies in transgenic plants).

[0254] Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host

cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293 cells as described, e.g., in Graham et al., *J. Gen Virol.* 36:59 (1977)); baby hamster kidney cells (BHK); mouse sertoli cells (TM4 cells as described, e.g., in Mather, *Biol. Reprod.* 23:243-251 (1980)); monkey kidney cells (CV1); African green monkey kidney cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK; buffalo rat liver cells (BRL 3A); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); TRI cells, as described, e.g., in Mather et al., *Annals N.Y. Acad. Sci.* 383:44-68 (1982); MRC 5 cells; and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including $DHFR^-$ CHO cells (Urlaub et al., *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); and myeloma cell lines such as Y0, NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, *see, e.g.*, Yazaki and Wu, *Methods in Molecular Biology, Vol. 248* (B.K.C. Lo, ed., Humana Press, Totowa, NJ), pp. 255-268 (2003).

Exemplary Assays

Binding assays and other assays

[0255] In some embodiments, an antibody provided herein is tested for its antigen binding activity, e.g., by known methods such as ELISA, Western blot, etc.

[0256] In some embodiments, competition assays may be used to identify an antibody that competes with an IL-4 antibody described herein for binding to IL-4. In some embodiments, competition assays may be used to identify an antibody that competes with an IL-4/IL-13 bispecific antibody described herein for binding to IL-4 and/or IL-13. In certain embodiments, such a competing antibody binds to the same epitope (e.g., a linear or a conformational epitope) that is bound by an antibody that comprises a VH amino acid sequence comprising SEQ ID NO: 9 and a VL amino acid sequence comprising SEQ ID NO: 10 for binding IL-4. In certain embodiments, such a competing antibody binds to the same epitope (e.g., a linear or a conformational epitope) that is bound by an antibody that comprises a VH amino acid sequence comprising SEQ ID NO: 19 and a VL amino acid sequence comprising SEQ ID NO: 20 for binding IL-13. In certain embodiments, such a competing antibody binds to the same epitope (e.g., a linear or a conformational epitope) that is bound by an antibody that comprises a VH amino acid sequence comprising SEQ ID NO: 49 and a VL amino acid sequence comprising SEQ ID NO: 48 for binding IL-13. Detailed exemplary methods for mapping an epitope to which an antibody binds are provided in

Morris (1996) "Epitope Mapping Protocols," in *Methods in Molecular Biology* vol. 66 (Humana Press, Totowa, NJ).

[0257] In an exemplary competition assay, immobilized IL-4 is incubated in a solution comprising a first labeled antibody that binds to IL-4 (e.g., an antibody that comprises a VH amino acid sequence comprising SEQ ID NO: 9 and a VL amino acid sequence comprising SEQ ID NO: 10) and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to IL-4. The second antibody may be present in a hybridoma supernatant. As a control, immobilized IL-4 is incubated in a solution comprising the first labeled antibody but not the second unlabeled antibody. After incubation under conditions permissive for binding of the first antibody to IL-4, excess unbound antibody is removed, and the amount of label associated with immobilized IL-4 is measured. If the amount of label associated with immobilized IL-4 is substantially reduced in the test sample relative to the control sample, then that indicates that the second antibody is competing with the first antibody for binding to IL-4. *See* Harlow and Lane (1988) *Antibodies: A Laboratory Manual* ch.14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

[0258] In a further exemplary competition assay, immobilized IL-13 is incubated in a solution comprising a first labeled antibody that binds to IL-13 (e.g., an antibody that comprises a VH amino acid sequence comprising SEQ ID NO: 19 and a VL amino acid sequence comprising SEQ ID NO: 20, or an antibody that comprises a VH amino acid sequence comprising SEQ ID NO: 49 and a VL amino acid sequence comprising SEQ ID NO: 48) and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to IL-13. The second antibody may be present in a hybridoma supernatant. As a control, immobilized IL-13 is incubated in a solution comprising the first labeled antibody but not the second unlabeled antibody. After incubation under conditions permissive for binding of the first antibody to IL-13, excess unbound antibody is removed, and the amount of label associated with immobilized IL-13 is measured. If the amount of label associated with immobilized IL-13 is substantially reduced in the test sample relative to the control sample, then that indicates that the second antibody is competing with the first antibody for binding to IL-13.

Activity assays

[0259] In some embodiments, assays are provided for identifying anti-IL-4 antibodies and anti-IL-4/IL-13 bispecific antibodies having biological activity. Biological activity may include, e.g., inhibition of IL-4 binding to an IL-4 receptor, inhibition of IL-4-induced STAT6

phosphorylation, inhibition of IL-4 induced cell proliferation, inhibition of IL-4-induced class switching of B cells to IgE, activity in asthma, and activity in IPF. In some embodiments, biological activities include, e.g., inhibition of IL-13 binding to an IL-13 receptor (for example, a heterodimeric receptor comprising IL-4Rα and IL-13Rα1), inhibition of IL-13-induced STAT6 phosphorylation, inhibition of IL-13-induced cell proliferation, inhibition of IL-13-induced class switching of B cells to IgE, inhibition of IL-13-induced mucus production, activity in asthma, and activity in IPF. Antibodies having such biological activity *in vivo* and/or *in vitro* are also provided. Nonlimiting exemplary assays for testing for such biological activities are described herein and/or are known in the art.

Immunoconjugates

[0260] In some embodiments, immunoconjugates comprising an anti-IL-4 antibody or an anti-IL-4/IL-13 bispecific antibody conjugated to one or more cytotoxic agents is provided. Nonlimiting exemplary such cytotoxic agents include chemotherapeutic agents or drugs, growth inhibitory agents, toxins (e.g., protein toxins, enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof), and radioactive isotopes.

[0261] In some embodiments, an immunoconjugate is an antibody-drug conjugate (ADC) in which an antibody is conjugated to one or more drugs, including but not limited to a maytansinoid (*see, e.g.*, U.S. Patent Nos. 5,208,020, 5,416,064 and European Patent EP 0 425 235 B1); an auristatin such as monomethylauristatin drug moieties DE and DF (MMAE and MMAF) (*see, e.g.*, U.S. Patent Nos. 5,635,483 and 5,780,588, and 7,498,298); a dolastatin; a calicheamicin or derivative thereof (*see, e.g.*, U.S. Patent Nos. 5,712,374, 5,714,586, 5,739,116, 5,767,285, 5,770,701, 5,770,710, 5,773,001, and 5,877,296; Hinman et al., *Cancer Res.* 53:3336-3342 (1993); and Lode et al., *Cancer Res.* 58:2925-2928 (1998)); an anthracycline such as daunomycin or doxorubicin (*see, e.g.*, Kratz et al., *Current Med. Chem.* 13:477-523 (2006); Jeffrey et al., *Bioorganic & Med. Chem. Letters* 16:358-362 (2006); Torgov et al., *Bioconj. Chem.* 16:717-721 (2005); Nagy et al., *Proc. Natl. Acad. Sci. USA* 97:829-834 (2000); Dubowchik et al., *Bioorg. & Med. Chem. Letters* 12:1529-1532 (2002); King et al., *J. Med. Chem.* 45:4336-4343 (2002); and U.S. Patent No. 6,630,579); methotrexate; vindesine; a taxane such as docetaxel, paclitaxel, larotaxel, tesetaxel, and ortataxel; a trichothecene; and CC1065.

[0262] In some embodiments, an immunoconjugate comprises an antibody as described herein conjugated to an enzymatically active toxin or fragment thereof, including but not limited to diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from Pseudomonas aeruginosa), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin,

Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes.

[0263] In some embodiments, an immunoconjugate comprises an antibody as described herein conjugated to a radioactive atom to form a radioconjugate. A variety of radioactive isotopes are available for the production of radioconjugates. Examples include At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu. When the radioconjugate is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example tc99m or I123, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, MRI), such as iodine-123 again, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron.

[0264] Conjugates of an antibody and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., *Science* 238:1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. *See, e.g.*, WO94/11026. The linker may be a "cleavable linker" facilitating release of a cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chari et al., *Cancer Res.* 52:127-131 (1992); U.S. Patent No. 5,208,020) may be used.

[0265] The immunuoconjugates or ADCs herein expressly contemplate, but are not limited to such conjugates prepared with cross-linker reagents including, but not limited to, BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (e.g., from Pierce Biotechnology, Inc., Rockford, IL., U.S.A).

Methods and Compositions for Diagnostics and Detection

[0266] In certain embodiments, any of the anti-IL-4 antibodies provided herein is useful for detecting the presence of IL-4 in a biological sample. In certain embodiments, any of the anti-IL-4/IL-13 bispecific antibodies provided herein is useful for detecting the presence of IL-4 and/or IL-13 in a biological sample. The term "detecting" as used herein encompasses quantitative or qualitative detection. In certain embodiments, a biological sample comprises a cell or tissue, such as serum, plasma, nasal swabs, bronchoalveolar lavage fluid, and sputum.

[0267] In some embodiments, an anti-IL-4 antibody for use in a method of diagnosis or detection is provided. In a further aspect, a method of detecting the presence of IL-4 in a biological sample is provided. In certain embodiments, the method comprises contacting the biological sample with an anti-IL-4 antibody as described herein under conditions permissive for binding of the anti-IL-4 antibody to IL-4, and detecting whether a complex is formed between the anti-IL-4 antibody and IL-4. Such method may be an *in vitro* or *in vivo* method. In some embodiments, an anti-IL-4 antibody is used to select subjects eligible for therapy with an anti-IL-4 antibody or anti-IL-4/IL-13 bispecific antibody, or any other TH2 pathway inhibitor, e.g. where IL-4 is a biomarker for selection of patients.

[0268] In some embodiments, an anti-IL-4/IL-13 bispecific antibody for use in a method of diagnosis or detection is provided. In a further aspect, a method of detecting the presence of IL-4 and/or IL-13 in a biological sample is provided. In certain embodiments, the method comprises contacting the biological sample with an anti-IL-4/IL-13 bispecific antibody as described herein under conditions permissive for binding of the anti-IL-4/IL-13 bispecific antibody to IL-4 and/or IL-13, and detecting whether a complex is formed between the anti-IL-4/IL-13 bispecific antibody and IL-4 and/or IL-13. Such method may be an *in vitro* or *in vivo* method. In some embodiments, an anti-IL-4/IL-13 bispecific antibody is used to select subjects eligible for therapy with an anti-IL-4/IL-13 bispecific antibody, or any other TH2 pathway inhibitor, e.g. where IL-4 and/or IL-13 is a biomarker for selection of patients.

[0269] Exemplary disorders that may be diagnosed using an anti-IL-4 antibody of anti-IL-4/IL-13 bispecific antibody are provided herein.

[0270] In certain embodiments, labeled anti-IL-4 antibodies are provided. In certain embodiments, labeled anti-IL-4/IL-13 bispecific antibodies are provided. Labels include, but are not limited to, labels or moieties that are detected directly (such as fluorescent, chromophoric, electron-dense, chemiluminescent, and radioactive labels), as well as moieties, such as enzymes or ligands, that are detected indirectly, e.g., through an enzymatic reaction or

molecular interaction. Exemplary labels include, but are not limited to, the radioisotopes ^{32}P, ^{14}C, ^{125}I, ^{3}H, and ^{131}I, fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luceriferases, e.g., firefly luciferase and bacterial luciferase (U.S. Patent No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase, β-galactosidase, glucoamylase, lysozyme, saccharide oxidases, e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free radicals, and the like.

Pharmaceutical Formulations

[0271] Pharmaceutical formulations of an anti-IL-4 antibody and/or an anti-IL-4/IL-13 bispecific antibody as described herein are prepared by mixing such antibody having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include insterstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX®, Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US Patent Publication Nos.

2005/0260186 and 2006/0104968. In some embodiments, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.

[0272] Exemplary lyophilized antibody formulations are described in US Patent No. 6,267,958. Aqueous antibody formulations include those described in US Patent No. 6,171,586 and WO2006/044908, the latter formulations including a histidine-acetate buffer.

[0273] The formulation herein may also contain more than one active ingredients as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, it may be desirable to further provide a controller and/or TH2 pathway inhibitor with the anti-IL-4 antibody and/or anti-IL-4/IL-13 bispecific antibody. Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended.

[0274] Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

[0275] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g. films, or microcapsules.

[0276] The formulations to be used for *in vivo* administration are generally sterile. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes.

Therapeutic Methods and Compositions

[0277] Any of the anti-IL-4 antibodies provided herein may be used in therapeutic methods. Any of the anti-IL-4/IL-13 bispecific antibodies provided herein may be used in therapeutic methods.

[0278] In certain embodiments, an anti-IL-4 antibody and/or anti-IL-4/IL-13 bispecific antibody for use as a medicament is provided. In certain embodiments, an anti-IL-4 antibody and/or anti-IL-4/IL-13 bispecific antibody for use in treating asthma, IPF, a respiratory disorder, an eosinophilic disorder, an IL-13 mediated disorder, or an IL-4 mediated disorder is provided. In certain embodiments, an anti-IL-4 antibody and/or anti-IL-4/IL-13 bispecific antibody for use in a method of treatment is provided. In certain embodiments, an anti-IL-4 antibody or anti-IL-4/IL-13 bispecific antibody is provided for use in a method of treating an

individual having asthma, a respiratory disorder, an eosinophilic disorder, an IL-13 mediated disorder, or an IL-4 mediated disorder comprising administering to the individual an effective amount of the anti-IL-4 antibody or anti-IL-4/IL-13 bispecific antibody. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described below.

[0279] An "individual" according to any of the above embodiments is preferably a human.

[0280] In some embodiments, use of an anti-IL-4 antibody and/or an anti-IL-4/IL-13 bispecific antibody in the manufacture or preparation of a medicament is provided. In one embodiment, the medicament is for treatment of asthma, a respiratory disorder, an eosinophilic disorder, an IL-13 mediated disorder, or an IL-4 mediated disorder. In a further embodiment, the medicament is for use in a method of treating asthma, IPF, a respiratory disorder, an eosinophilic disorder, an IL-13 mediated disorder, or an IL-4 mediated disorder comprising administering to an individual having asthma, a respiratory disorder, an eosinophilic disorder, an IL-13 mediated disorder, or an IL-4 mediated disorder an effective amount of the medicament. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described below.

[0281] In some embodiments, pharmaceutical formulations comprising any of the anti-IL-4 antibodies and/or anti-IL-4/IL-13 bispecific antibodies described herein are provided, e.g., for use in any of the above therapeutic methods. In some embodiments, a pharmaceutical formulation comprises any of the anti-IL-4 antibodies and/or anti-IL-4/IL-13 bispecific antibodies provided herein and a pharmaceutically acceptable carrier. In some embodiments, a pharmaceutical formulation comprises any of the anti-IL-4 antibodies and/or anti-IL-4/IL-13 bispecific antibodies provided herein and at least one additional therapeutic agent, e.g., as described below.

[0282] Antibodies provided herein can be used either alone or in combination with other agents in a therapy. For instance, an antibody provided herein may be co-administered with at least one additional therapeutic agent. In certain embodiments, an additional therapeutic agent is a TH2 inhibitor. In certain embodiments, an additional therapeutic is a controller of asthma inflammation, such as a corticosteroid, leukotriene receptor antagonist, LABA, corticosteroid/LABA combination composition, theophylline, cromolyn sodium, nedocromil sodium, omalizumab, LAMA, MABA (e.g., bifunctional muscarinic antagonist-beta2 Agonist), 5-Lipoxygenase Activating Protein (FLAP) inhibitor, or enzyme PDE-4 inhibitor.

[0283] Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and separate administration, in which case, administration of the anti-IL-4 antibody and/or anti-IL-4/IL-13 bispecific antibody can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent or agents. In some embodiments, administration of the anti-IL-4 antibody and/or anti-IL-4/IL-13 bispecific antibody and administration of an additional therapeutic agent occur within about one month, or within about one, two or three weeks, or within about one, two, three, four, five, or six days, of each other.

[0284] In some embodiments, an anti-IL-4 antibody and/or anti-IL-4/IL-13 bispecific antibody is used in treating cancer, such as glioblastoma or non-Hodgkin's lymphoma. In some embodiments, antibodies provided herein can also be used in combination with radiation therapy.

[0285] An anti-IL-4 antibody and/or anti-IL-4/IL-13 bispecific antibody (and any additional therapeutic agent) can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Dosing can be by any suitable route, e.g. by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

[0286] An anti-IL-4 antibody and/or anti-IL-4/IL-13 bispecific antibody would be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The antibody need not be, but is optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of antibody present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration

routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

[0287] For the prevention or treatment of disease, the appropriate dosage of an anti-IL-4 antibody and/or anti-IL-4/IL-13 bispecific antibody (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the type of antibody, the severity and course of the disease, whether the antibody is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The antibody is suitably administered to the patient at one time or over a series of treatments. One skilled in the art can determine a suitable dose of an antibody depending on the type and severity of the disease. Nonlimiting exemplary dosing for anti-IL-13 antibodies is described, e.g., in PCT Publication No. WO 2012/083132. General guidance for dosing of antibodies can be found, for example, in Bai et al., *Clinical Pharmacokinetics*, 51: 119-135 (2012) and Deng et al., *Expert Opin. Drug Metab. Toxicol.* 8(2):141-160 (2012). The progress of the antibody therapy may be monitored by conventional techniques and assays.

[0288] It is understood that any of the above formulations or therapeutic methods may be carried out using an immunoconjugate in place of or in addition to an anti-IL 4 antibody or anti-IL-4/IL-13 bispecific antibody.

Articles of Manufacture

[0289] In some embodiments, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an anti-IL-4 antibody and/or anti-IL-4/IL-13 bispecific antibody. The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises an anti-IL-4 antibody and/or anti-IL-4/IL-13 bispecific antibody; and (b) a second container with a composition contained therein, wherein the composition

comprises a further cytotoxic or otherwise therapeutic agent. In some embodiments, the article of manufacture may further comprise a package insert indicating that the compositions can be used to treat a particular condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

[0290] It is understood that any of the above articles of manufacture may include an immunoconjugate in place of or in addition to an anti-IL-4 antibody or anti-IL-4/IL-13 bispecific antibody.

EXAMPLES

[0291] The following are examples of methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

EXAMPLE 1 – Certain Methods and Reagents

Surface Plasmon Resonance (SPR) BIAcore affinity measurement

[0292] The binding kinetics of the anti-IL-4, anti-IL-13 and anti-IL-4/IL-13 bispecific antibodies were measured using surface plasmon resonance (SPR) on a Biacore 3000 instrument (GE Healthcare). Anti-human Fc (GE Healthcare) was immobilized on a CM5 sensor chip via amine-based coupling using manufacturer provided protocol. Antibody was captured at a level of 1200 resonance units (RU).

[0293] Bispecific binding was measured to human IL-4, cyno IL-4, human IL-13, human IL-13 R130Q (SEQ ID NO: 31), and cyno IL-13 at concentrations of 0, 3.13, 6.25, 12.50, 25.0, and 50.0 nM. Sensograms for binding of cytokine were recorded using an injection time of 2 minutes with a flow rate of 30 µl/min, at a temperature of 25 °C, and with a running buffer of 10 mM HEPES, pH 7.4, 150 mM NaCl, and 0.005 % Tween 20. After injection, disassociation of the cytokine from the antibody was monitored for 1000 seconds in running buffer. The surface was regenerated between binding cycles with a 60 µl injection of 3 M Magnesium Chloride. After subtraction of a blank which contained running buffer only, sensograms observed for cytokine binding to anti-IL-13/anti-IL-4 bispecific antibody were analyzed using a 1:1 Langmuir binding model with software supplied by the manufacturer to calculate the kinetics and binding constants.

Surface Plasmon Resonance (SPR) BIAcore binding competition assay

[0294] Inhibition of human IL-13Rα2 binding to human IL-13 by anti-IL-4/ IL-13 bispecific antibody was tested using surface plasmon resonance (SPR) measurements on a Biacore 3000 instrument (GE Healthcare). Human IL-13 was immobilized on a CM5 sensor chip using the manufacturer's protocol for amine-based coupling. IL-13 was immobilized at a level of 985 resonance units (RU) on flow cell 4 (FC4), and unreacted sites were subsequently blocked using 1 M ethanolamine-HCl. FC3 was used as a reference cell for measurements, and it was prepared by activation followed by subsequent blocking with ethanolamine. Sensograms for binding of IL-13Rα2 (histidine-tagged recombinant human IL-13Rα2 made and purified according to standard methods in the art) were recorded using an injection time of 2 minutes with a flow rate of 30 µl/min, at a temperature of 25°C, and with a running buffer of 10mM HEPES, pH 7.4, 150 mM NaCl, and 0.005% Tween 20. To determine the binding constant for IL-13Rα2 binding to IL-13, sensograms for a series of solutions of IL-13Rα2 varying in concentration (2-fold dilutions) from 12.5 to 200 nM were recorded. After injection, disassociation of the receptor from the cytokine was monitored for 600 seconds in running buffer. The surface was regenerated between binding cycles with a 60 µl injection of 10 mM Glycine-HCl pH 1.7.

[0295] To assess the binding of IL-13Rα2 to IL-13 in the presence of anti-IL-4/ IL-13 bispecific antibody, an injection of 60 µl of 250 nM anti-IL-4/ IL-13 bispecific antibody was added as an additional step to assess the binding of receptor to cytokine in the presence of competing antibody. After subtraction of a blank which contained running buffer only, sensograms observed for receptor binding to cytokine in the absence and presence of competing antibody were analyzed using a 1:1 Langmuir binding model with software supplied by the manufacturer to calculate the kinetics and binding constants.

ELISA binding competition assay

[0296] To determine whether an antibody inhibits IL-4 binding to IL-4 receptor (IL-4R), an ELISA assay was used. In a 96 well plate, a 150 µg/mL (1000 nM) solution of the antibody was serially diluted three fold in assay buffer (phosphate buffered saline [PBS], pH 7.5, containing 0.05% Tween 20 and 0.5% bovine serum albumin [BSA]) to provide a range of 0.0009, 0.003, 0.008, 0.02, 0.07, 0.21, 0.62, 1.9, 5.6, 16.7, 50.0, and 150 µg/mL (0.0056, 0.017, 0.05, 0.15, 0.46, 1.37, 4.12, 12.3, 37, 111, 333, and 1000 nM, respectively). The volume of each dilution was 35 µL. To each well, 35 µL of a 11.6 ng/mL (780 pM) solution of biotinylated IL 4 was added. The mixture was incubated for 40 minutes at room temperature. Following incubation,

the contents of the well were transferred to a 96 well Nunc Maxisorp plate (Roskilde, Denmark) that was coated overnight with 50 μL of a 2.0 μg/mL solution of soluble IL-4R protein (R&D Systems, Cat. No. 230-4R/CF) in PBS and blocked with PBS containing 1% BSA. After a 40 minute incubation, the plate was washed five times in wash buffer (1X PBS containing 0.05% Tween 20). Each well then received 50 μL of a streptavidin horseradish peroxidase solution (Caltag Laboratories, Invitrogen; Carlsbad, CA) and was incubated for 40 minutes. Following five washes with wash buffer, 50 μL of tetramethylbenzidine (TMB) substrate (KPL; Gaithersburg, MD) was added to each well. After several minutes, 50 μL of a 1 N solution of HCl was added to stop the reaction. The plate was read at 450 nM using a Spectra Max 340 plate reader (Molecular Devices; Sunnyvale, CA). For each sample, the optical density (OD) reading at 450 nM was plotted against concentration. Curves were plotted in Kaleidagraph (Synergy Software; Reading, PA) and fitted using a 4 parameter fit or plotted point to point.

[0297] To determine whether an antibody inhibits IL-13 binding to IL-13Rα1 receptor, an ELISA assay was carried out substantially as described above, except biotinylated IL-13 R130Q (SEQ ID NO: 31) was used in place of biotinylated IL-4, and soluble IL-13Rα1-Fc protein (R&D Systems, Cat. No. 146-IR-100) was used in place of soluble IL-4R-Fc.

[0298] To determine whether an antibody inhibits IL-13 binding to IL-13Rα2 receptor, an ELISA assay was carried out substantially as described above, except biotinylated IL-13 was used in place of biotinylated IL-4, and soluble IL-13Rα2-Fc protein (R&D Systems, Cat. No. 614-IR-100) was used in place of soluble IL-4R-Fc.

Plasmid construction and expression of antibodies

[0299] Antibodies were cloned into expression vectors described previously (Simmons et al., 2002, *J Immunol Methods* 263, 133–147). The STII signal sequence with a translation initiation strength of one for both the heavy chain and light chain preceded the sequence coding for the mature antibody. For protein expression an overnight culture in a suitable W3110 derivative (Reilly and Yansura, 2010, Antibody Engineering (Berlin, Heidelberg: Springer Berlin Heidelberg)) was grown at 30°C in LB (100 μg/ml carbenicillin), diluted 1:100 into CRAP media (100 μg/ml carbenicillin) and grown for 24 hours at 30°C. For larger preparations, cultures were grown in 10 L fermenters, e.g., as previously described (Simmons et al., 2002, *J Immunol Methods* 263, 133–147).

[0300] For SDS-PAGE analysis under non-reducing conditions 200 μl of overnight culture was harvested and resuspended in 100 μl of NR-lysis buffer (88 μl PopCulture Reagent (Novagen), 10 μl 100 mM iodoacetamide, 2 μl lysonase reagent (EMD

Biosciences)). After incubation for 10 minutes at room temperature, samples were spun for 2' at 9300 rcf and 50 µl supernatant transferred into a fresh tube and mixed with the same volume of 2x SDS sample buffer (Invitrogen). Before loading 10 µl of the sample on NuPAGE 4-12 % Bis-Tris/MES gels (Invitrogen), samples were heated for 5' at 95 °C and spun for 1' at 16000 rcf. Gels were transferred by iBlot (Invitrogen) onto nitrocellulose membrane, immunoblotted with IRDye800CW conjugated anti-Human IgG F(c) antibody (Rockland) and imaged with a LiCOR Odyssey Imager.

[0301] For total reduced cell samples, the cell pellet was resuspended in R-lysis buffer (10 µl 1M DTT, 88 µl PopCulture Reagent (Novagen), 2 µl lysonase) and incubated for 10 minutes at room temperature before samples were mixed with 2x SDS sample buffer. Western blots were images as described before with the exception that IRDye800CW conjugated anti-human antibody (Rockland) was used for immunodetection.

Purification and assembly of bispecific antibodies

[0302] *E. coli* whole cell broth was homogenized using a Niro-Soavi homogenizer from GEA (Bedford, NH, U.S.A). The resulting homogenate was then extracted by addition of polyethyleneimine flocculent to a final concentration of 0.4 %, diluted with purified water and mixed for 16 hours at room temperature. The extract was cleared by centrifugation and after filtration using a 0.2 µm sterile filter cooled to 15°C and loaded on a pre-equilibrated (25mM Tris, 25mM NaCl 5mM EDTA pH 7.1) Protein A column. The column was washed with equilibration buffer and 0.4 M potassium phosphate pH 7.0 and finally eluted with 100 mM acetic acid pH 2.9. The Protein A pools were then combined in an assembly reaction.

[0303] The separate half antibody Protein A pools were conditioned with 0.2 M arginine, pH adjusted using 1.5 M Tris base to pH 8.0, combined and L-reduced glutathione (GSH) was added in a 200 x molar excess over bispecific antibody and incubated at 20°C for 48 hours. After incubation, the assembled bispecific was purified by an anion exchange chromatography step and a cation exchange chromatography step. The cation exchange eluate was concentrated and buffer exchanged into final formulation buffer.

Analytical characterization of antibodies by intact and reduced mass spectrometric analysis

[0304] Reduced and intact masses of bispecifics were obtained by LC/MS analysis using an Agilent 6210 ESI-TOF mass spectrometer coupled with a nano-Chip-LC system. The bispecific samples, with and without prior TCEP reduction, at about 5 ng antibodies per injection, were desalted by RP-HPLC for direct online MS analysis. The resulting spectra for both reduced and non-reduced samples exhibited a distribution of multiply charged protein

ions and the spectra were deconvoluted to zero charge state using the MassHunter Workstation software/Qualitative Analysis B.03.01 (Agilent Technologies Inc. 2009).

Analytical size-exclusion chromatography

[0305] Size variants were separated using a TosoHaas TSK G3000SW$_{XL}$ column (7.8 x 300 mm) eluted isocratically with a mobile phase consisting of 0.2 M potassium phosphate and 0.25 M potassium chloride (pH 6.2). The separation was conducted at room temperature with a flow rate of 0.5 mL/min. The column effluent was monitored at 280 nm. Relative percentage of peak areas for high molecular weight species (HMWS), main peak, and low molecular weight species (LMWS) was performed by using the Chromeleon Software v6.80 SR11 from Dionex Corporation.

Capillary electrophoresis-sodium dodecyl sulfate analysis (CE-SDS)

[0306] The bispecific samples were first diluted with citrate-phosphate buffer pH 6.6 and treated with SDS and N-ethylmaleimide at 70°C for 3 minutes. Upon cooling, samples were labeled at 50°C for 10 minutes with 3-(2-furoyl)quinoline-2-carboxaldehyde) (FQ) in the presence of excess potassium cyanide. The labeling reaction was quenched by buffer exchange then treated with 1% SDS. Non-reduced samples were heated at 70°C for 5 minutes. Reduced samples were treated with 50 mM Dithiothreitol (DTT) at 70°C for 10 minutes.

[0307] Both non-reduced and reduced samples were analyzed by CE-SDS using a Beckman PA 800 CE system with a 50 μm diameter uncoated fused-silica capillary. Samples were injected electrokinetically (40 seconds at 5 kV), and separation was performed at a constant voltage of 15 kV in reversed polarity for 35 minutes. Capillary temperature was maintained at 40°C. The migration of labeled components was monitored by LIF detection; the excitation was at 488 nm, and the emission was monitored at 600 nm.

Cell culture (TF-1 cells)

[0308] Human TF-1 (erythroleukemic cells, R&D Systems, Minneapolis, MN) were cultured in a humidified incubator at 37°C with 5% CO2 in growth media containing RPMI 1640 (Genentech Media Preparation Facility, South San Francisco, CA) containing 10% heat inactivated fetal bovine serum (FBS) (Catalog No. SH30071.03, HyClone Laboratories, Inc., Logan, UT); and 1X Penicillin:Streptomycin:Glutamine (Catalog No. 10378-016, Gibco Invitrogen Corp., Carlsbad, CA) and 2 ng/mL rhGM-CSF (Catalog No. 215-GM, R&D Systems, Minneapolis, MN). Assay media is growth media without 2 ng/mL rhGM-CSF. Cytokines were added to the assay media as specified, at the following final concentrations:

0.2ng/ml human IL-4 (Catalog No. 204-IL, R&D Systems, Minneapolis, MN), 10ng/ml human IL-13 (Genentech, So. San Francisco, CA), 10ng/ml human IL-13 R130Q (Genentech, So. San Francisco, CA), 2ng/ml cynomolgus monkey IL-4 (Genentech, So. San Francisco, CA), and 20ng/ml cynomolgus monkey IL-13 (Genentech, So. San Francisco, CA).

EXAMPLE 2 – Generation of Antibodies the Bind IL-4

[0309] A panel of antibodies that selectively bind human interleukin-4 (IL-4) was generated using commercially-available human IL-4 (R&D Systems, Minneapolis, MN). Each hind footpad of 5 BALB/c mice was injected with 0.5 μg IL-4 resuspended in 25 μl total of monophosphoryl-lipid A and trehalose dicorynomycolate (MPL™ + TDM)-based adjuvant (Corixa, Hamilton, MT) in phosphate-buffered saline (PBS) at 3- to 4-day intervals. Serum samples were taken after 7 boosts and titers determined by enzyme-linked immunosorbant assay (ELISA) to identify mice with a positive immune response to IL-4. Animals were boosted twice more via footpad (0.5 μg in 25 μl/footpad), intraperitoneal cavity (2 μg in 100 μl), and intravenous (1 μg in 50 μl) routes using adjuvant in PBS. Three days after the final boost, animals which showed positive serum titers by ELISA were sacrificed, and a single cell suspension of splenocytes was fused with the mouse myeloma cell line P3X63Ag.U.1 (American Type Culture Collection, Manassas, VA) using electrofusion (Cyto Pulse Sciences, Inc., Glen Burnie, MD). Fused hybridoma cells were selected from unfused splenic, popliteal node or myeloma cells using hypoxanthin-aminopterin-thymidine (HAT) selection in Medium D from the ClonaCell® hybridoma selection kit (StemCell Technologies, Inc., Vancouver, BC, Canada). Hybridoma cells were cultured in Medium E from the ClonaCell® hybridoma selection kit, and cell culture supernatants were used for further characterization and screening. To screen the 1921 hybridoma cell lines generated, enzyme-linked immunosorbant assay (ELISA) was performed generally as described earlier (Baker, K.N., et al., *Trends Biotechnol.* 20, 149–156 (2002)).

[0310] We identified clone 19C11, which bound to human IL-4 with an affinity of ≤10 pM, as determined by surface plasmon resonance (SPR) analysis. To determine whether 19C11 blocks binding of human IL-4 to IL-4Rα, biotinylated IL-4 (0.17 nM) was premixed with 50 μl of serially diluted supernatants of IgG (1000, 200, 40, 8, 1.6, and 0.32 nM, final concentration) from clone 19C11 or a control antibody. Following a 30 minute incubation at room temperature, the mixture was transferred to a Nunc Maxisorp plate containing immobilized soluble human IL-4Rα (R&D Systems, Minneapolis, MN). For immobilization, soluble human IL-4Rα was immobilized by coating the plates with 2 μg/ml of IL-4Rα in

phosphate buffered saline (PBS) overnight at 4°C. The plates were blocked with 200 μL of a 0.5% solution of bovine serum albumin (Sigma, St. Louis, MO) diluted in PBS prior to adding antibody/IL-4. After addition of the antibody/IL-4 mixture, the plates were incubated for 60 minutes at room temperature. Following the incubation, the plates were washed 3 times with PBS containing 0.05% Polysorbate 20 (Sigma). Horseradish peroxidase conjugated to streptavidin (Jackson ImmunoResearch, West Grove, PA) was diluted 1:5000 in the assay buffer and 100 μL was added to each well. Following a 30 minute incubation at room temperature, the plates were washed as described above. 100 μL of the TMB substrate was added and the plate was incubated for 5 to 15 minutes. Reactions were stopped by the addition of 1N Phosphoric Acid. The ELISA plates were read at OD450 using a Spectra Max 340 plate reader (Molecular Devices, Sunnyvale, CA. Curves were plotted using Kaleidagraph graphing software (Synergy Software, Reading, PA).

[0311] To determine whether 19C11 blocks IL-4-induced proliferation of TF-1 cells, serial dilutions of purified 19C11 or irrelevant control antibody were incubated with IL-4 and TF-1 cells. Following a 48 hour incubation, each sample received ^{3}H-thymidine and after a 4 hour incubation incorporation of ^{3}H-thymidine was determined.

[0312] 19C11 blocked binding of biotinylated IL-4 to IL-4Rα (Fig. 1A), suggesting an epitope on IL-4 that overlaps with a region involved in binding to IL-4Rα. 19C11 also inhibited IL-4-induced proliferation of TF-1 cells (Fig. 1B). The IC50 for blocking IL-4-induced proliferation of TF-1 cells was determined to be 0.014 μg/ml, and the IC90 was determined to be 0.07 μg/ml (data not shown). 19C11 was subsequently humanized by grafting the hypervariable region into a human Vkappa-1/VHIII acceptor framework with select point mutations. The binding affinity, epitope, and cellular activity of 19C11 were conserved in the humanization process (data not shown).

EXAMPLE 3 – Humanization of 19C11

[0313] The hypervariable regions (HVRs) from mu19C11 were grafted into the human VL kappa I (huKI), VL kappa III (huKIII), VH subgroup I (huVH1) and VH subgroup III (huVHIII) consensus acceptor frameworks to generate CDR grafts (19C11-κ1 graft, 19C11-κ3 graft, 19C11-VH1 graft, 19C11-VH3 graft) (*see* Figures 10 to 13). In the VL domain the following regions were grafted: positions 24-34 (HVRL1, SEQ ID NO: 15), 50-56 (HVRL2, SEQ ID NO: 16) and 89-97 (HVRL3, SEQ ID NO: 17). In the VH domain, positions 26-35b (HVRH1, SEQ ID NO: 12), 49-65 (HVRH2, SEQ ID NO: 13) and 95-102 (HVRH3, SEQ ID NO: 14) were grafted.

[0314] The 19C11-grafts were generated by Kunkel mutagenesis as IgG expression constructs using separate oligonucleotides for each hypervariable region. Correct clones were identified by DNA sequencing. To potentially enhance the affinity and function of the 19C11-grafts, certain murine vernier framework positions were restored in the VH domain grafts (*see* Figures 12 and 13). Specifically, positions 67, 69 and 71 of 19C11-VH1 graft, and positions 69, 71 and 78 of 19C11-VH3 graft were diversified to generate 19C11-VH1.L, 19C11-VH1.FFL, 19C11-VH3.LA, and 19C11-VH3. FLA. In addition, mutations D62S and F63V were introduced into CDR-H2 of 19C11-VH3.LA to generate 19C11-VH3.LA.SV (*see* Figure 13).

[0315] For screening purposes, IgG variants were initially produced in 293 cells in 6-well plates. Vectors coding for VL and VH (2 μg each) were transfected into 293 cells using the FuGene system. 6 μl of FuGene was mixed with 100 μl of DMEM media containing no FBS and incubated at room temperature for 5 minutes. Each chain (2 μg) was added to this mixture and incubated at room temperature for 20 minutes and then transferred to 6-well plates for transfection overnight at 37°C in 5% CO_2. The following day the media containing the transfection mixture was removed and replaced with 2 ml cell culture media, e.g., DMEM containing FBS. Cells were incubated for an additional 5 days, after which the media was harvested at 1000 rpm for 5 minutes and sterile filtered using a 0.22 μm low protein-binding filter. Samples are stored at 4°C.

[0316] Affinity determinations were performed by surface plasmon resonance using a BIAcore™-A100. Anti-human Fcγ antibody (approximately ~7000 RU) was immobilized in 10 mM sodium acetate pH 4.8 on a CM5 sensor chip. Humanized 19C11 IgG variants expressed in 293 cells were captured by anti-human Fcγ antibody. Recombinant IL-4 was then injected at a flow rate of 30 μL/min. After each injection the chip was regenerated using 3 M $MgCL_2$. Binding response was corrected by subtracting a control flow cell from humanized 19C11 variant IgG flow cells. A 1:1 Languir model of simultaneous fitting of k_{on} and k_{off} was used for kinetics analysis.

[0317] Twelve different humanized 19C11 variants were made, combining each of the humanized light chains (19C11-κ1 graft, 19C11-κ3 graft) with each of the humanized heavy chains (19C11-VH1 graft, 19C11-VH1.L, 19C11-VH1.FFL, 19C11-VH3 graft, 19C11-VH3.LA, and 19C11-VH3. FLA). The twelve humanized 19C11 variants were tested for IL-4 affinity by SPR, along with a chimeric 19C11 in which the mouse variable regions were combined with human IgG constant regions (Figure 14). Most of the variants retained an

affinity for IL-4 of less than 10 pM, with the exception of 19C11-VH1 graft/κ1 graft, 19C11-VH3 graft/κ1 graft, 19C11-VH3.FLA /κ1 graft, and 19C11-VH3 graft/κ3 graft. 19C11-VH1.FFL/κ3 graft and 19C11-VH3.FLA/κ3 graft had an affinity for IL-4 of 11 pM.

[0318] 19C11-VH3.LA.SV/κ1 graft was selected for further study. The heavy chain and light chain variable region sequences for humanized antibody 19C11-VH3.LA.SV/κ1 graft (referred to in the Examples below as anti-IL-4) are shown in SEQ ID NOs: 9 and 10, respectively. The heavy chain hypervariable regions (HVRs) for antibody 19C11-VH3.LA.SV/κ1 graft are shown in SEQ ID NOs: 12 to 14, and the light chain HVRs are shown in SEQ ID NOs: 15 to 17.

EXAMPLE 4 – Generation of IL-4 / IL-13 IgG1 Bispecific Antibody

[0319] We previously established a technology to generate human IgG1 bispecific antibodies with two different light chains in *E. coli* (Yu et al., 2011, *Sci Transl Med* 3, 84ra44). The method utilizes knobs-into-holes technology (Ridgway et al., 1996, *Protein Eng.* 9, 617–621; Atwell et al., 1997, *J Mol Biol* 270, 26–35) to promote hetero-dimerization of immunoglobulin heavy chains. To enable the use of two different light chains without light chain mispairing, we cultured each arm as a hemimer in separate *E. coli* cells. We applied this approach to generate the anti-IL-4/IL-13 bispecific antibody by subcloning the anti-IL-4 and anti-IL-13 parental antibodies into vectors allowing the expression of the anti-IL-4 arm as a human IgG1 hole and of the anti-IL-13 arm as a human IgG1 knob. The sequence of the IgG1 knob constant region is shown in SEQ ID NO: 34 and the sequence of the IgG1 hole constant region is shown in SEQ ID NO: 35.

[0320] We based the anti-IL-13 Fab of the bispecific antibody on lebrikizumab, which has been previously generated and characterized. *See, e.g.*, PCT Publication No. WO 2005/062967 A2. Lebrikizumab binds soluble human IL-13 with a Biacore-derived Kd that is lower than the detection limit of 10 pM. Binding of lebrikizumab to IL-13 does not inhibit binding of the cytokine to IL-13Rα1, but does block the subsequent formation of the heterodimeric signaling competent IL-4Rα /IL-13Rα1 complex (Ultsch, M. et al., 2013, *J. Mol. Biol.*, dx.doi.org/10.1016/j.jmb.2013.01.024; Corren et al., 2011, *N. Engl. J. Med.* 365, 1088–1098).

[0321] For antibody expression, *E. coli* strain 64B4 was used. An overnight culture was grown at 30°C in LB (100 μg/ml carbenicillin), diluted 1:100 into 5 ml CRAP media (100 μg/ml carbenicillin) (Simmons et al., 2002, *J. Immunol. Methods*, 263: 133-147) and grown for 24 hours at 30°C. After expression, the soluble fractions were subjected to SDS-PAGE

followed by anti-Fc immunostaining to analyze the formation of half-antibody species. The knob and hole mutations both result in a predominant half-antibody species. For scale-up to 10L fermenters, initial starter cultures (500 ml) were grown into stationary phase and used to inoculate 10L fermentations (Simmons et al., 2002, *J. Immunol. Methods*, 263: 133-147).

[0322] Initial expression of anti-IL-13 IgG1 knob hemimer in *E. coli* was lower than expected. It has previously been shown that random mutagenesis and/or replacing hydrophobic surface residues of a Fab sequence can lead to improved Fab stability and folding (Forsberg et al., 1997, *J. Biol. Chem.*, 272: 12430-12436; Demarest et al., 2006, *Protein Eng. Des. Sel.*, 19: 325-336; Kugler et al., 2009, *Protein Eng. Des. Sel.*, 22: 135-147).

[0323] Variants were expressed in *E. coli* cells, and non-reducing whole cell extracts were analyzed by non-reducing SDS-PAGE followed by anti-Fc immunoblot. The hemimer band was quantified using an Odyssey® (LiCOR Biosciences) and normalized to the lebrikizumab signal.

[0324] Several changes in the heavy chain and light chain were found to improve hemimer yield and/or folding. One of the changes, M4L in the light chain, was selected. In addition, a Q1E change was introduced in the heavy chain. The two changes were combined in a single hemimer, and the resulting hemimer was found to have improved yield and folding over the wild-type hemimer. The sequence of the lebrikizumab Q1E heavy chain variable region is shown in SEQ ID NO: 19 and the sequence of the lebrikizumab M4L light chain variable region is shown in SEQ ID NO: 20. Those variable regions were used to construct the anti-IL-4/IL-13 IgG1 bispecific antibody.

[0325] The intact bispecific antibody was assembled from isolated half-antibodies by redox-chemistry using methods previously described, for example, in U.S. Patent Publication No. 2011/0287009 and International Patent Application No. PCT/US2012/059810.

EXAMPLE 5 – Generation of IL-4 / IL-13 IgG4 Bispecific Antibody

[0326] After establishing the production of an anti-IL-4/IL-13 bispecific antibody of human IgG1 isotype, we changed the bispecific platform to the human IgG4 isotype. We wished to make the anti-IL-4/IL-13 bispecific antibody as a human IgG4 antibody in order to match the isotype of lebrikizumab, the anti-IL-13 antibody which has shown clinical benefit in the treatment of moderate-to-severe uncontrolled asthma (Corren et al., 2011, *N. Engl. J. Med.* 365, 1088–1098).

[0327] In contrast to IgG1, the heavy-light interchain disulfide of IgG4 is formed by non-consecutive disulfides. This non-consecutive disulfide linkage-pattern is not commonly

observed for *E. coli* proteins (Berkmen, 2005, *J. Biol. Chem.* 280, 11387-11394). In addition, the hinge region of IgG4 is destabilized by an S228 residue, and the CH3 dimer interface of IgG4 contains a destabilizing R409 residue (Dall'Acqua et al., 1998, *Biochemistry* 37, 9266-9273) (EU numbering convention). We designed several constructs to dissect the impact of the IgG4 Fc region sequence, the inter-chain disulfide pattern, and the CH3 R409 on the functional expression of the half-antibodies in *E. coli* and subsequent assembly to a bispecific molecule. In each case, we introduced a stabilizing S228P mutation in the hinge region to attenuate Fab arm exchange after assembly (Stubenrauch et al., 2010, *Drug Metab. Dispos.* 38, 84-91). We first grafted the IgG4 Fc region with corresponding knob/hole mutations (knob: T366W; hole: T366S, L368A, Y407V) onto the IgG1 Fab in order to assess the impact of the IgG4 Fc region on functional expression of the half-antibody. For both antibodies, anti-IL-4 and anti-IL-13, this yielded similar amounts of disulfide-bonded material as the IgG1 isotype (Figures 2C and 2D), indicating that the differences between the isotypes in the Fc region do not impact functional half-antibody expression in *E. coli*. We next converted the entire constant region of the heavy chain to the IgG4 subclass. While this resulted in a reduction in functionally expressed half-antibody, it demonstrated that *E. coli* is in principle capable of forming intramolecular disulfides in the constant region of the antibody from non-consecutive cysteines.

[0328] Since position 409 may be important for the CH3 stability (Dall'Acqua et al., 1998, *Biochemistry* 37, 9266–9273) and the impact of R409 for a downstream assembly process was uncertain at this stage, we also designed a construct with an R409K mutation, to recreate the CH3 interface found in the IgG1 isotype. For both antibodies, this partially rescued the slight drop in functional expression of the IgG4 isotype (Figures 2C and 2D).

EXAMPLE 6 – Assembly and Purification of IL-4 / IL-13 Bispecific Antibodies

[0329] To compare the assembly of the different bispecific antibody constructs, we grew cultures expressing half-antibodies as IgG1, IgG4 and IgG4R409K. After purification of the half-antibodies by Protein A chromatography, the hemimer pairs were mixed, and the intact bispecific antibody formed by a redox chemistry step of the heterodimerized knob/hole pairs. Excess half-antibody was removed by anion and cation-exchange chromatography steps. After the final chromatography step the material was formulated at 45 g/l in 0.2 M Arginine Succinate pH 5.5, 0.02 % Polysorbate-20. To confirm that the assembled antibodies shifted from the half-antibody species to a stable intact antibody, we characterized them by size exclusion chromatography. All three constructs eluted with a retention time corresponding to

an intact, 150 kDa antibody (Figure 3A). Furthermore no significant amounts of aggregated species (0.6/0.4/0.4 % for IgG1/IgG4/IgG4R409K) and only trace amounts of low molecular weight species (0.2/0/4.4 % for IgG1/IgG4/IgG4R409K) were detected, suggesting that both isotypes can be used to assemble antibodies of low aggregation propensity.

[0330] One of the steps during bispecific assembly is the formation of the hinge-disulfides. Since size exclusion chromatography cannot resolve the oxidation state of the interchain disulfides, we subjected the antibodies to capillary electrophoresis-sodium dodecyl sulfate analysis (CE-SDS) and found that all three formats formed hinge-disulfides with similar efficiency. For IgG1, IgG4 and IgG4R409K, 89.3 %, 91.4 %, and 86.7 % of the material was observed in the fully-oxidized conformation, respectively (Figure 3B). We next reduced the samples and reanalyzed them by CE-SDS to determine the respective ratios of light to heavy chains (Figure 3C). All three formats had a similar and expected distribution of light (31.3/31.4/30.9 % for IgG1/IgG4/IgG4R409K) and heavy chains (65.8/64.9/65.4 % for IgG1/IgG4/IgG4R409K), further confirming the existence of a natural antibody conformation.

[0331] To ensure that heterodimeric species were generated during the assembly process, we analyzed the final bispecific molecules by mass spectrometry. The intact and reduced masses are summarized in Table 2, Figure 4 and Table 3. For all three bispecific antibodies, the experimental masses matched closely the theoretical masses, and we were not able to detect any masses corresponding to homodimeric species. A reverse-phase HPLC assay further confirmed that the antibodies were bispecific, with no evidence of homodimeric antibodies (data not shown).

Table 2: Mass Spectrometric Analysis of non-reduced anti-IL-4/IL-13 Bispecific Antibodies

	Theoretical Mass (Da)	Experimental Mass (Da)
anti-IL-4/IL-13 IgG1 Bispecific	145298.4	145304.5
anti-IL-4 IgG1 homodimer	144798.6	n.o.
anti-IL-13 IgG1 homodimer	145798.3	n.o.
anti-IL-4/IL-13 IgG4 Bispecific	144923.7	144929.6
anti-IL-4 IgG4 homodimer	144423.9	n.o.
anti-IL-13 IgG4 homodimer	145423.5	n.o.
anti-IL-4/IL-13 $IgG4_{R409K}$ Bispecific	144867.7	144874.0
anti-IL-4 $IgG4_{R409K}$ homodimer	144367.8	n.o.
anti-IL-13 $IgG4_{R409K}$ homodimer	145367.5	n.o.

n.o. not observed

Table 3: Mass Spectrometric Analysis of reduced anti-IL-4/IL-13 Bispecific Antibodies

	Theoretical Mass (Da)	Experimental Mass (Da)
anti-IL-4 LC IgG1	23522	23521
anti-IL-4 HC IgG1	48893	48893
anti-IL-13 LC IgG1	23815	23815
anti-IL-13 HC IgG1	49100	49099
anti-IL-4 LC IgG4	23522	23523
anti-IL-4 HC IgG4	48706	48708
anti-IL-13 LC IgG4	23815	23816
anti-IL-13 HC IgG4	48913	48914
anti-IL-4 LC $IgG4_{R409K}$	23522	23523
anti-IL-4 HC $IgG4_{R409K}$	48678	48679
anti-IL-13 LC $IgG4_{R409K}$	23815	23816
anti-IL-13 HC $IgG4_{R409K}$	48885	48886

LC light chain, HC heavy chain

[0332] Since we could not detect any significant differences in the assembly of R409 and R409K IgG4 bispecific knobs-into-holes antibodies, all further studies utilized the wildtype (R409) IgG4 bispecific antibody format.

EXAMPLE 7 – Biochemical Characterization of IL-4 / IL-13 Bispecific Antibodies

[0333] We next characterized the IgG1 and IgG4 bispecific antibodies to assess whether their binding affinities to IL-4 and IL-13, as well as their ability to block the binding of IL-4 and IL-13 to their receptors, were comparable. The affinities of the IgG1 and IgG4 bispecific antibodies for IL-4 and IL-13 were measured by Biacore as described in Example 1 and were found to be comparable (Table 4) and similar to those of the parental antibodies, indicating that the ability to bind ligand is not impacted by the bispecific format or the isotype.

[0334] Anti-IL-4/IL-13 bispecific antibody binds with high affinity to human IL-13, human IL-13 R130Q (SEQ ID NO: 31), and cyno IL-13. Dissociation constants of 0.056, 0.142, and 0.048 (nM) were calculated for those cytokines, respectively. Kinetic constants are provided in Table 4. Additional SPR experiments showed the anti-IL-4/IL-13 bispecific

antibody binds with high affinity to human IL-4 and cyno IL-4. Dissociation constants of 0.046 and 0.076 nM were calculated for those cytokines, respectively. Kinetic constants are provided in Table 4.

Table 4: Binding kinetics of anti-IL-4/IL-13 bispecific antibodies

Isotype	Ligand	$K_{on}/10^4$ ($M^{-1}s^{-1}$)	$k_{off}/10^{-4}$ (s^{-1})	K_d (nM)
IgG1	human IL-4	134.4±49.8	0.848±0.05	0.068±0.020
IgG4	human IL-4	287.00±4.58	1.327±0.058	0.046±0.001
IgG1	human IL-13	71.4±4.0	0.170±0.119	0.023±0.015
IgG4	human IL-13	53.73±2.1	0.301±0.109	0.056±0.020
IgG4	human IL-13 R130Q	1.84±0.13	0.262±0.036	0.142±0.013
IgG4	cyno IL-4	201.67±39.15	1.507±0.153	0.076±0.013
IgG4	cyno IL-13	60.80±4.94	0.283±0.202	0.048±0.036

[0335] To ensure that the bispecific molecule can block binding of cytokine to its receptor, ELISA binding competition assays substantially as described in Example 1 were used. Anti-IL-4/ IL-13 bispecific antibody inhibited biotinylated human IL-4 (5.8 ng/mL) direct binding to human IL-4R (*see* Figure 15). A decrease in biotinylated IL-4 binding to IL-4R was observed at 0.035 to 25 μg/mL (0.23 to 167 nM) of bispecific antibody.

[0336] In contrast, anti-IL-4/ IL-13 bispecific antibody did not inhibit biotinylated human IL-13 (0.625 μg/mL) direct binding to human IL-13Rα1 (*see* Figure 16). No decrease in biotinylated human IL-13 binding to IL-13Rα1 was observed with the addition of bispecific antibody at the concentrations tested.

[0337] Anti-IL-4/ IL-13 bispecific did not substantially inhibit biotinylated human IL-13 (0.056 μg/mL) direct binding to human IL-13Rα2 (*see* Figure 17). A partial decrease in biotinylated IL-13 binding to IL-13Rα2 was observed.

[0338] SPR was used to observe the binding of IL-13Rα2 to IL-13 as described in Example 1. Sensograms were collected for injection of a series of concentrations of IL-13Rα2 over immobilized IL-13. Based on the sensograms, a binding constant (Kd) of 0.365 nM (k_{on} = 24.27x10^4±0.49 Ms^{-1}, k_{off} = 0.891x10^{-4}±0.026 s^{-1}) was observed. Anti-IL-4/IL-13 bispecific antibody was previously shown to bind IL-13 with high affinity (Kd = 56 pM) in separate SPR experiments (see Table 4). To test the inhibition of IL-13Rα2 binding to IL-13, 250 nM anti-IL-4/IL-13 bispecific antibody was injected over the immobilized IL-13 prior to injection of IL-13Rα2. Binding of the bispecific antibody did not prevent association of IL-

13Rα2 with the immobilized IL-13 (*see* Figure 18). The Kd for binding of IL-13Rα2 to the bispecific antibody:IL-13 complex was 1.09 nM (k_{on} = 10.06x10^4±0.56 Ms^{-1}, k_{off} = 1.10x10^{-4}±0.12 s^{-1}). Presaturation of immobilized IL-13 with bispecific antibody only modestly disrupted IL-13Rα2 binding to IL-13 and indicates that the bispecific antibody does not significantly inhibit IL-13 binding to IL-13Rα2.

[0339] Thus, similar to the parental anti-IL-4 and anti-IL-13 antibodies, the bispecific antibody fully inhibited binding of IL-4 to IL-4Rα, and did not substantially inhibit binding of IL-13 to IL-13Rα1 or IL-13Rα2. These findings suggest that the binding epitope and monovalent affinity for each IL-13 and IL-4 arm was conserved in the bispecific antibodies.

EXAMPLE 8 – Neutralization of IL-4 and IL-13 Activity in a Cellular Assay

[0340] The activity of both anti-IL-4/IL-13 IgG1 and anti-IL-4/IL-13 IgG4 bispecific antibodies was assessed in an *in vitro* cellular assay in which human IL-4 and IL-13 induce the proliferation of TF-1 cells. The ability of each bispecific antibody to block proliferation of TF-1 cells induced by human IL-4 and human IL-13 alone and in combination was evaluated as described below.

[0341] Antibodies were serially diluted 3.3 fold in 50 µl of assay media containing cytokines in a 96 well tissue culture plate (Catalog No. 353072, Falcon BD, Franklin Lakes, NJ). Plates were incubated for 30 minutes at 37°C. TF-1 cells were washed twice in assay media and resuspended at a final volume of 2.5 x 10^5 cells /ml. 50µl of cells were added to each well for a total volume of 100 µl. Plates were incubated for 4 days in a humidified incubator at 37°C with 5% CO_2, before the addition of 1 µCi of ^{3}H thymidine per well. After an additional 4 hour incubation, proliferation was measured by cell-associated ^{3}H thymidine incorporation using a liquid scintillation counter. Results from duplicate samples are expressed as mean values. Graphs were generated using KaleidaGraph (Synergy Software, Reading, PA).

[0342] Both anti-IL-4/IL-13 IgG1 and anti-IL-4/IL-13 IgG4 bispecific antibodies inhibited human IL-4- and IL-13-induced proliferation of TF-1 cells in a dose-dependent manner, with no significant differences in the IC_{50} for *in vitro* neutralization between the two different bispecific antibodies (Figure 5 and Table 5).

Table 5: IC_{50} of TF-1 proliferation inhibition assays for anti-IL-4/IL-13 bispecific antibodies

	IC_{50} (µg/ml)		
	IL-4	IL-13	IL-4 + IL-13
IgG1 Bispecific	0.06	0.03	0.07
IgG4 Bispecific	0.05	0.03	0.05

[0343] A similar analysis was carried out to determine if anti-IL-4/IL-13 IgG1 and anti-IL-4/IL-13 IgG4 bispecific antibodies inhibited cynomolgus monkey IL-4- and IL-13-induced proliferation of TF-1 cells in a dose-dependent manner (Figure 6).

EXAMPLE 9 – Pharmacokinetic Studies in Cynomolgus Monkeys

[0344] We assessed the *in vivo* pharmacokinetics of the IgG4 and IgG1 anti-IL-4/IL-13 bispecific antibodies following single intravenous (IV) or subcutaneous (SC) administration to cynomolgus monkeys. The pharmacokinetic (PK) studies in cynomolgus monkeys were approved by the Institutional Animal Care and Use Committee (IACUC). The PK study with anti-IL-4/IL-13 IgG4 was conducted at Charles River Laboratories (CRL) Preclinical Services (Reno, NV). A total of 15 female cynomolgus monkeys (2.2 – 2.6 kg) from CRL stock were randomly assigned to five groups (n = 3/group). Animals in group 1 were given an intravenous (IV) and subcutaneous (SC) dose of the control vehicle. Animals in groups 2, 3, and 4 were given a single IV bolus dose of anti-IL-4/IL-13 IgG4 at 10, 30, and 100 mg/kg, respectively. Animals in group 5 were given a SC dose of anti-IL-4/IL-13 IgG4 at 10 mg/kg.

[0345] The PK study with anti-IL-4/IL-13 IgG1 was conducted at Shin Nippon Biomedical Laboratories (SNBL) USA (Everett, WA). A total of 12 female cynomolgus monkeys (2.4 – 3.1 kg) from SNBL stock were randomly assigned to four groups (n = 3/group). Animals in group 1 were given an IV dose of the control vehicle. Animals in groups 2, 3, and 4 were given a single IV bolus dose of anti-IL-4/IL-13 IgG1 at 10, 30, and 60 mg/kg, respectively.

[0346] For both studies, serum samples were collected at various time points out to 4-5 weeks post dose and concentrations of anti-IL-4/IL-13 IgG4 or anti-IL-4/IL-13 IgG1 and were assessed by ELISA with limit of quantitation of 0.078 μg/mL and anti-therapeutic antibodies (ATA) by bridging ELISA. For PK data calculations, Study Day 1 was converted to PK Day 0 to indicate the start of dose administration. All time points after the in life dosing day are calculated as Study Day minus 1. The serum concentration data for each animal were analyzed using 2 compartment analysis with WinNonlin®, Version 5.2.1 (Pharsight; Mountain View, CA).

[0347] The serum concentration-time profiles of anti-IL-4/IL-13 IgG4 and anti-IL-4/IL-13 IgG1 bispecific antibodies exhibited biphasic disposition with linear pharmacokinetics over the dose range tested (Figures 7A and 7B). The initial volume of central compartment for both antibodies was similar to the serum volume indicating limited distribution. Both

antibodies had a relatively slow clearance (CL) and a long terminal half-life as expected for human IgG4 and IgG1 antibodies in cynomolgus monkeys (Mean CL = 5.79 to 6.70 mL/day/kg for anti-IL-4/IL-13 IgG4 and 3.59 to 4.09 mL/day/kg for anti-IL-4/IL-13 IgG1). Based on the area-under-the curve (AUC) calculated for the 10 mg/kg dose groups, the SC bioavailability of the anti-IL-4/IL-13 IgG4 antibody was 95.1%. The presence of anti-therapeutic antibodies (ATA) was detected in 50% of the anti-IL-4/IL-13 IgG4 dosed animals, including all 3 animals in the 100 mg/kg IV dose group, and appeared to be associated with the increased elimination of anti-IL-4/IL-13 IgG4 after day 14). There was a low incidence of ATA detected in anti-IL-4/IL-13 IgG1 treated animals which did not appear to affect the PK. Overall the pharmacokinetics of both anti-IL-4/IL-13 IgG4 and anti-IL-4/IL-13 IgG1 bispecific antibodies were similar and comparable to that of other humanized IgG1and IgG4 monoclonal antibodies in cynomolgus monkeys.

EXAMPLE 10 – Lung Partitioning in a Cynomolgus Monkey Asthma Model

[0348] We evaluated potential differences in the lung partitioning of IgG4 vs. IgG1 anti-IL-4/IL-13 bispecific antibodies in a cynomolgus monkey model of asthma. In this asthma model, cynomolgus monkeys that were naturally sensitized to Ascaris suum (*A. suum*) received an aerosol challenge of *A. suum* extract to elicit allergic inflammatory responses that mimic those of asthmatics exposed to allergens.

[0349] The lung partitioning study in cynomolgus monkeys was approved by IACUC. This study comparing anti-IL-4/IL-13 IgG4 and anti-IL-4/IL-13 IgG1 was conducted at CRL, Preclinical Services (Reno, NV). The study consisted of two different sessions. In the first session, cynomolgus monkeys (3-10 kg) from CRL stock received a baseline aerosol challenge with *Ascaris suum* (*A. suum*) to determine the suitability of the *A. suum* challenge to elicit appropriate airway responses in each animal. The animals were monitored for signs of distress throughout the challenge period and were not given antibodies during this session. Four weeks later, the second session was initiated and a total of 7 male cynomolgus monkeys were randomly assigned to two groups (n = 3 in IgG4 group; n=4 in IgG1 group). These monkeys then received 10 mg/kg of either anti-IL-4/IL-13 IgG4 or anti-IL-4/IL-13 IgG1 via an IV bolus dose on Study Day 1 and Study Day 8. Subsequently, the animals were challenged via aerosol inhalation with *A. suum* on Study Day 9. At various time points up to 23 days post dose, bronchoalveolar lavage (BAL) fluid and serum samples were collected and analyzed for anti-IL-4/IL-13 IgG4 or anti-IL-4/IL-13 IgG1 concentrations by ELISA with limit of quantitation of 0.078 μg/mL. For data calculations, Study Day 1 was converted to PK

Day 0 to indicate the start of dose administration. All time points after the in life dosing day are calculated as Study Day minus 1. Urea and albumin were measured in BAL and serum to estimate epithelial lining fluid (ELF) concentrations and to correct for inflammation induced vascular leakage, respectively. Ascaris specific IgE was also measured in the serum by ELISA. Dilution factors were estimated using BAL and serum urea concentration data as described by Rennard et al., 1986, *J. Appl. Physiol.*, 60(2): 532-538.

[0350] We compared the serum concentrations to epithelial lining fluid (ELF) concentrations of anti-IL-4/IL-13 IgG4 and anti-IL-4/3 IgG1 antibodies following IV administration of 10 mg/kg on Study Days 1 and 8 and a lung challenge with *A. suum* extract on Study Day 9. IgG concentration values in the ELF were derived by correcting BAL fluid IgG concentration data for dilution inherent to the BAL fluid collection procedure as described, e.g., in Rennard et al., 1986, *J. Appl. Physiol.*, 60(2): 532-538. The serum to lung partitioning of anti-IL-4/IL-13 IgG4 and anti-IL-4/IL-13 IgG1 bispecific antibodies were comparable throughout the length of the study (Figure 8). Prior to the allergen challenge, ELF concentrations for both antibodies were approximately 1% - 4% of IgG serum concentrations, indicating that only a small fraction of the systemic antibody reached the ELF. Inhalation challenge with *A. suum* on Study Day 9 appeared to result in increased lung partitioning for both antibodies. However, upon normalizing IgG concentrations to albumin concentrations in the ELF and comparing these values to serum IgG concentrations, the data suggested that the increased ELF IgG concentrations following the respiratory challenge were due to non-specific macromolecular vascular leakage induced by the challenge.

EXAMPLE 11 – Anti-IL-4, Anti-IL-13, and Anti-IL-4/IL-13 Antibody Efficacy in a Mouse Allergic Airway Inflammation and Asthma Model

[0351] Eight BALB/c mice (Charles River Laboratories) were used in this study. On day 0 all mice were intraperitoneally (IP) immunized with 50 μg trinitrophenyl-ovalbumin (TNP-OVA) in 2mg alum in 100 μl sterile PBS. Starting on day 35 post immunization, all mice were aerosol challenged daily for 7 consecutive days with 1% TNP-OVA in PBS for 30 minutes via a nebulizer. Starting on day 37, mice were treated daily with monoclonal antibodies (mAbs), administered IP 4 hours prior to each aerosol challenge for 7 days as shown in Figure 9A.

[0352] On day 42, all mice were bled retroorbitally under anesthesia for 200 μl serum terminally (to measure TNP-OVA-specific IgE, IgG1, and antibody serum concentrations achieved during study). Mice were orbitally bled under isoflurane anesthesia to obtain serum

samples for TNP-OVA specific immunoglobulin and serum TARC (thymus and activation regulated chemokine) measurements by ELISA. Bronchoalveolar lavage fluid samples were collected for differential counts. Lungs were perfused with cold PBS then analyzed by FACS. Lungs were minced into pieces, then mashed through a metal mash to obtain single cells suspensions, then filtered through vial 0.7 μm nylon filter. Lung samples are resuspended in 5 ml. A fixed volume of cell suspension was added to a fixed concentration of FITC labeled fluorescent beads and analyzed on a flow cytometer, collecting 5000 bead events per sample to obtain cell counts. For quantitative and phenotypic analysis of lungs, 3 million lung cells per sample were stained with fluorochrome-labeled mAbs against surface leukocyte markers (CD44-FTC, CD4-APC, CCR3-Pe and CD4-APC, or CD11c-FITC, CD11b-PE and Gr-1-APC; BD Biosciences, San Jose, CA). Samples were run on a BD FACSCalibur (BD, San Jose, CA) and analyzed on Flowjo software (Ashland, OR).

[0353] The results of that experiment are shown in Figures 9B to 9E. Administration of anti-IL-4/IL-13 bispecific antibody suppressed lung eosinophils to a greater extent than anti-IL-4 antibody ($p=0.0381$), and appeared to suppress lung eosinophils to a greater extent than anti-IL-13 antibody, although the difference did not reach statistical significance ($p=0.1803$) (Figure 9B). Similarly, administration of anti-IL-4/IL-13 bispecific antibody suppressed eosinophils in bronchoalveolar lavage fluid to a greater extent than either anti-IL-4 antibody ($p=0.0031$) or anti-IL-13 antibody ($p=0.0135$) (Figure 9C). Administration of either anti-IL-4 antibody or anti-IL-4/IL-13 bispecific antibody appeared to reduce TNP-OVA-specific IgE compared to control treatment, although the results did not reach statistical significance (Figure 9D). Finally, administration of anti-IL-4/IL-13 bispecific antibody suppressed serum TARC levels to a greater extent than either anti-IL-4 antibody or anti-IL-13 antibody ($p<0.0001$ and $p=0.0323$, respectively) (Figure 9E).

DISCUSSION

[0354] Here we have applied the previously developed knobs-into-holes bispecific antibody platform to generate human IgG1 and human IgG4 bispecific antibodies against the cytokines IL-4 and IL-13. Given the overlapping and unique biologies of IL-4 and IL-13, as well as the activities of anti-IL-13 antibodies in the treatment of moderate-to-severe asthmatics, a bispecific antibody targeting both IL-4 and IL-13 may be an improved therapy over anti-IL-13 for the treatment of asthma. The data presented in Example 11 above is supportive of this hypothesis. Our anti-IL-4/IL-13 bispecific antibody is an extension of the anti-IL-13 antibody lebrikizumab, which showed clinical efficacy in a Phase II study in

moderate-to-severe uncontrolled asthma. Since lebrikizumab is a human IgG4 antibody, we used the knobs-into-holes bispecific antibody platform with human IgG4 in order to match the isotype of our anti-IL-4/IL-13 bispecific antibody to that of lebrikizumab.

[0355] One of the key differences between human IgG1 and IgG4 isotypes is the CH3 dimer interface, which affects the dimer stability. Differences are driven by position 409. Our results demonstrate that the knobs-into-holes mutations are compatible with Arg409 in the CH3 domain of IgG4, both in terms of expression as half-antibodies as well as assembly into a bispecific antibody. We could not detect any significant differences in the assembly efficiency or in the quality of final antibody material between the two different isotypes.

[0356] While the expression of human antibodies of various isotypes is well-established in mammalian cells, there have been fewer attempts to express different human antibody isotypes in *E. coli*, and thus, the expression of full-length or half antibodies of human IgG4 isotype in *E. coli* is not as well-documented. Here we demonstrate for these anti-IL-4/IL-13 bispecific antibodies that human IgG4 hemimers can be successfully expressed in large quantities in *E. coli* cells and assembled into bispecific antibodies as readily as human IgG1 bispecific antibodies.

[0357] One of the hallmarks of the knobs-into-holes technology is the retention of the biophysical properties of the monovalent parental antibody in a final bispecific molecule. Both the IgG1 and IgG4 bispecific antibodies retained the target epitope and binding properties of the parental Fab, including high affinity to the IL-4 or IL-13 target cytokine, leading to high potency in *in vitro* cellular assays.

[0358] Pharmacokinetic studies in cynomolgus monkeys demonstrated slow clearance and similar terminal half-lives for both IgG1 and IgG4 bispecific antibodies. In addition, both IgG1 and IgG4 bispecific antibodies partitioned comparably from the serum to the lung at levels that may enable the complete neutralization of pathogenic IL-4 and IL-13 in the lung, which is important for the treatment of asthma. Although the IgG4 bispecific appeared to have a higher rate of ATA compared to the IgG1 bispecific in cynomolgus monkeys, given the small number of animals used in our studies, as well as the lack of a clear relationship between the immunogenicity of humanized antibodies in cynomolgus monkeys vs. humans, we cannot make any conclusions about the relative immunogenicity of our anti-IL-4/IL-13 IgG4 and IgG1 bispecific antibodies in humans. It should be noted, however, that aside from the CDR regions of the antibody Fab's, our bispecific antibodies consist of fully human IgG1 and IgG4 sequences that should exhibit minimal immunogenicity in humans. Thus, the

bispecific antibodies that we have generated are good candidates for clinical development for the treatment of asthma as well as IPF and other respiratory disorders. Furthermore, based on the in vivo data presented herein, methods of treating human disorders, such as asthma, IPF and other respiratory disorders, would naturally follow.

[0359] Antibodies of different human isotypes can have very different *in vitro* and *in vivo* properties resulting from differences in binding to serum complement proteins and Fcγ receptors on immune effector cells (Nirula, A. et al., 2011, *Curr Opin Rheumatol* **23**, 119–124). In particular, antibodies of human IgG1 isotype effectively activate the complement system and engage Fcγ receptors to trigger antibody-dependent cellular cytoxicity (ADCC), whereas antibodies of human IgG4 isotype do not activate the complement system and have reduced ADCC. Importantly, these properties in antibody effector function require antibody glycosylation that is generated during expression in mammalian cells. Antibodies produced in bacterial cells such as *E. coli* lack antibody effector function (Jung, S.T. et al., 2011, *Curr. Opin. Biotechnol.* **22**, 858–867; Simmons, L.C., et al., 2002, *J Immunol Methods* **263**, 133–147) regardless of isotype, due to a lack of antibody glycosylation. Although the bispecific antibodies produced in this study were produced in *E. coli* and therefore lacked glycosylation and Fc effector function, the bispecific antibodies described herein may also be produced in mammalian cells. This approach may effectively extend the knobs-into-holes bispecific antibody platform for these antibodies to include fully glycosylated bispecific anti-IL-4/IL-13 of human IgG1 and IgG4 antibody isotypes and may in turn provide a broad range of therapeutic bispecific antibodies with differing effector functions.

[0360] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all patent and scientific literature cited herein are expressly incorporated in their entirety by reference.

TABLE OF SEQUENCES

SEQ ID NO:	Description	Sequence
1	mu19C11 VH	QIQLVQSGPE LKKPGETVKI SCKASGYTFT DYSMHWMKQA PGKGLKWMVW INTETGEPTY ADDFKGRFAF SLETSANTAY LKINNLKNED TATYFCARGG IFYGMDYWGQ GTSVTVSS
2	mu19C11 VL	SIVMTQTPKF LLISAGDRVT ITCKASQSVI NDAAWYQQKP GQSPRLLIYY TSHRYTGVPD RFTGSGYGTD FTFTISTVQA EDLAVYFCQQ DYTSPWTFGG GTKLEIKR
3	hu19C11 VH1 graft	QVQLVQSGAE VKKPGASVKV SCKASGYTFT DYSMHWVRQA PGQGLEWMVW INTETGEPTY ADDFKGRVTI TRDTSTSTAY LELSSLRSED TAVYYCARGG IFYGMDYWGQ GTLVTVSS
4	hu19C11 VH1.L graft	QVQLVQSGAE VKKPGASVKV SCKASGYTFT DYSMHWVRQA PGQGLEWMVW INTETGEPTY ADDFKGRVTI TLDTSTSTAY LELSSLRSED TAVYYCARGG IFYGMDYWGQ GTLVTVSS
5	hu19C11 VH1.FFL graft	QVQLVQSGAE VKKPGASVKV SCKASGYTFT DYSMHWVRQA PGQGLEWMVW INTETGEPTY ADDFKGRFTF TLDTSTSTAY LELSSLRSED TAVYYCARGG IFYGMDYWGQ GTLVTVSS
6	hu19C11 VH3 graft	EVQLVESGGG LVQPGGSLRL SCAASGYTFT DYSMHWVRQA PGKGLEWVVW INTETGEPTY ADDFKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG IFYGMDYWGQ GTLVTVSS
7	hu19C11 VH3.FLA graft	EVQLVESGGG LVQPGGSLRL SCAASGYTFT DYSMHWVRQA PGKGLEWVVW INTETGEPTY ADDFKGRFTF SLDNSKNTAY LQMNSLRAED TAVYYCARGG IFYGMDYWGQ GTLVTVSS
8	hu19C11 VH3. LA graft	EVQLVESGGG LVQPGGSLRL SCAASGYTFT DYSMHWVRQA PGKGLEWVVW INTETGEPTY ADDFKGRFTI SLDNSKNTAY LQMNSLRAED TAVYYCARGG IFYGMDYWGQ GTLVTVSS
9	hu19C11 VH3. LA.SV graft	EVQLVESGGG LVQPGGSLRL SCAASGYTFT DYSMHWVRQA PGKGLEWVVW INTETGEPTY ADSVKGRFTI SLDNSKNTAY LQMNSLRAED TAVYYCARGG IFYGMDYWGQ GTLVTVSS
10	hu19C11 VL κ1 graft	DIQMTQSPSS LSASVGDRVT ITCKASQSVI NDAAWYQQKP GKAPKLLIYY TSHRYTGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ DYTSPWTFGQ GTKVEIKR
11	hu19C11 VL κ3 graft	EIVLTQSPAT LSLSPGERAT LSCKASQSVI NDAAWYQQKP GQAPRLLIYY TSHRYTGIPA RFSGSGSGTD FTLTISSLEP EDFAVYYCQQ DYTSPWTFGQ GTKVEIKR
12	19C11 HVRH1	GYTFTDYSMH
13	19C11 HVRH2.SV	VWINTETGEPTYADSVKG
14	19C11 HVRH3	GGIFYGMDY
15	19C11 HVRL1	KASQSVINDAA
16	19C11 HVRL2	YTSHRYT
17	19C11 HVRL3	QQDYTSPWT
18	19C11 HVRH2	VWINTETGEPTYADDFKG
56	lebrikizumab VH	QVTLRESGPA LVKPTQTLTL TCTVSGFSLS AYSVNWIRQP PGKALEWLAM IWGDGKIVYN SALKSRLTIS KDTSKNQVVL TMTNMDPVDT ATYYCAGDGY YPYAMDNWGQ GSLVTVSS
57	lebrikizumab VL	DIVMTQSPDS LSVSLGERAT INCRASKSVD SYGNSFMHWY QQKPGQPPKL LIYLASNLES GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQQNNEDPR TFGGGTKVEI KR
19	lebrikizumab VH Q1E	EVTLRESGPA LVKPTQTLTL TCTVSGFSLS AYSVNWIRQP PGKALEWLAM IWGDGKIVYN SALKSRLTIS KDTSKNQVVL TMTNMDPVDT ATYYCAGDGY YPYAMDNWGQ GSLVTVSS
20	lebrikizumab VL M4L	DIVLTQSPDS LSVSLGERAT INCRASKSVD SYGNSFMHWY QQKPGQPPKL LIYLASNLES GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQQNNEDPR TFGGGTKVEI KR
21	lebrikizumab HVRH1	GFSLSAYSVNW
60	lebrikizumab HVRH1 (alternate)	AYSVN

22	lebrikizumab HVRH2	MIWGDGKIVYNSALKS
23	lebrikizumab HVRH3	DGYYPYAMDN
24	lebrikizumab HVRL1	RASKSVDSYGNSFMH
25	lebrikizumab HVRL2	LASNLES
26	lebrikizumab HVRL3	QQNNEDPRT
27	human IL-4 precursor (Swiss-Prot Accession No. P05112.1)	MGLTSQLLPP LFFLLACAGN FVHGHKCDIT LQEIIKTLNS LTEQKTLCTE LTVTDIFAAS KNTTEKETFC RAATVLRQFY SHHEKDTRCL GATAQQFHRH KQLIRFLKRL DRNLWGLAGL NSCPVKEANQ STLENFLERL KTIMREKYSK CSS
28	human IL-4, mature form (without signal sequence)	HKCDIT LQEIIKTLNS LTEQKTLCTE LTVTDIFAAS KNTTEKETFC RAATVLRQFY SHHEKDTRCL GATAQQFHRH KQLIRFLKRL DRNLWGLAGL NSCPVKEANQ STLENFLERL KTIMREKYSK CSS
29	human IL-13 precursor (Swiss-Prot Accession No. P35225.2)	MALLLT TVIALTCLGG FASPGPVPPS TALRELIEEL VNITQNQKAP LCNGSMVWSI NLTAGMYCAA LESLINVSGC SAIEKTQRML SGFCPHKVSA GQFSSLHVRD TKIEVAQFVK DLLLHLKKLF REGRFN
30	human IL-13, mature form (without signal sequence)	SP GPVPPSTALR ELIEELVNIT QNQKAPLCNG SMVWSINLTA GMYCAALESL INVSGCSAIE KTQRMLSGFC PHKVSAGQFS SLHVRDTKIE VAQFVKDLLL HLKKLFREGR FN
31	human IL-13 R130Q mature form	LTCLGGFASP GPVPPSTALR ELIEELVNIT QNQKAPLCNG SMVWSINLTA GMYCAALESL INVSGCSAIE KTQRMLSGFC PHKVSAGQFS SLHVRDTKIE VAQFVKDLLL HLKKLFREGQ FN
32	cynomolgus monkey IL-13 precursor (GenBank Accession No. ABG75889.1)	MALLLTMVIA LTCLGGFASP SPVPPSTALK ELIEELVNIT QNQKAPLCNG SMVWSINLTA GVYCAALESL INVSGCSAIE KTQRMLNGFC PHKVSAGQFS SLRVRDTKIE VAQFVKDLLV HLKKLFREGQ FN
33	cynomolgus monkey IL-4 precursor (Swiss-Prot Accession No. P79339.2); mature form is amino acids 25-153	MGLTSQLLPP LFFLLACAGN FVHGHKCDIT LQEIIKTLNS LTEQKTLCTK LTITDILAAS KNTTEKETFC RAATVLRQFY SHHEKDTRCL GATAQQFHRH KQLIRFLKRL DRNLWGLAGL NSCPVKEANQ STLENFLERL KTIMREKYSK CSS
34	IgG1 T366W heavy chain constant region	ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP KSCDKTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSREE MTKNQVSLWC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV

		LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK
35	IgG1 T366S/ L368A/Y407V heavy chain constant region	ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP KSCDKTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSREE MTKNQVSLSC AVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLV SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK
36	IgG4 T366W / S228P heavy chain constant region	ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTKT YTCNVDHKPS NTKVDKRVES KYGPPCPPCP APEFLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSQED PEVQFNWYVD GVEVHNAKTK PREEQFNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS SIEKTISKAK GQPREPQVYT LPPSQEEMTK NQVSLWCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSRL TVDKSRWQEG NVFSCSVMHE ALHNHYTQKS LSLSLGK
37	IgG4 T366S/ L368A/Y407V/ S228P heavy chain constant region	ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTKT YTCNVDHKPS NTKVDKRVES KYGPPCPPCP APEFLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSQED PEVQFNWYVD GVEVHNAKTK PREEQFNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS SIEKTISKAK GQPREPQVYT LPPSQEEMTK NQVSLSCAVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLVSRL TVDKSRWQEG NVFSCSVMHE ALHNHYTQKS LSLSLGK
38	hu19C11 IgG4 T366S/ L368A/Y407V/ S228P heavy chain	EVQLVESGGG LVQPGGSLRL SCAASGYTFT DYSMHWVRQA PGKGLEWVVW INTETGEPTY ADSVKGRFTI SLDNSKNTAY LQMNSLRAED TAVYYCARGG IFYGMDYWGQ GTLVTVSSAS TKGPSVFPLA PCSRSTSEST AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTKTYT CNVDHKPSNT KVDKRVESKY GPPCPPCPAP EFLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSQEDPE VQFNWYVDGV EVHNAKTKPR EEQFNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKGLPSSI EKTISKAKGQ PREPQVYTLP PSQEEMTKNQ VSLSCAVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLVSRLTV DKSRWQEGNV FSCSVMHEAL HNHYTQKSLS LSLGK
39	hu19C11 light chain	DIQMTQSPSS LSASVGDRVT ITCKASQSVI NDAAWYQQKP GKAPKLLIYY TSHRYTGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ DYTSPWTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
40	lebrikizumab Q1E IgG4 T366W/S228P heavy chain	EVTLRESGPA LVKPTQTLTL TCTVSGFSLS AYSVNWIRQP PGKALEWLAM IWGDGKIVYN SALKSRLTIS KDTSKNQVVL TMTNMDPVDT ATYYCAGDGY YPYAMDNWGQ GSLVTVSSAS TKGPSVFPLA PCSRSTSEST AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTKTYT CNVDHKPSNT KVDKRVESKY GPPCPPCPAP EFLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSQEDPE VQFNWYVDGV EVHNAKTKPR EEQFNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKGLPSSI EKTISKAKGQ PREPQVYTLP PSQEEMTKNQ VSLWCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSRLTV DKSRWQEGNV FSCSVMHEAL HNHYTQKSLS LSLGK
41	lebrikizumab M4L light chain	DIVLTQSPDS LSVSLGERAT INCRASKSVD SYGNSFMHWY QQKPGQPPKL LIYLASNLES GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQQNNEDPR TFGGGTKVEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV THQGLSSPVT KSFNRGEC

58	lebrikizumab IgG4 T366W/ S228P heavy chain	QVTLRESGPA LVKPTQTLTL TCTVSGFSLS AYSVNWIRQP PGKALEWLAM IWGDGKIVYN SALKSRLTIS KDTSKNQVVL TMTNMDPVDT ATYYCAGDGY YPYAMDNWGQ GSLVTVSSAS TKGPSVFPLA PCSRSTSEST AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTKTYT CNVDHKPSNT KVDKRVESKY GPPCPPCPAP EFLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSQEDPE VQFNWYVDGV EVHNAKTKPR EEQFNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKGLPSSI EKTISKAKGQ PREPQVYTLP PSQEEMTKNQ VSLWCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSRLTV DKSRWQEGNV FSCSVMHEAL HNHYTQKSLS LSLGK
59	lebrikizumab light chain	DIVMTQSPDS LSVSLGERAT INCRASKSVD SYGNSFMHWY QQKPGQPPKL LIYLASNLES GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQQNNEDPR TFGGGTKVEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV THQGLSSPVT KSFNRGEC
42	IL-13 epitope, amino acids 68 to 75 of SEQ ID NO: 29 (amino acids 50 to 57 of SEQ ID NO: 30)	ESLINVSG
43	IL-13 epitope, amino acids 63 to 74 of SEQ ID NO: 29 (amino acids 45 to 56 of SEQ ID NO: 30)	YCAALESLINVS
44	anti-IL-13 mu11H4 VL	DIVLTQSPAS LAVSLGQRAT ISCRASQSVS TSSYSYMNWY QQTPGQPPKL LIKYASNLES GIPARFSGSG SGTDFTLNIH PVEEEDTATY YCQHSWEIPY TFGGGT
45	anti-IL-13 mu11H4 VH	QVTLKESGPG ILQPSQTLSL TCSFSGFSLS TSDMGVGWIR QPSGKGLEWL AHIWWDDVKR YNPALKSRLT ISKDTSSSQV FLKIASVDTA DTATYYCARI GTNYGYDGLF DYWGQGTTLT VSS
46	anti-IL-13 hu11H4v6 light chain	DIVMTQSPDS LAVSLGERAT INCRASQSVS TSSYSYMNWY QQKPGQPPKL LIKYASNLES GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQHSWEIPY TFGQGTKVEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV THQGLSSPVT KSFNRGEC
47	anti-IL-13 hu11H4v6 heavy chain	EVQLVESGPA LVKPTQTLTL TCTFSGFSLS TSDMGVGWIR QPPGKALEWL AHIWWDDVKR YNPALKSRLT ISKDTSKNQV VLTMTNMDPV DTATYYCARI GTNYGYDALF DYWGQGTLVT VSSASTKGPS VFPLAPSSKS TSGGTAALGC LVKDYFPEPV TVSWNSGALT SGVHTFPAVL QSSGLYSLSS VVTVPSSSLG TQTYICNVNH KPSNTKVDKK VEPKSCDKTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS REEMTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK
48	anti-IL-13 hu11H4v6 VL	DIVMTQSPDS LAVSLGERAT INCRASQSVS TSSYSYMNWY QQKPGQPPKL LIKYASNLES GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQHSWEIPY TFGQGTKVEI K
49	anti-IL-13 hu11H4v6 VH	EVQLVESGPA LVKPTQTLTL TCTFSGFSLS TSDMGVGWIR QPPGKALEWL AHIWWDDVKR YNPALKSRLT ISKDTSKNQV VLTMTNMDPV DTATYYCARI GTNYGYDALF DYWGQGTLVT VSS

50	hu11H4v6 HVRH1	GFSLSTSDMGVG
51	hu11H4v6 HVRH2	AHIWWDDVKRYNPALKS
52	hu11H4v6 HVRH3	ARIGTNYGYDALFDY
53	hu11H4v6 HVRL1	RASQSVSTSSYSYMN
54	hu11H4v6 HVRL2	YASNLES
55	hu11H4v6 HVRL3	QHSWEIPYT

CLAIMS:

1. A multispecific antibody comprising an antigen-binding domain that comprises a first VH/VL unit that specifically binds IL-4 and a second VH/VL unit that specifically binds IL-13, wherein the antibody:

a) inhibits binding of IL-4 to IL-4 receptor alpha (IL-4Rα),

b) inhibits IL-4-induced proliferation of cells *in vitro*, and/or

c) inhibits IL-13-induced proliferation of cells *in vitro*.

2. The multispecific antibody of claim 1, wherein the first VH/VL unit comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 14, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 17, and HVR-H2 comprising the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 18.

3. The multispecific antibody of claim 1 or claim 2, wherein the first VH/VL unit comprises HVR-H1 comprising the amino acid sequence of SEQ ID NO: 12, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 18, and HVR-H3 comprising the amino acid sequence of SEQ ID NO: 14.

4. The multispecific antibody of any one of claims 1 to 3, wherein the first VH/VL unit comprises HVR-L1 comprising the amino acid sequence of SEQ ID NO: 15, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 16, and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 17.

5. The multispecific antibody of any one of claims 1 to 4, wherein the first VH/VL unit comprises (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 9; (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 10; or (c) a VH sequence as in (a) and a VL sequence as in (b).

6. The multispecific antibody of any one of claims 1 to 5, wherein the first VH/VL unit comprises a VH sequence selected from SEQ ID NOs: 1 and 3 to 9.

7. The multispecific antibody of any one of claims 1 to 6, wherein the first VH/VL unit comprises a VL sequence selected from SEQ ID NOs: 2, 10, and 11.

8. The multispecific antibody of claim 1, wherein the first VH/VL unit comprises the VH sequence of SEQ ID NO: 9 and the VL sequence of SEQ ID NO: 10.

9. The multispecific antibody of any one of claims 1 to 8, wherein the second VH/VL unit comprises:

a) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26, and HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22; or

b) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 52, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 55, and HVR-H2 comprising the amino acid sequence of SEQ ID NO: 51.

10. The multispecific antibody of any one of claims 1 to 9, wherein the second VH/VL unit comprises:

a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 21 or the amino acid sequence of SEQ ID NO: 60, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22, and HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23; or

b) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 50, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 51, and HVR-H3 comprising the amino acid sequence of SEQ ID NO: 52.

11. The multispecific antibody of any one of claims 1 to 10, wherein the second VH/VL unit comprises:

a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 24, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 25, and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26; or

b) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 53, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 54, and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 55.

12. The multispecific antibody of any one of claims 1 to 11, wherein the second VH/VL unit comprises:

a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 19;

b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 20;

c) a VH sequence as in (a) and a VL sequence as in (b);

d) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 49;

e) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 48;

f) a VH sequence as in (d) and a VL sequence as in (e).

13. The multispecific antibody of any one of claims 1 to 12, wherein the second VH/VL unit comprises the VH sequence of SEQ ID NO: 19, 56, or 49.

14. The multispecific antibody of any one of claims 1 to 13, wherein the second VH/VL unit comprises the VL sequence of SEQ ID NO: 20, 57, or 48.

15. The multispecific antibody of any one of claims 1 to 14, wherein the second VH/VL unit comprises the VH sequence of SEQ ID NO: 19 or 56 and the VL sequence of SEQ ID NO: 20 or 57; or the VH sequence of SEQ ID NO: 49 and the VL sequence of SEQ ID NO: 48.

16. The multispecific antibody of any one of claims 1 to 15, wherein the antibody competes for binding to IL-4 with an antibody comprising a VH sequence of SEQ ID NO: 9 and a VL sequence of SEQ ID NO: 10.

17. The multispecific antibody of any one of claims 1 to 16, wherein the antibody competes for binding to IL-13 with an antibody comprising a VH sequence of SEQ ID NO: 19 and a VL sequence of SEQ ID NO: 20, or with an antibody comprising a VH sequence of SEQ ID NO: 49 and a VL sequence of SEQ ID NO: 48.

18. The multispecific antibody of any one of claims 1 to 17, wherein the antibody binds an epitope within amino acids 77 to 89 of SEQ ID NO: 29, or within amino acids 82 to 89 of SEQ ID NO: 29.

19. A multispecific antibody comprising a first VH/VL unit that specifically binds IL-4 and a second VH/VL unit that specifically binds IL-13, wherein the first VH/VL unit comprises the VH sequence of SEQ ID NO: 9 and the VL sequence of SEQ ID NO: 10, and the second VH/VL unit comprises the VH sequence of SEQ ID NO: 19 and the VL sequence of SEQ ID NO: 20.

20. The multispecific antibody of any one of the preceding claims, wherein the antibody is an IgG antibody.

21. The multispecific antibody of claim 20, wherein the antibody is an IgG1 or IgG4 antibody.

22. The multispecific antibody of claim 21, wherein the antibody is an IgG4 antibody.

23. The multispecific antibody of any one of the preceding claims, wherein the antibody comprises a first heavy chain constant region and a second heavy chain constant region, wherein the first heavy chain constant region comprises a knob mutation and the second heavy chain constant region comprises a hole mutation.

24. The multispecific antibody of claim 23, wherein the first heavy chain constant region is fused to the heavy chain variable region portion of a VH/VL unit that binds IL-4.

25. The multispecific antibody of claim 23 or claim 24, wherein the second heavy chain constant region is fused to the heavy chain variable region portion of a VH/VL unit that binds IL-13.

26. The multispecific antibody of claim 23, wherein the first heavy chain constant region is fused to the heavy chain variable region portion of a VH/VL unit that binds IL-13.

27. The multispecific antibody of claim 23 or claim 26, wherein the second heavy chain constant region is fused to the heavy chain variable region portion of a VH/VL unit that binds IL-4.

28. The multispecific antibody of any one of claims 23 to 27, wherein the antibody is an IgG1 antibody and wherein the knob mutation comprises a T366W mutation.

29. The multispecific antibody of any one of claims claim 23 to 28, wherein the antibody is an IgG1 antibody and wherein the hole mutation comprises at least one, at least two, or three mutations selected from T366S, L368A, and Y407V.

30. The multispecific antibody of any one of claims 23 to 27, wherein the antibody is an IgG4 antibody and wherein the knob mutation comprises a T366W mutation.

31. The multispecific antibody of any one of claims 23 to 27 and 30, wherein the antibody is an IgG4 antibody and wherein the hole mutation comprises at least one, at least two, or three mutations selected from T366S, L368A, and Y407V mutations.

32. The multispecific antibody of claim 23, wherein the antibody comprises a first heavy chain constant region comprising the sequence of SEQ ID NO: 34.

33. The multispecific antibody of claim 23 or claim 32, wherein the antibody comprises a second heavy chain constant region comprising the sequence of SEQ ID NO: 35.

34. The multispecific antibody of claim 23, wherein the antibody comprises a first heavy chain constant region comprising the sequence of SEQ ID NO: 36.

35. The multispecific antibody of claim 23 or claim 34, wherein the antibody comprises a second heavy chain constant region comprising the sequence of SEQ ID NO: 37.

36. A multispecific antibody that binds IL-4 and IL-13, wherein the antibody comprises a first heavy chain comprising the sequence of SEQ ID NO: 38, a first light chain comprising the sequence of SEQ ID NO: 39, a second heavy chain comprising the sequence of SEQ ID NO: 40, and a second light chain comprising the sequence of SEQ ID NO: 41.

37. An isolated antibody that binds IL-4, wherein the antibody comprises:

(a) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 14, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 17, and HVR-H2 comprising the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 18; or

(b) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 12, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 18, and HVR-H3 comprising the amino acid sequence of SEQ ID NO: 14; or

(c) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 15, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 16, and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 17; or

(d) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 9; or

(e) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 10.

38. The isolated antibody of claim 37, wherein the antibody comprises HVR-H1 comprising the amino acid sequence of SEQ ID NO: 12, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 18, HVR-H3 comprising the amino acid sequence of SEQ ID NO: 14, HVR-L1 comprising the amino acid sequence of SEQ ID NO: 15, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 16, and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 17.

39. The isolated antibody of claim 37 or claim 38, wherein the antibody comprises a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 9 and a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 10.

40. The isolated antibody of any one of claims 37 to 39, wherein the antibody comprises a VH sequence selected from SEQ ID NOs: 1 and 3 to 9.

41. The isolated antibody of any one of claims 37 to 40, wherein the antibody comprises a VL sequence selected from SEQ ID NOs: 2, 10, and 11.

42. An isolated antibody comprising the VH sequence of SEQ ID NO: 9 and the VL sequence of SEQ ID NO: 10.

43. An isolated nucleic acid encoding:

(a) the antibody of any one of claims 1 to 42;

(b) the first VH/VL unit of the multispecific antibody of any one of claims 1 to 34; or

(c) the second VH/VL unit of the multispecific antibody of any one of claims 1 to 34.

44. A host cell comprising the nucleic acid of claim 43.

45. The host cell of claim 44, wherein the host cell is an *E. coli* cell or a CHO cell.

46. A method of producing an antibody comprising culturing the host cell of claim 44 or claim 45.

47. An immunoconjugate comprising the antibody of any one of claims 1 to 42 and a cytotoxic agent.

48. A pharmaceutical formulation comprising the antibody of any one of claims 1 to 42 and a pharmaceutically acceptable carrier.

49. The antibody of any one of claims 1 to 42 for use as a medicament.

50. The antibody of any one of claims 1 to 42 for use in treating an eosinophilic disorder, an IL-13 mediated disorder, an IL-4 mediated disorder, or a respiratory disorder.

51. The antibody of claim 50, wherein the eosinophilic disorder is selected from asthma, severe asthma, chronic asthma, atopic asthma, atopic dermatitis, allergy, allergic rhinitis, non-allergic rhinitis, contact dermatitis, erythema multiform, bullous skin disease, psoriasis, eczema, rheumatoid arthritis, juvenile chronic arthritis, chronic eosinophilic pneumonia, allergic bronchopulmonary aspergillosis, coeliac disease, Churg-Strauss syndrome (periarteritis nodosa plus atopy), eosinophilic myalgia syndrome, hypereosinophilic syndrome, oedematous reactions including episodic angioedema, helminth infections, urticaria, onchocercal dermatitis, eosinophil-associated gastrointestinal disorders, eosinophilic esophagitis, eosinophilic gastritis, eosinophilic gastroenteritis, eosinophilic enteritis, eosinophilic colitis, ulcerative colitis, Whipple's disease, nasal micropolyposis, nasal polyposis, aspirin intolerance, obstructive sleep apnea, Crohn's disease, scleroderma, endomyocardial fibrosis, fibrosis, inflammatory bowel disease, idiopathic interstitial pneumonia, eosinophilic pneumonia, hypersensitivity pneumonitis, goblet cell metaplasia, pulmonary fibrosis, idiopathic pulmonary fibrosis (IPF), pulmonary fibrosis secondary to sclerosis, chronic obstructive pulmonary disease (COPD), hepatic fibrosis, uveitis, cancer, glioblastoma, Hodgkin's lymphoma, and non-Hodgkin's lymphoma.

52. The antibody of claim 50, wherein the IL-13 mediated disease is selected from atopic dermatitis, allergic rhinitis, asthma, fibrosis, inflammatory bowel disease, Crohn's disease, lung inflammatory disorders, pulmonary fibrosis, idiopathic pulmonary fibrosis (IPF), chronic obstructive pulmonary disease (COPD), hepatic fibrosis, cancer, glioblastoma, and non-Hodgkin's lymphoma.

53. The antibody of claim 50, wherein the IL-4 mediated disease is selected from atopic dermatitis, allergic rhinitis, asthma, fibrosis, inflammatory bowel disease, Crohn's disease, lung inflammatory disorders, pulmonary fibrosis, idiopathic pulmonary fibrosis (IPF), chronic obstructive pulmonary disease (COPD), hepatic fibrosis, cancer, glioblastoma, and non-Hodgkin's lymphoma.

54. The antibody of claim 50, wherein the respiratory disorder is selected from asthma, allergic asthma, non-allergic asthma, bronchitis, chronic bronchitis, chronic obstructive pulmonary disease (COPD), emphysema, cigarette-induced emphysema, airway inflammation, cystic fibrosis, pulmonary fibrosis, allergic rhinitis, and bronchiectasis.

55. Use of an antibody of any one of claims 1 to 42 in the manufacture of a medicament for treating an eosinophilic disorder, an IL-13 mediated disorder, an IL-4 mediated disorder, or a respiratory disorder.

56. The use of claim 55, wherein the eosinophilic disorder is selected from asthma, severe asthma, severe asthma, chronic asthma, atopic asthma, atopic dermatitis, allergy, allergic rhinitis, non-allergic rhinitis, contact dermatitis, erythema multiform, bullous skin disease, psoriasis, eczema, rheumatoid arthritis, juvenile chronic arthritis, chronic eosinophilic pneumonia, allergic bronchopulmonary aspergillosis, coeliac disease, Churg-Strauss syndrome (periarteritis nodosa plus atopy), eosinophilic myalgia syndrome, hypereosinophilic syndrome, oedematous reactions including episodic angioedema, helminth infections, urticaria, onchocercal dermatitis, eosinophil-associated gastrointestinal disorders, eosinophilic esophagitis, eosinophilic gastritis, eosinophilic gastroenteritis, eosinophilic enteritis, eosinophilic colitis, ulcerative colitis, Whipple's disease, nasal micropolyposis, nasal polyposis, aspirin intolerance, obstructive sleep apnea, Crohn's disease, scleroderma, endomyocardial fibrosis, fibrosis, inflammatory bowel disease, idiopathic interstitial pneumonia, eosinophilic pneumonia, hypersensitivity pneumonitis, goblet cell metaplasia, pulmonary fibrosis, idiopathic pulmonary fibrosis (IPF), pulmonary fibrosis secondary to sclerosis, chronic obstructive pulmonary disease (COPD), hepatic fibrosis, uveitis, cancer, glioblastoma, Hodgkin's lymphoma, and non-Hodgkin's lymphoma.

57. The use of claim 55, wherein the IL-13 mediated disease is selected from atopic dermatitis, allergic rhinitis, asthma, fibrosis, inflammatory bowel disease, Crohn's disease, lung inflammatory disorders, pulmonary fibrosis, idiopathic pulmonary fibrosis (IPF), chronic obstructive pulmonary disease (COPD), hepatic fibrosis, cancer, glioblastoma, and non-Hodgkin's lymphoma.

58. The use of claim 55, wherein the IL-4 mediated disease is selected from atopic dermatitis, allergic rhinitis, asthma, fibrosis, inflammatory bowel disease, Crohn's disease, lung inflammatory disorders, pulmonary fibrosis, idiopathic pulmonary fibrosis (IPF), chronic obstructive pulmonary disease (COPD), hepatic fibrosis, cancer, glioblastoma, and non-Hodgkin's lymphoma.

59. The use of claim 55, wherein the respiratory disorder is selected from asthma, allergic asthma, non-allergic asthma, bronchitis, chronic bronchitis, chronic obstructive pulmonary disease (COPD), emphysema, cigarette-induced emphysema, airway inflammation, cystic fibrosis, pulmonary fibrosis, allergic rhinitis, and bronchiectasis.

60. A method of treating an individual with an eosinophilic disorder comprising administering to the individual an effective amount of an antibody of any one of claims 1 to 42.

61. The method of claim 60, wherein the eosinophilic disorder is selected from asthma, severe asthma, chronic asthma, atopic asthma, atopic dermatitis, allergy, allergic rhinitis, non-allergic rhinitis, contact dermatitis, erythema multiform, bullous skin disease, psoriasis, eczema, rheumatoid arthritis, juvenile chronic arthritis, chronic eosinophilic pneumonia, allergic bronchopulmonary aspergillosis, coeliac disease, Churg-Strauss syndrome (periarteritis nodosa plus atopy), eosinophilic myalgia syndrome, hypereosinophilic syndrome, oedematous reactions including episodic angioedema, helminth infections, urticaria, onchocercal dermatitis, eosinophil-associated gastrointestinal disorders, eosinophilic esophagitis, eosinophilic gastritis, eosinophilic gastroenteritis, eosinophilic enteritis, eosinophilic colitis, ulcerative colitis, Whipple's disease, nasal micropolyposis, nasal polyposis, aspirin intolerance, obstructive sleep apnea, Crohn's disease, scleroderma, endomyocardial fibrosis, fibrosis, inflammatory bowel disease, idiopathic interstitial pneumonia, eosinophilic pneumonia, hypersensitivity pneumonitis, goblet cell metaplasia, pulmonary fibrosis, idiopathic pulmonary fibrosis (IPF), pulmonary fibrosis secondary to sclerosis, chronic obstructive pulmonary disease (COPD), hepatic fibrosis, uveitis, cancer, glioblastoma, Hodgkin's lymphoma, and non-Hodgkin's lymphoma.

62. The method of claim 60, wherein the IL-13 mediated disease is selected from atopic dermatitis, allergic rhinitis, asthma, fibrosis, inflammatory bowel disease, Crohn's disease, lung inflammatory disorders, pulmonary fibrosis, idiopathic pulmonary fibrosis (IPF), chronic obstructive pulmonary disease (COPD), hepatic fibrosis, cancer, glioblastoma, and non-Hodgkin's lymphoma.

63. The method of claim 60, wherein the IL-4 mediated disease is selected from atopic dermatitis, allergic rhinitis, asthma, fibrosis, inflammatory bowel disease, Crohn's disease, lung inflammatory disorders, pulmonary fibrosis, idiopathic pulmonary fibrosis (IPF), chronic obstructive pulmonary disease (COPD), hepatic fibrosis, cancer, glioblastoma, and non-Hodgkin's lymphoma.

64. The method of claim 60, wherein the respiratory disorder is selected from asthma, allergic asthma, non-allergic asthma, bronchitis, chronic bronchitis, chronic obstructive pulmonary disease (COPD), emphysema, cigarette-induced emphysema, airway inflammation, cystic fibrosis, pulmonary fibrosis, allergic rhinitis, and bronchiectasis.

65. The method of any one of claims 60 to 64, further comprising administering to the individual a TH2 pathway inhibitor.

66. The method of claim 65, wherein the TH2 pathway inhibitor inhibits at least one target selected from ITK, BTK , IL-9, IL-5, IL-13, IL-4, OX40L, TSLP, IL-25, IL-33, IgE, IL-9 receptor, IL-5 receptor, IL-4 receptor alpha, IL-13receptoralpha1, IL-13receptoralpha2, OX40, TSLP-R, IL-7Ralpha, IL17RB, ST2, CCR3, CCR4, CRTH2, FcepsilonRI, FcepsilonRII/CD23, Flap, Syk kinase; CCR4, TLR9, CCR3, IL5, IL3, and GM-CSF.

67. The method of any one of claims 60 to 66, wherein the individual is suffering from moderate to severe asthma.

68. The method of any one of claims 60 to 66, wherein the individual is suffering from idiopathic pulmonary fibrosis.

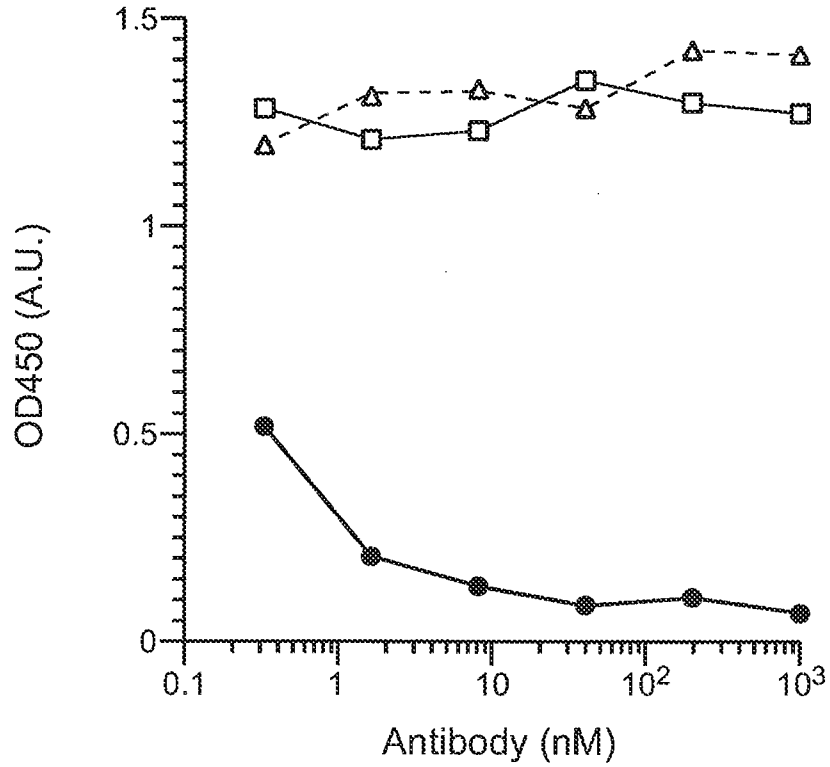
1.5
1
0.5
0
OD450 (A.U.)
0.1
1
10
10^2
10^3
Antibody (nM)

FIG. 1A

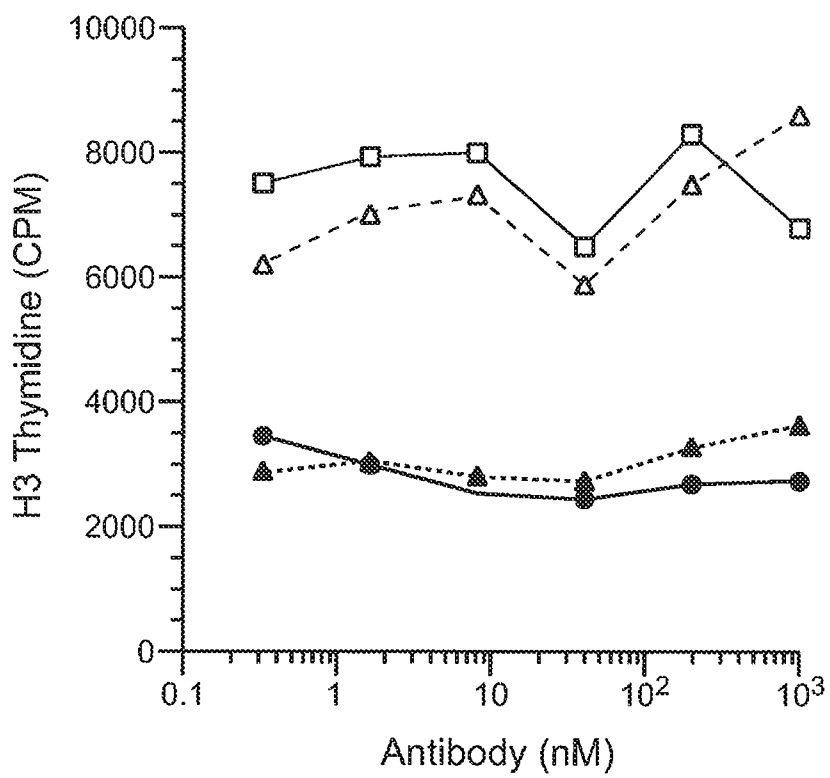
10000
8000
6000
4000
2000
0
H3 Thymidine (CPM)
0.1
1
10
10^2
10^3
Antibody (nM)

FIG. 1B

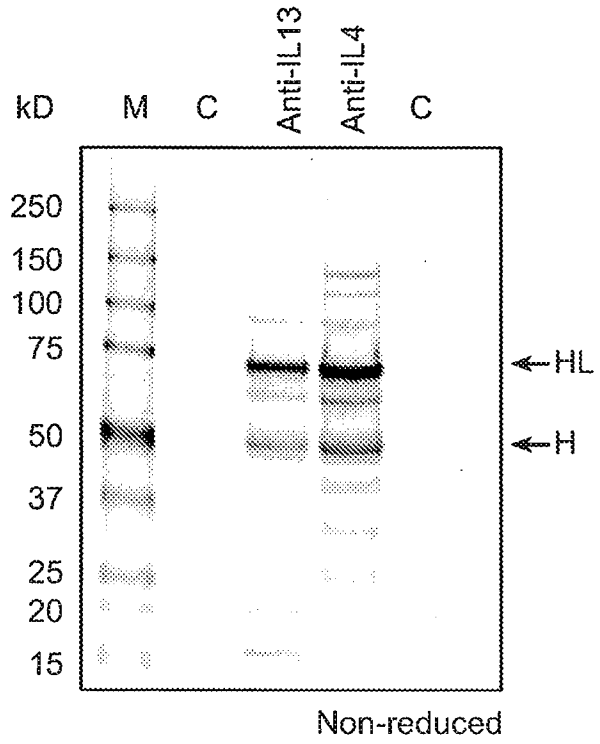

FIG. 2A

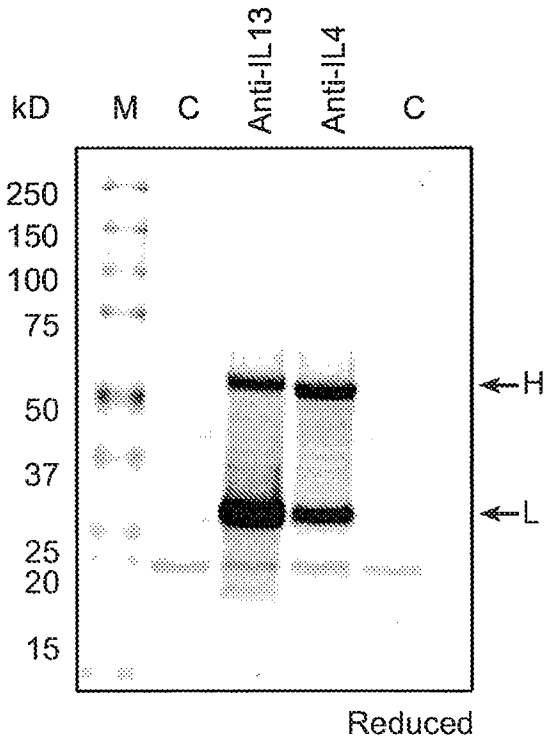

FIG. 2B

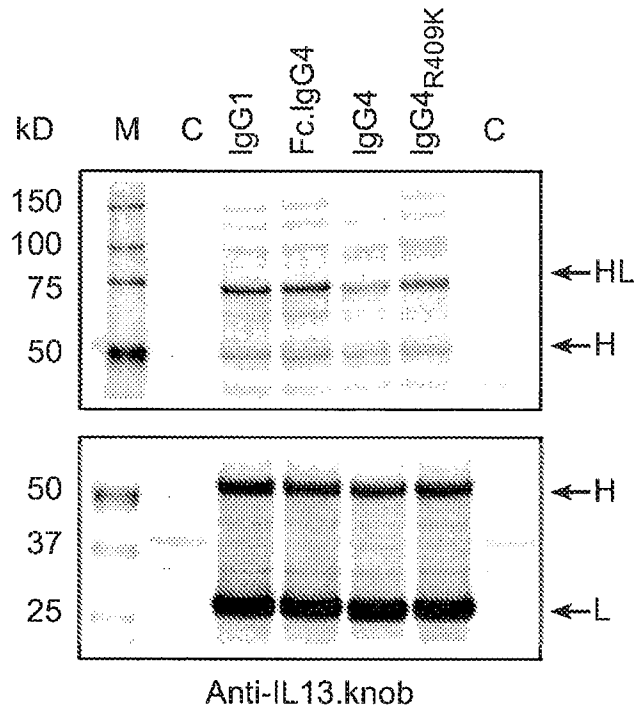

FIG. 2C

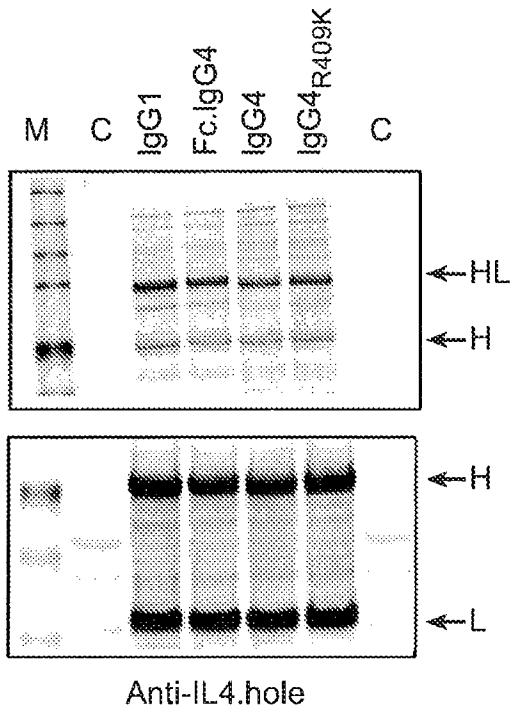

FIG. 2D

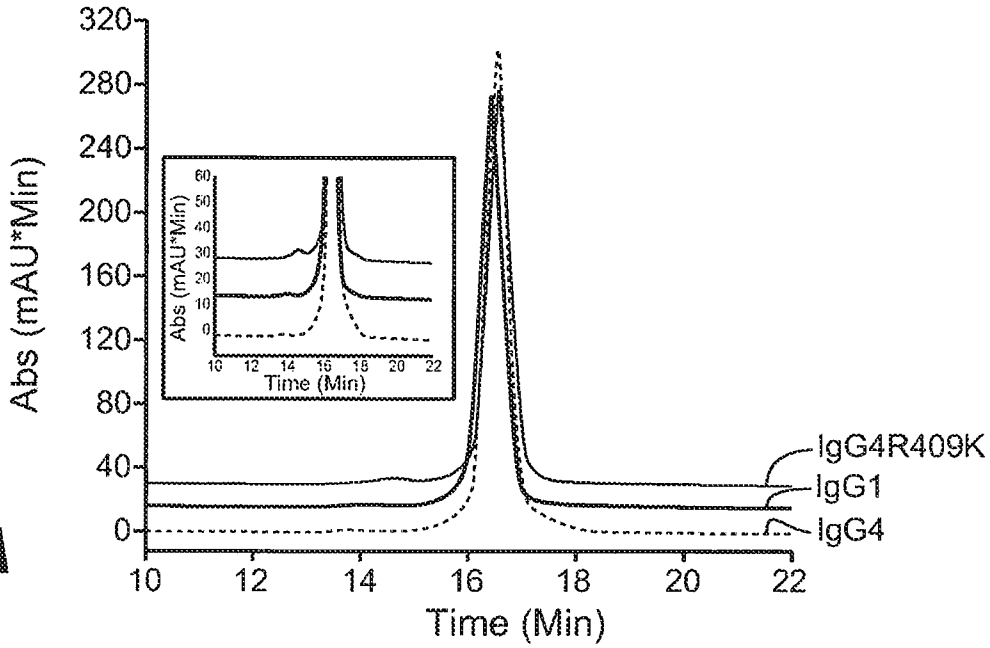

FIG. 3A

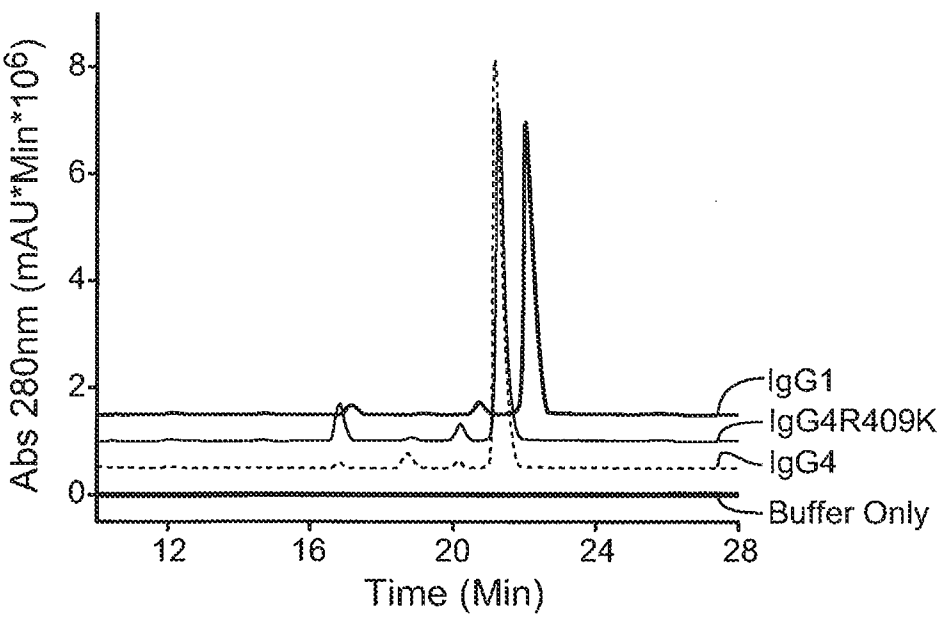

FIG. 3B

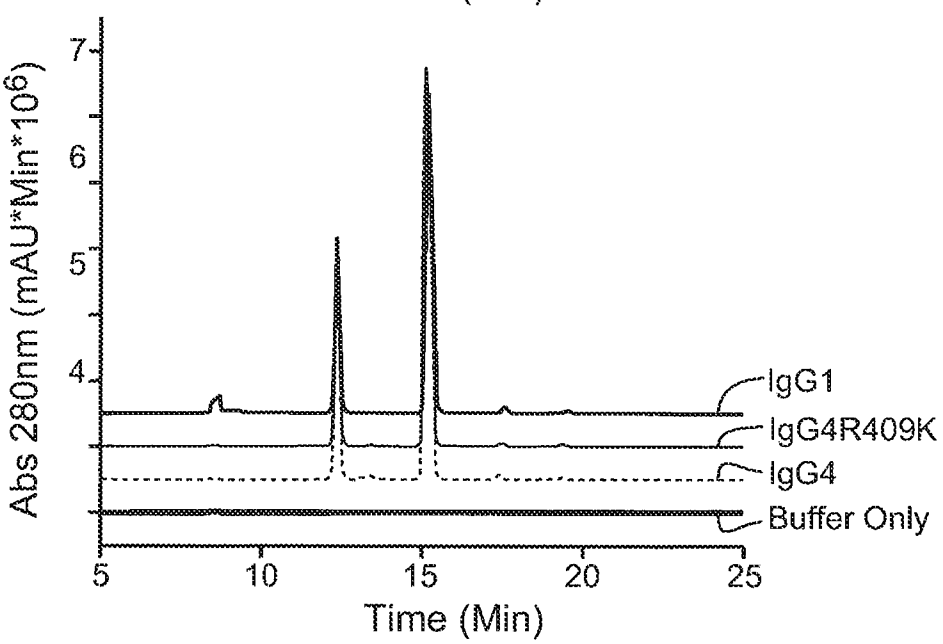

FIG. 3C

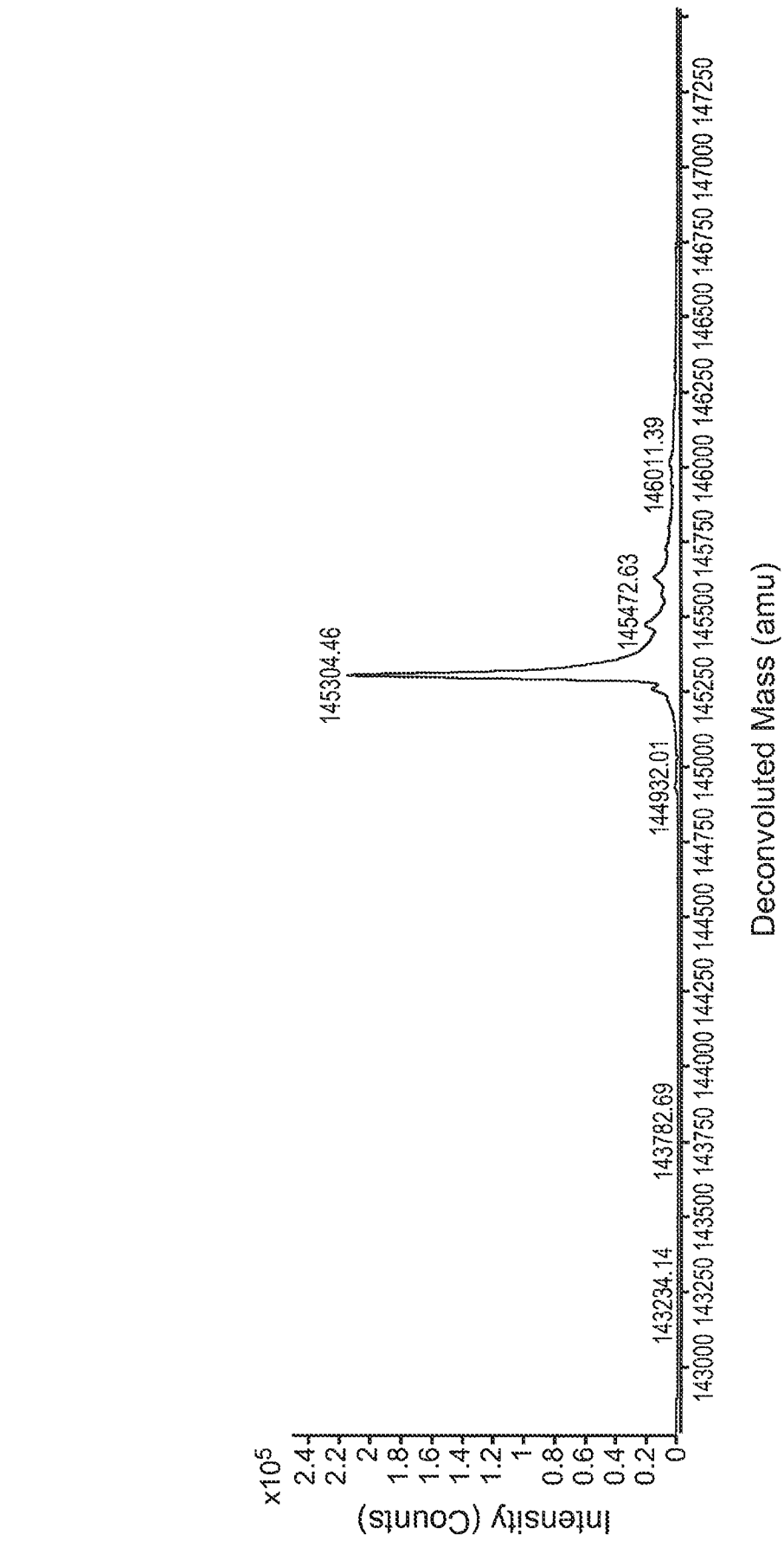
x10^5
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Intensity (Counts)
143000 143250 143500 143750 144000 144250 144500 144750 145000 145250 145500 145750 146000 146250 146500 146750 147000 147250
Deconvoluted Mass (amu)
143234.14
143782.69
144932.01
145304.46
145472.63
146011.39

FIG. 4A

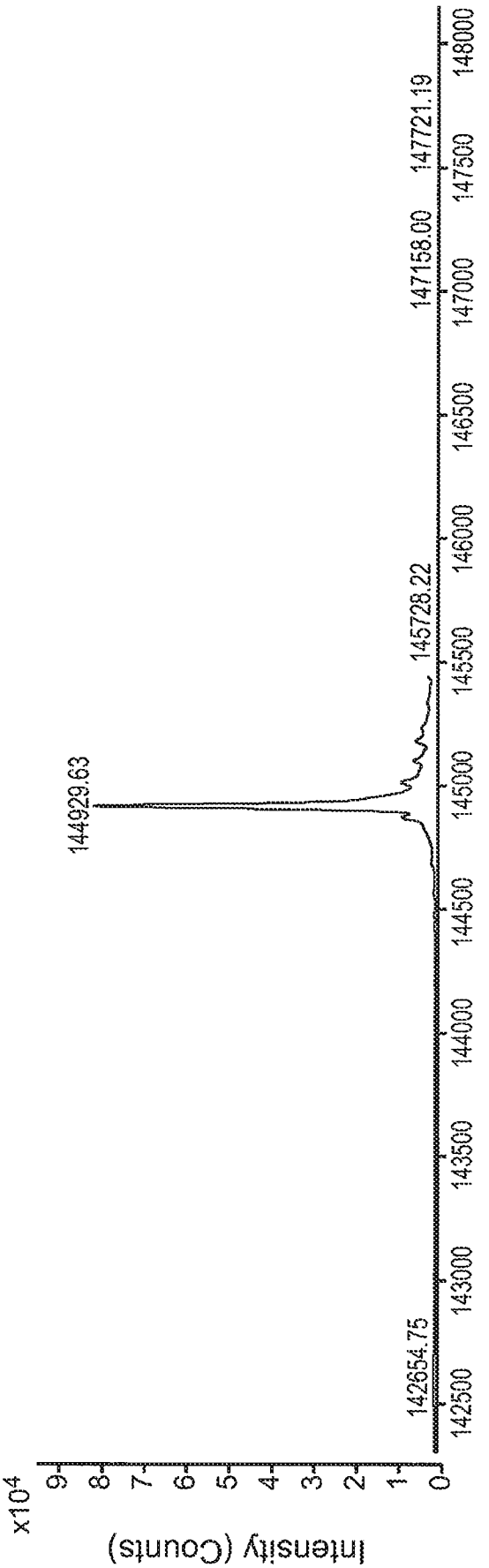
Intensity (Counts)
x10^4
9
8
7
6
5
4
3
2
1
0
142654.75
144929.63
145728.22
147158.00
147721.19
142500
143000
143500
144000
144500
145000
145500
146000
146500
147000
147500
148000
Deconvoluted Mass (amu)

FIG. 4B

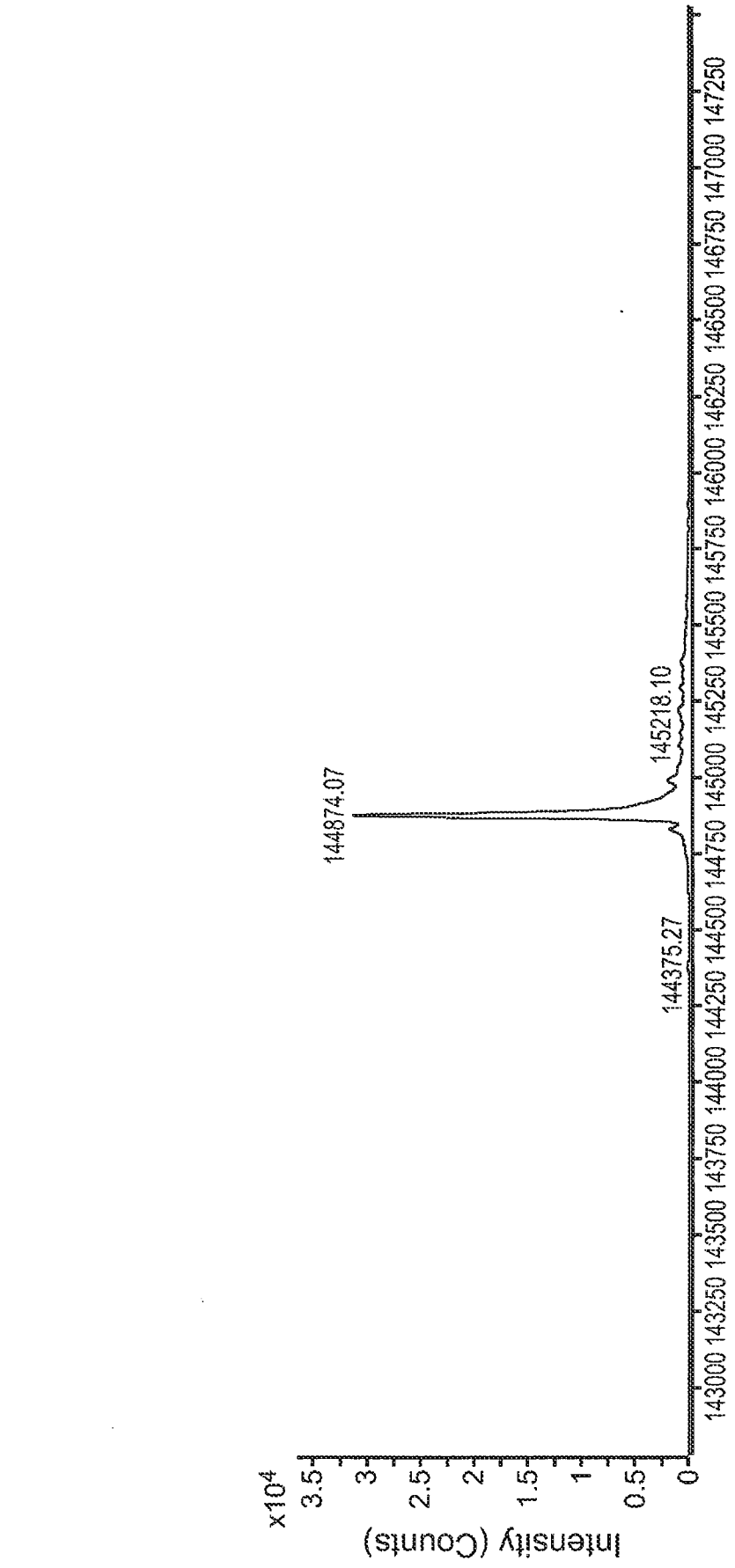
144874.07
145218.10
144375.27
Intensity (Counts)
x10^4
3.5
3
2.5
2
1.5
1
0.5
0
143000 143250 143500 143750 144000 144250 144500 144750 145000 145250 145500 145750 146000 146250 146500 146750 147000 147250
Deconvoluted Mass (amu)

FIG. 4C

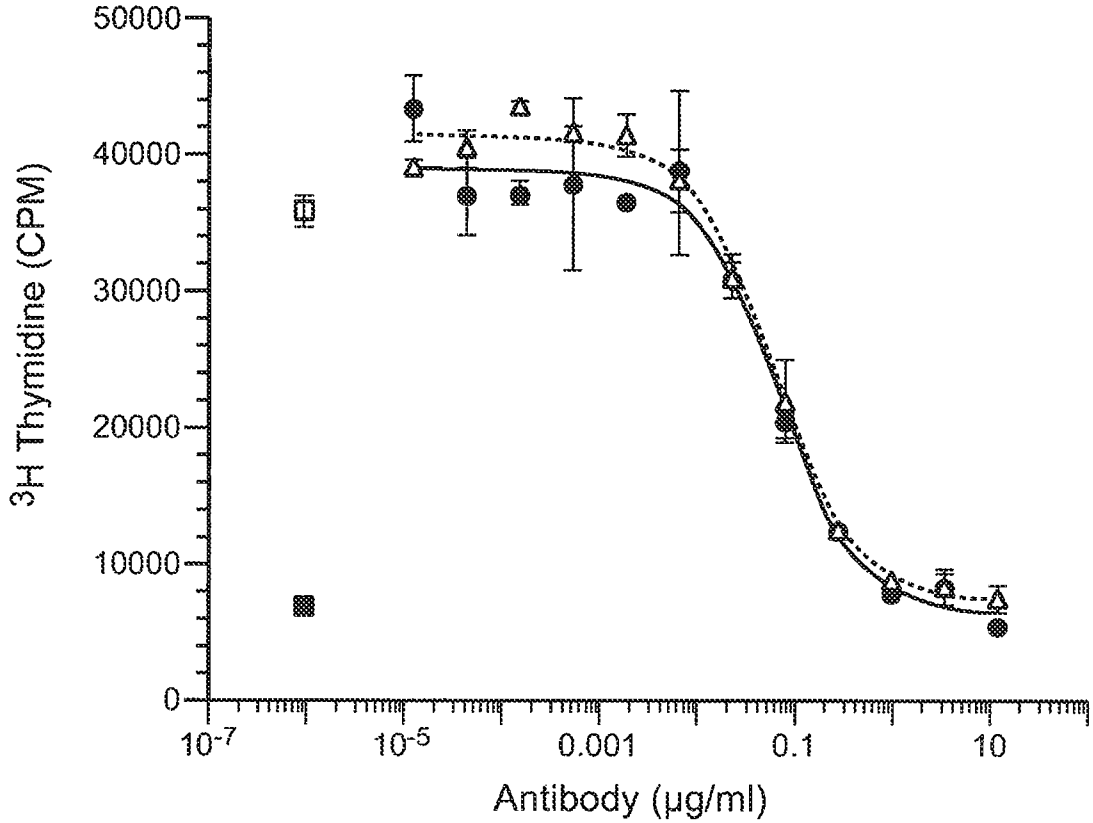
50000
40000
30000
20000
10000
0
^{3}H Thymidine (CPM)
10^{-7}
10^{-5}
0.001
0.1
10
Antibody (µg/ml)

FIG. 5A

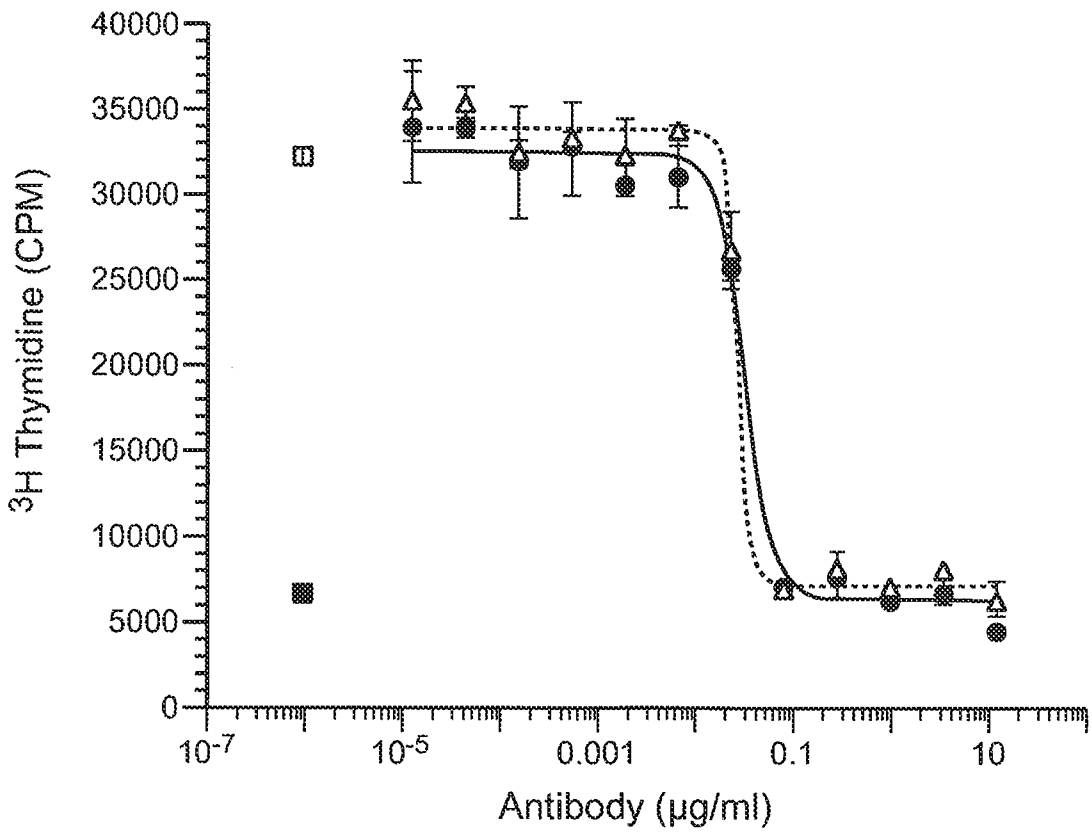
40000
35000
30000
25000
20000
15000
10000
5000
0
^{3}H Thymidine (CPM)
10^{-7}
10^{-5}
0.001
0.1
10
Antibody (µg/ml)

FIG. 5B

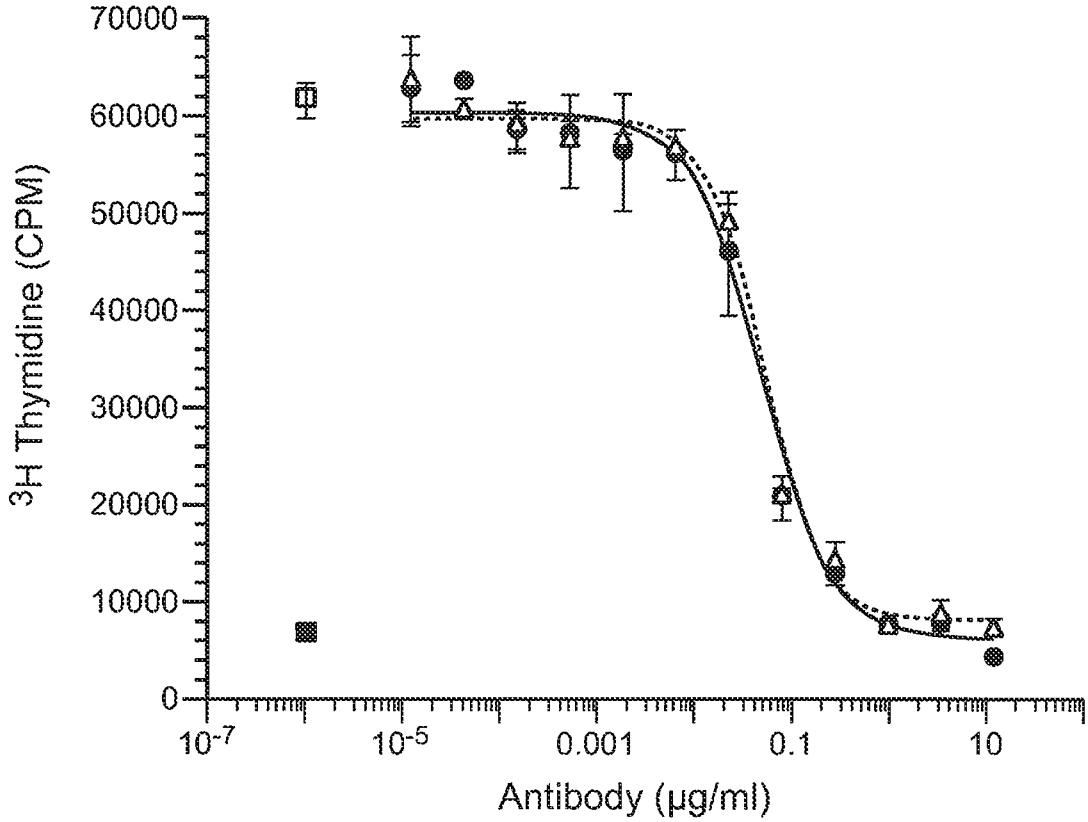
70000
60000
50000
40000
30000
20000
10000
0
^{3}H Thymidine (CPM)
10^{-7}
10^{-5}
0.001
0.1
10
Antibody (µg/ml)

FIG. 5C

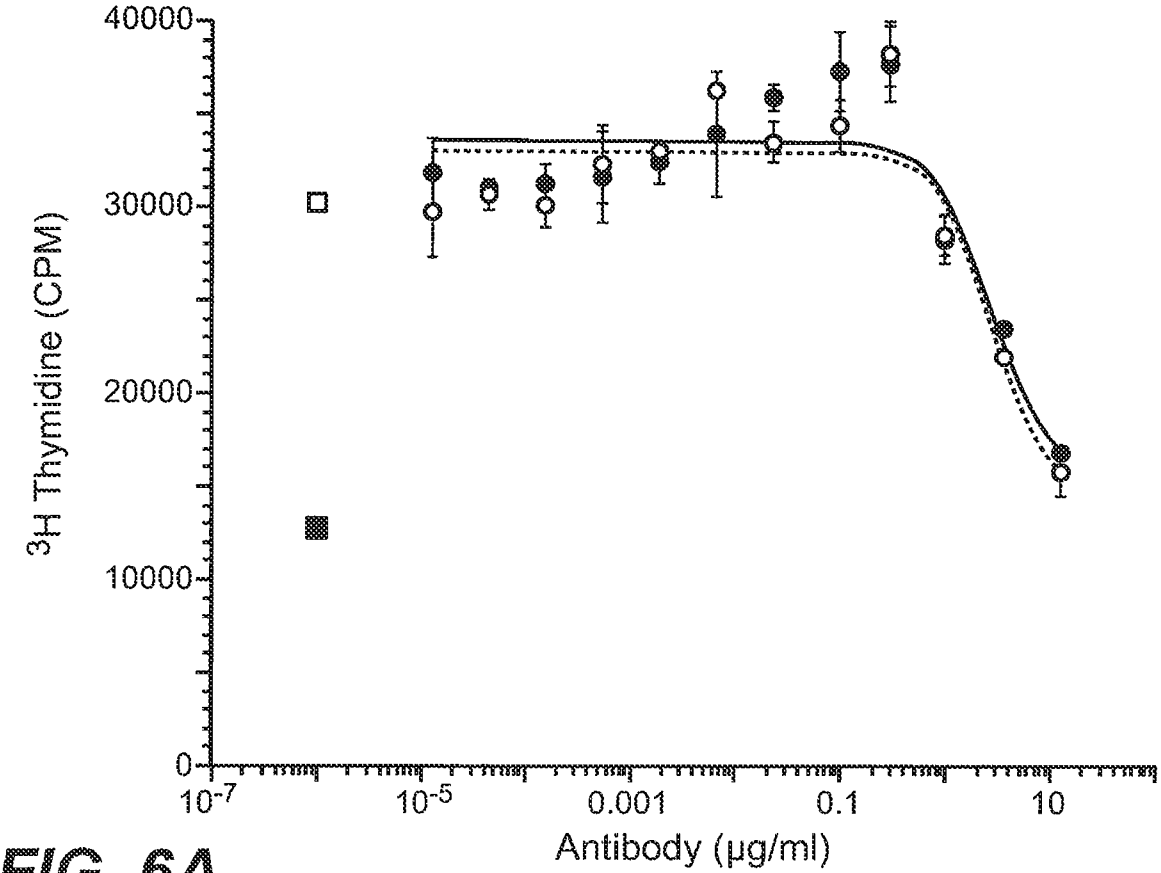
40000
30000
20000
10000
0
^{3}H Thymidine (CPM)
10^{-7}
10^{-5}
0.001
0.1
10
Antibody (µg/ml)

FIG. 6A

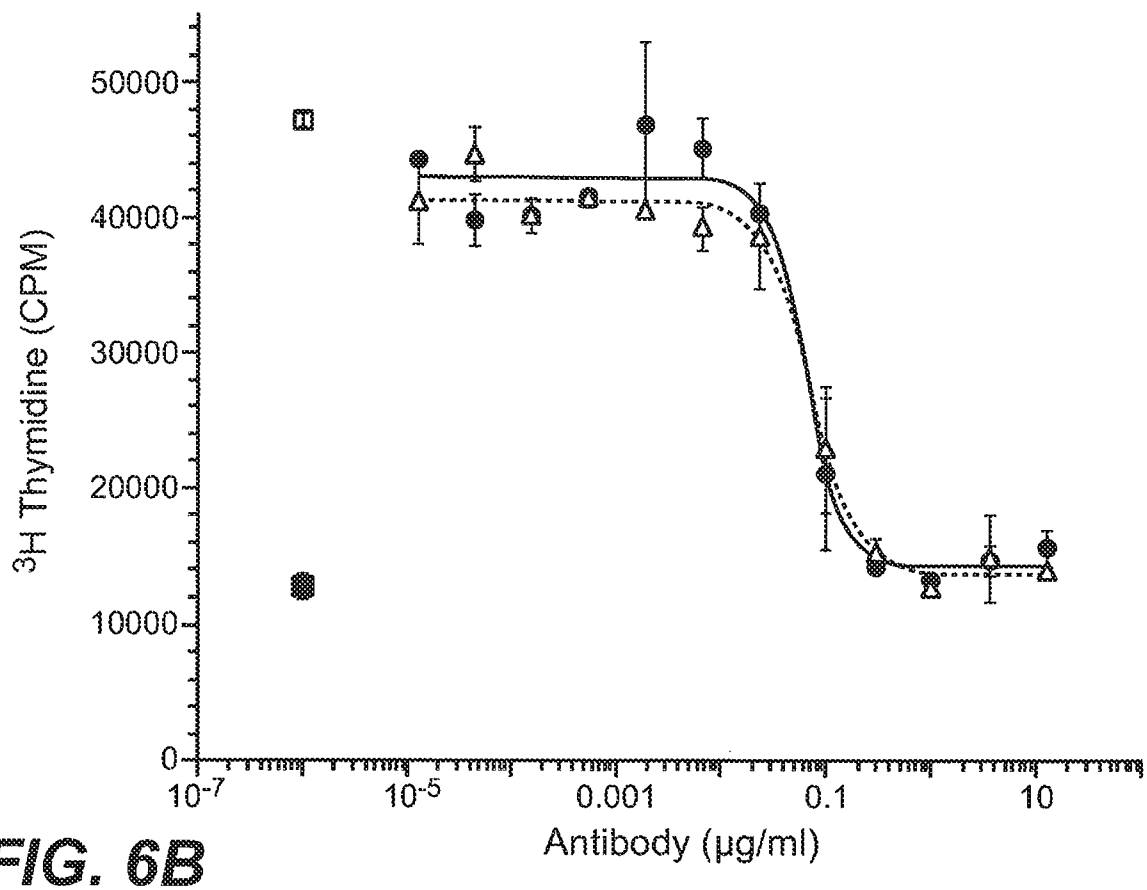
50000
40000
30000
20000
10000
0
^{3}H Thymidine (CPM)
10^{-7}
10^{-5}
0.001
0.1
10
Antibody (µg/ml)

FIG. 6B

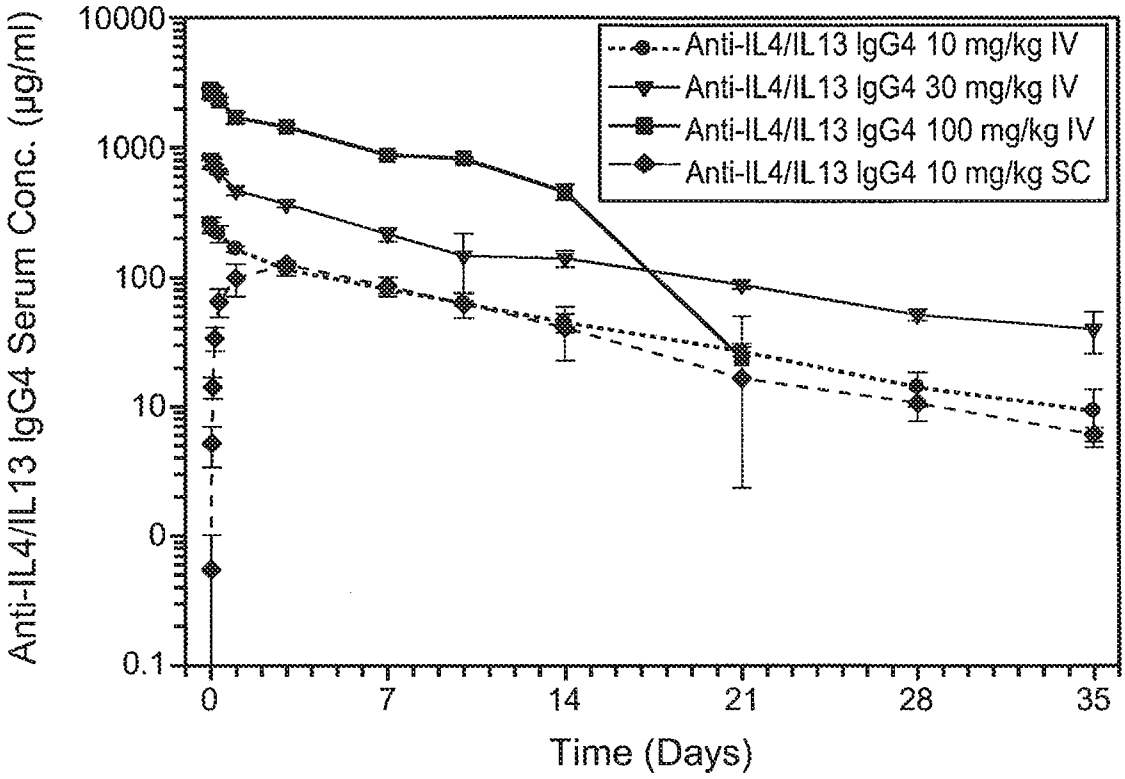
Anti-IL4/IL13 IgG4 Serum Conc. (µg/ml)
10000
1000
100
10
0
0.1
0
7
14
21
28
35
Time (Days)
Anti-IL4/IL13 IgG4 10 mg/kg IV
Anti-IL4/IL13 IgG4 30 mg/kg IV
Anti-IL4/IL13 IgG4 100 mg/kg IV
Anti-IL4/IL13 IgG4 10 mg/kg SC

FIG. 7A

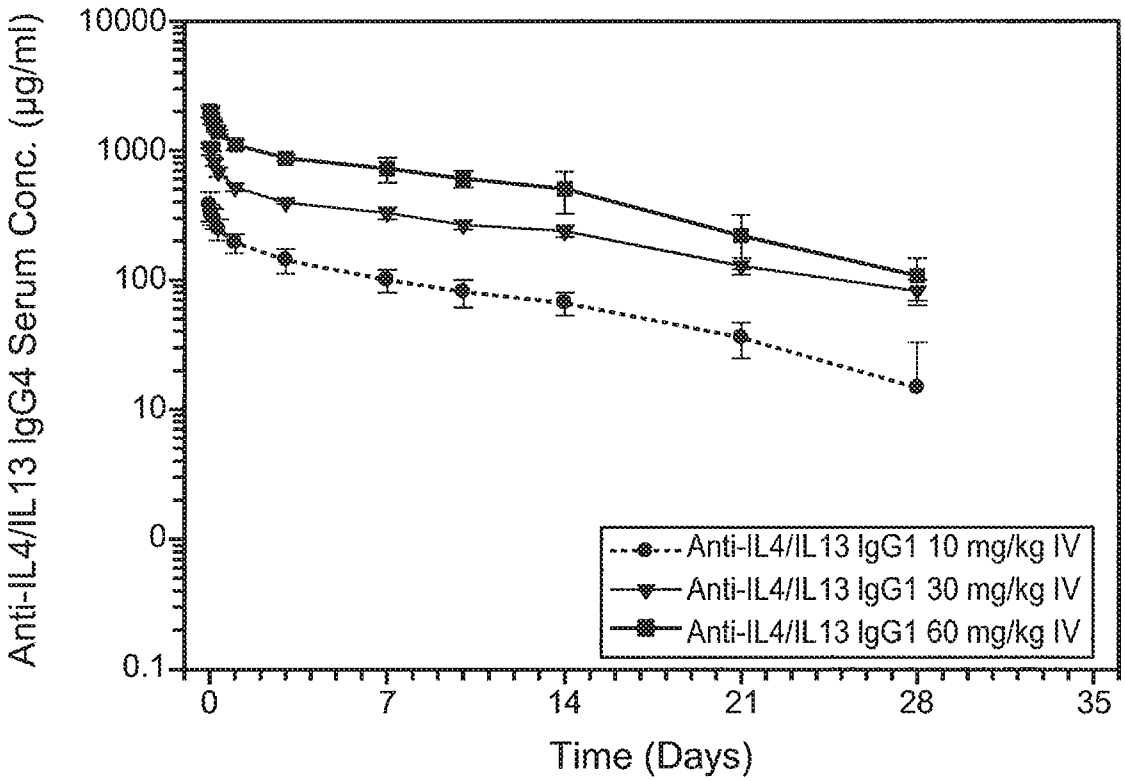
Anti-IL4/IL13 IgG4 Serum Conc. (µg/ml)
10000
1000
100
10
0
0.1
0
7
14
21
28
35
Time (Days)
Anti-IL4/IL13 IgG1 10 mg/kg IV
Anti-IL4/IL13 IgG1 30 mg/kg IV
Anti-IL4/IL13 IgG1 60 mg/kg IV

FIG. 7B

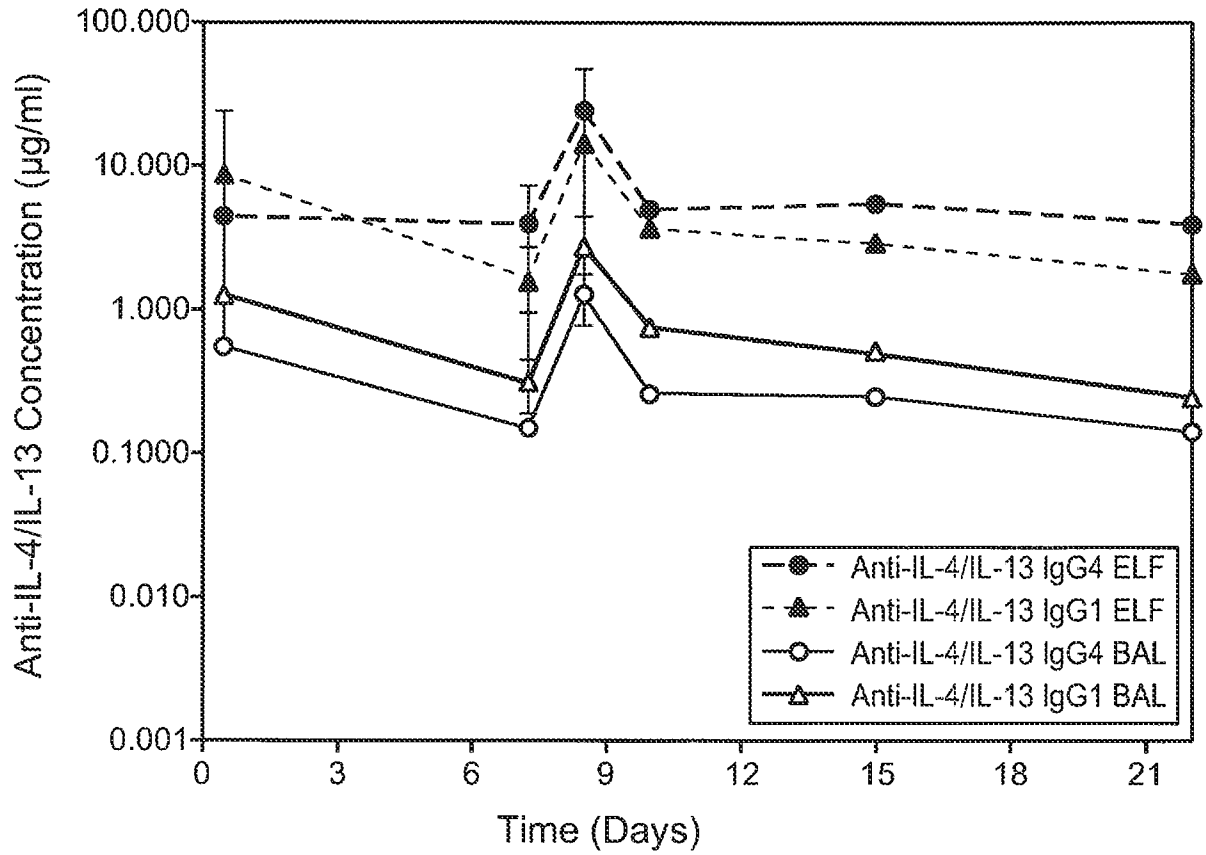
100.000
10.000
1.000
0.1000
0.010
0.001
Anti-IL-4/IL-13 Concentration (µg/ml)
0
3
6
9
12
15
18
21
Time (Days)
Anti-IL-4/IL-13 IgG4 ELF
Anti-IL-4/IL-13 IgG1 ELF
Anti-IL-4/IL-13 IgG4 BAL
Anti-IL-4/IL-13 IgG1 BAL

FIG. 8

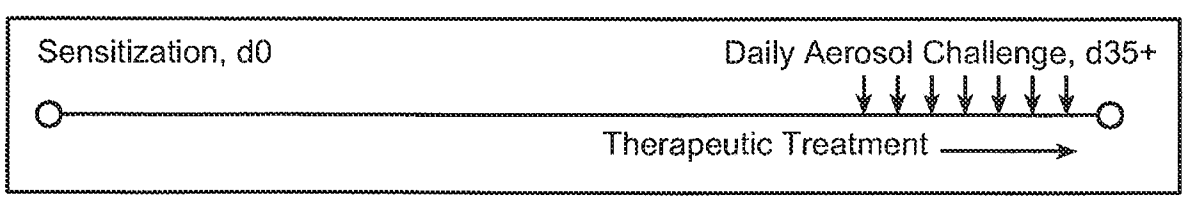
Sensitization, d0
Daily Aerosol Challenge, d35+
Therapeutic Treatment

FIG. 9A

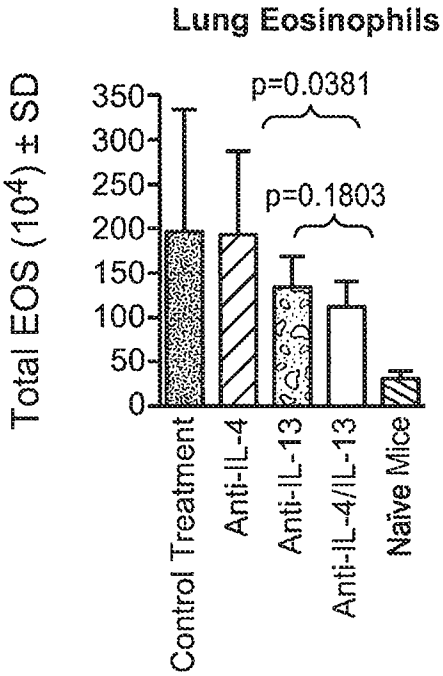

FIG. 9B

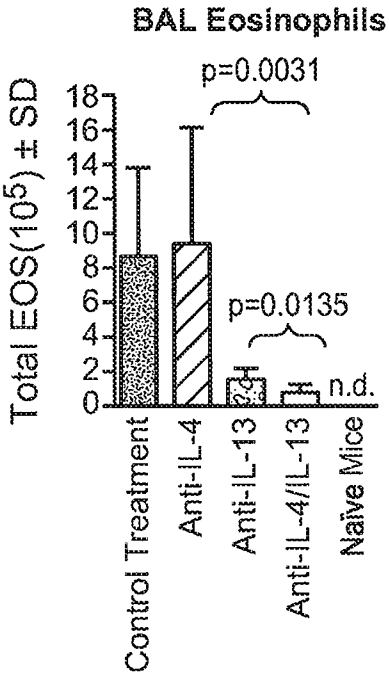

FIG. 9C

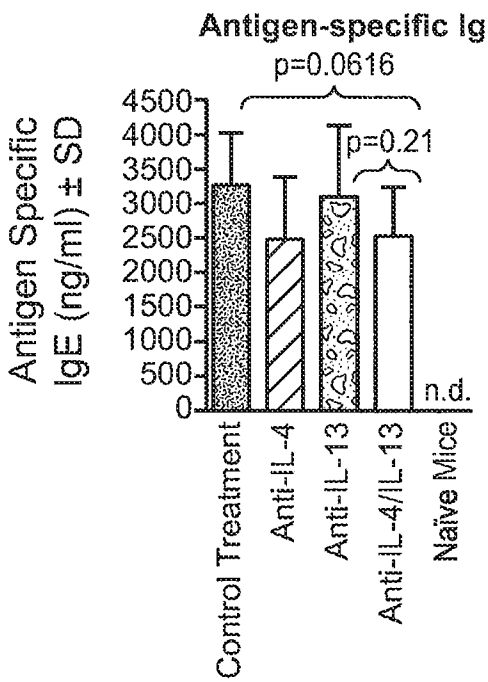

FIG. 9D

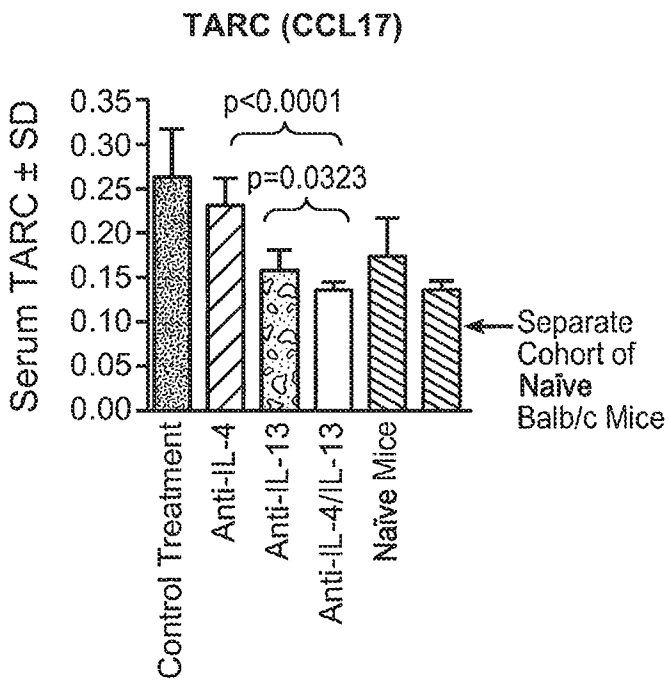

FIG. 9E

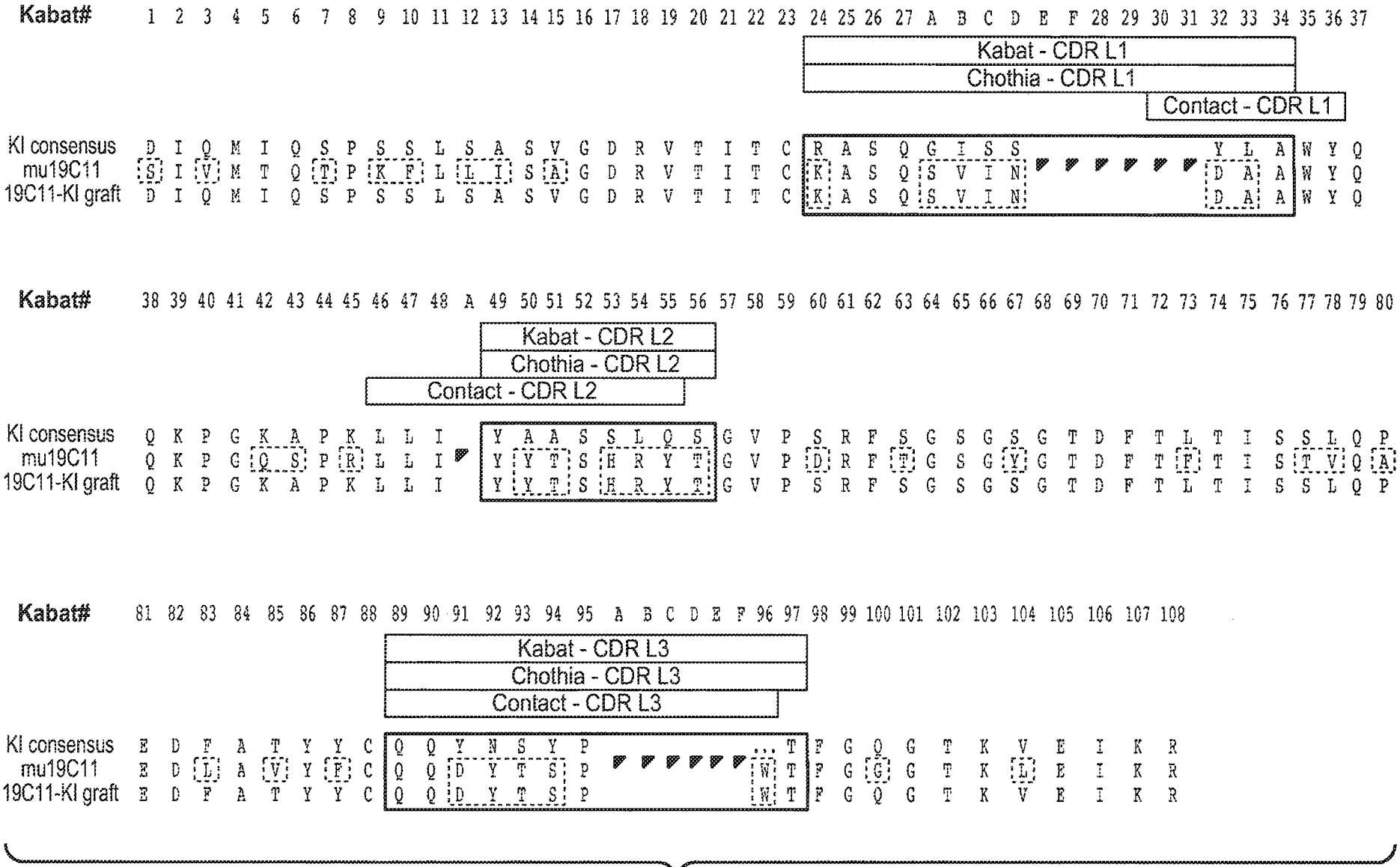
Kabat#
Kabat - CDR L1
Chothia - CDR L1
Contact - CDR L1
KI consensus
mu19C11
19C11-KI graft
Kabat#
Kabat - CDR L2
Chothia - CDR L2
Contact - CDR L2
KI consensus
mu19C11
19C11-KI graft
Kabat#
Kabat - CDR L3
Chothia - CDR L3
Contact - CDR L3
KI consensus
mu19C11
19C11-KI graft

FIG. 10

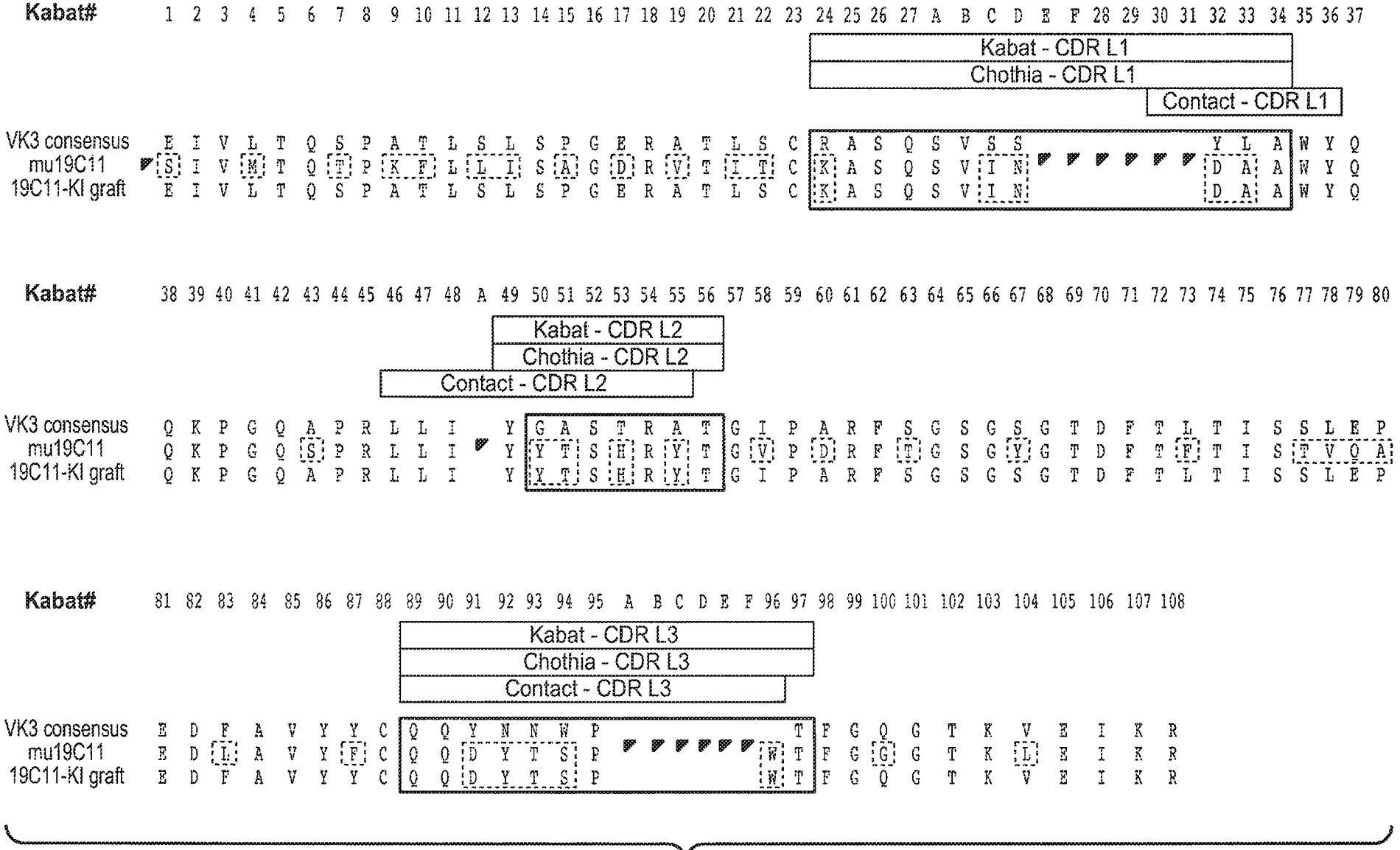
Kabat#
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 A B C D E F 28 29 30 31 32 33 34 35 36 37
Kabat - CDR L1
Chothia - CDR L1
Contact - CDR L1
VK3 consensus E I V L T Q S P A T L S L S P G E R A T L S C R A S Q S V S S Y L A W Y Q
mu19C11 S I V M T Q T P K F L L I S A G D R V T I T C K A S Q S V I N D A A W Y Q
19C11-KI graft E I V L T Q S P A T L S L S P G E R A T L S C K A S Q S V I N D A A W Y Q
Kabat#
38 39 40 41 42 43 44 45 46 47 48 A 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
Kabat - CDR L2
Chothia - CDR L2
Contact - CDR L2
VK3 consensus Q K P G Q A P R L L I Y G A S T R A T G I P A R F S G S G S G T D F T L T I S S L E P
mu19C11 Q K P G Q S P R L L I Y Y T S H R Y T G V P D R F T G S G Y G T D F T F T I S T V Q A
19C11-KI graft Q K P G Q A P R L L I Y Y T S H R Y T G I P A R F S G S G S G T D F T L T I S S L E P
Kabat#
81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 A B C D E F 96 97 98 99 100 101 102 103 104 105 106 107 108
Kabat - CDR L3
Chothia - CDR L3
Contact - CDR L3
VK3 consensus E D F A V Y Y C Q Q Y N N W P T F G Q G T K V E I K R
mu19C11 E D L A V Y F C Q Q D Y T S P W T F G G G T K L E I K R
19C11-KI graft E D F A V Y Y C Q Q D Y T S P W T F G Q G T K V E I K R

FIG. 11

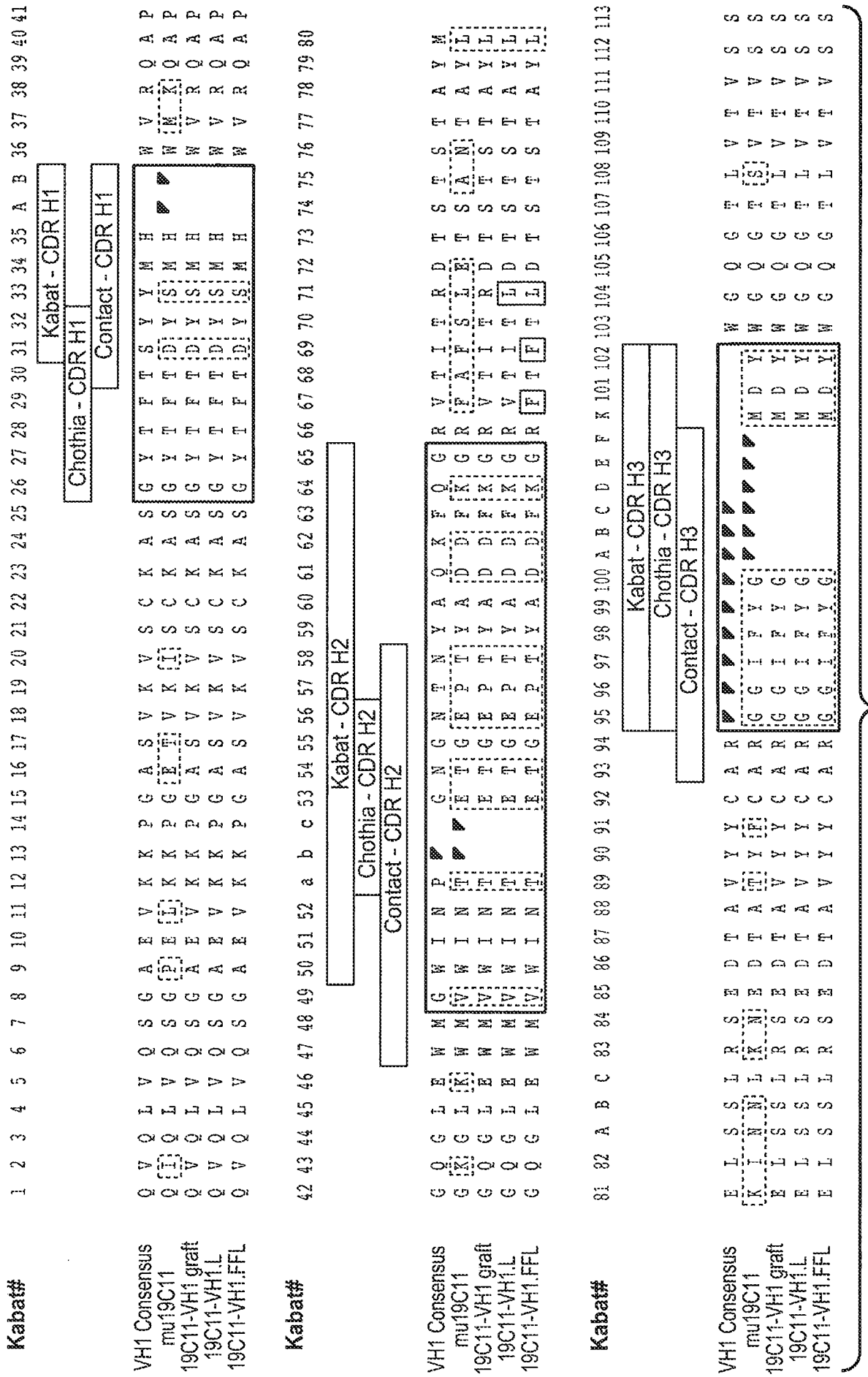
Kabat#
Kabat - CDR H1
Chothia - CDR H1
Contact - CDR H1
VH1 Consensus
mu19C11
19C11-VH1 graft
19C11-VH1.L
19C11-VH1.FFL
Kabat - CDR H2
Chothia - CDR H2
Contact - CDR H2
Kabat - CDR H3
Chothia - CDR H3
Contact - CDR H3

FIG. 12

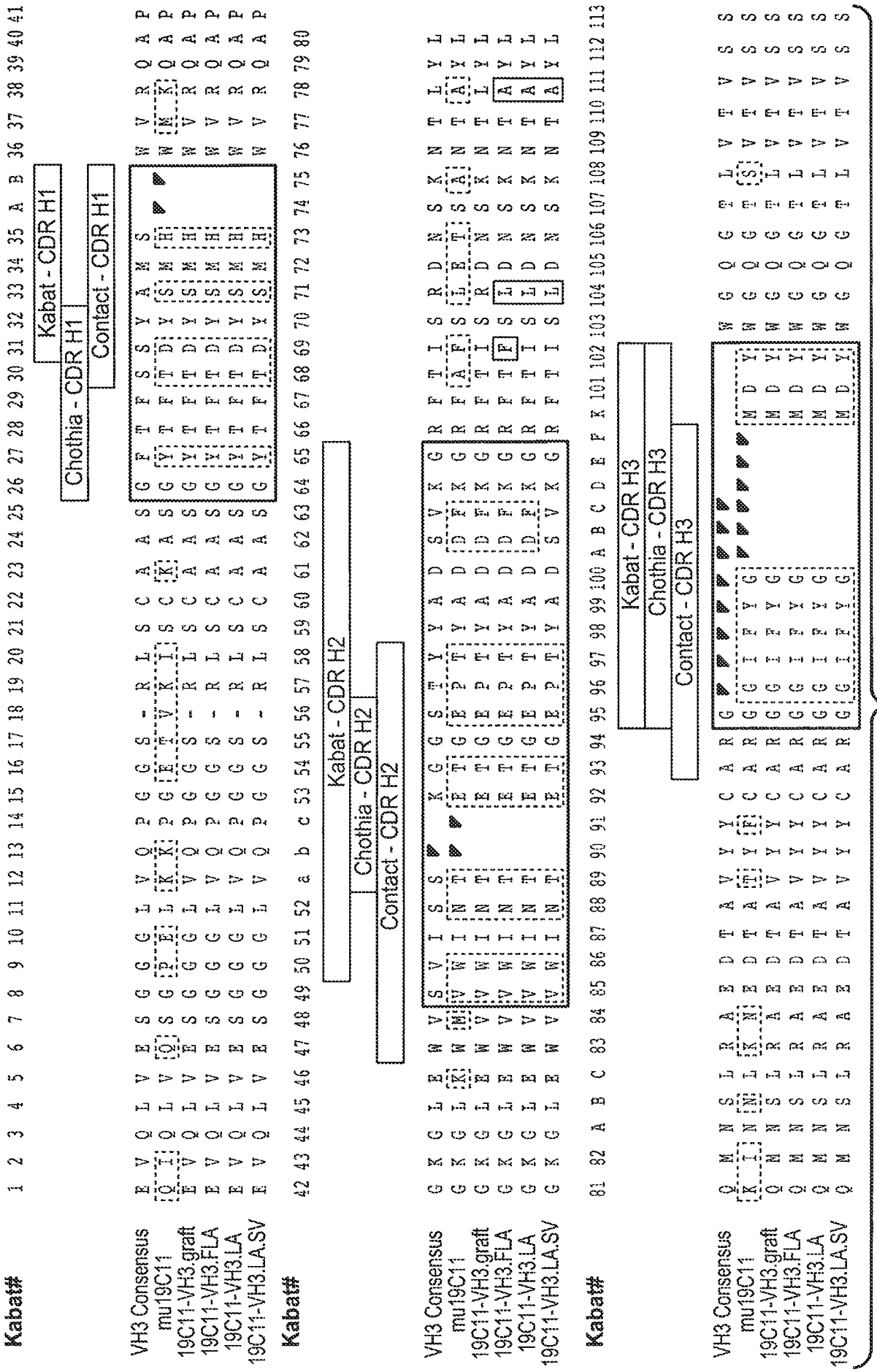
Kabat - CDR H1
Chothia - CDR H1
Contact - CDR H1
Kabat - CDR H2
Chothia - CDR H2
Contact - CDR H2
Kabat - CDR H3
Chothia - CDR H3
Contact - CDR H3
Kabat#
VH3 Consensus
mu19C11
19C11-VH3.graft
19C11-VH3.FLA
19C11-VH3.LA
19C11-VH3.LA.SV

FIG. 13

	HuKI Kd (pM)	HuKIII Kd (pM)
$HuVH_I$	24	<10
$HuVH_I$ (L)	<10	<10
$HuVH_I$ (FFL)	<10	11
$HuVH_{III}$	84	26
$HuVH_{III}$ (LA)	<10	<10
$HuVH_{III}$ (FLA)	140	11
19C11 Chimera	<10	

FIG. 14

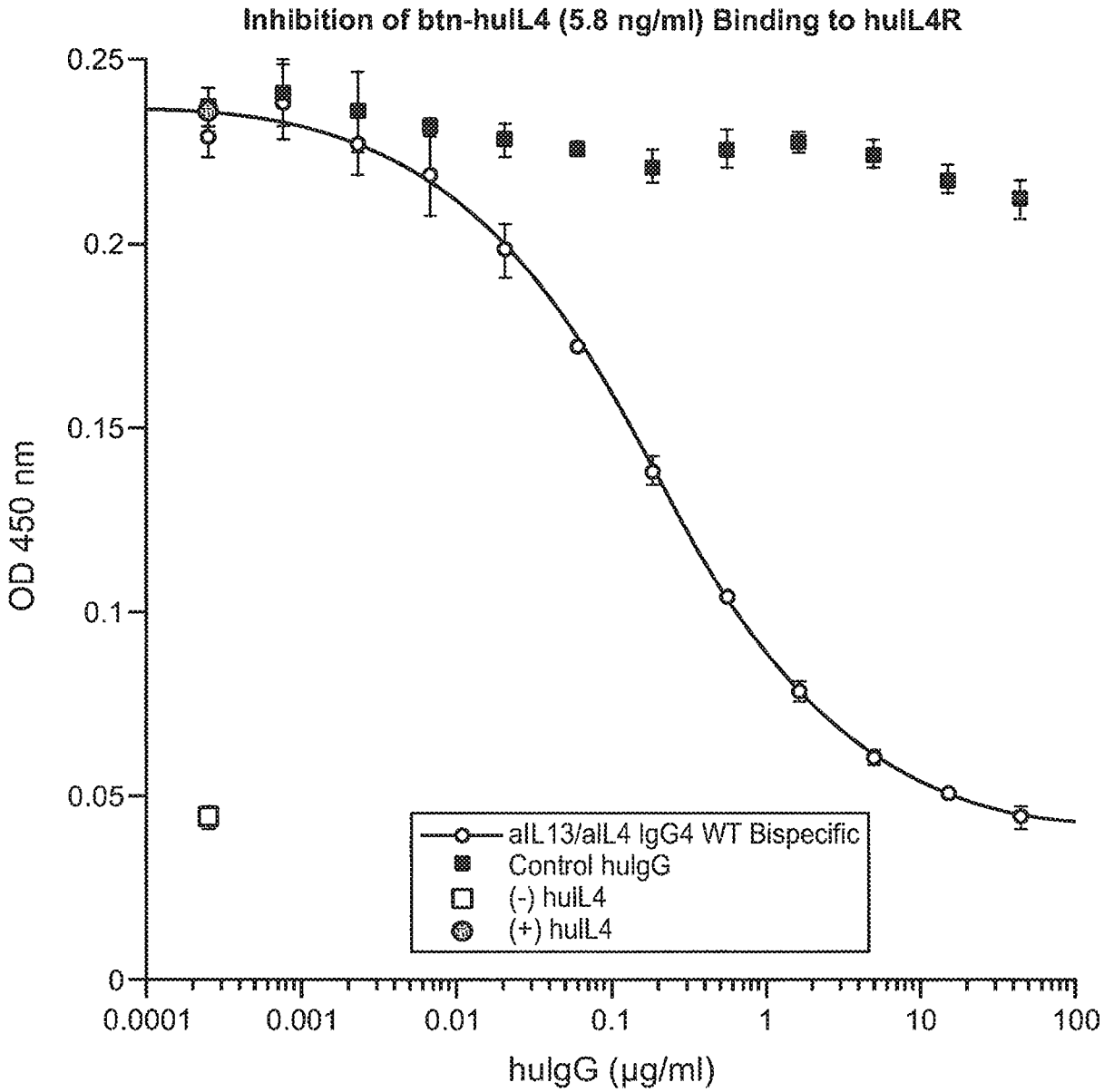
Inhibition of btn-hulL4 (5.8 ng/ml) Binding to hulL4R
OD 450 nm
0.25
0.2
0.15
0.1
0.05
0
0.0001
0.001
0.01
0.1
1
10
100
hulgG (µg/ml)
alL13/alL4 IgG4 WT Bispecific
Control hulgG
(-) hulL4
(+) hulL4

FIG. 15

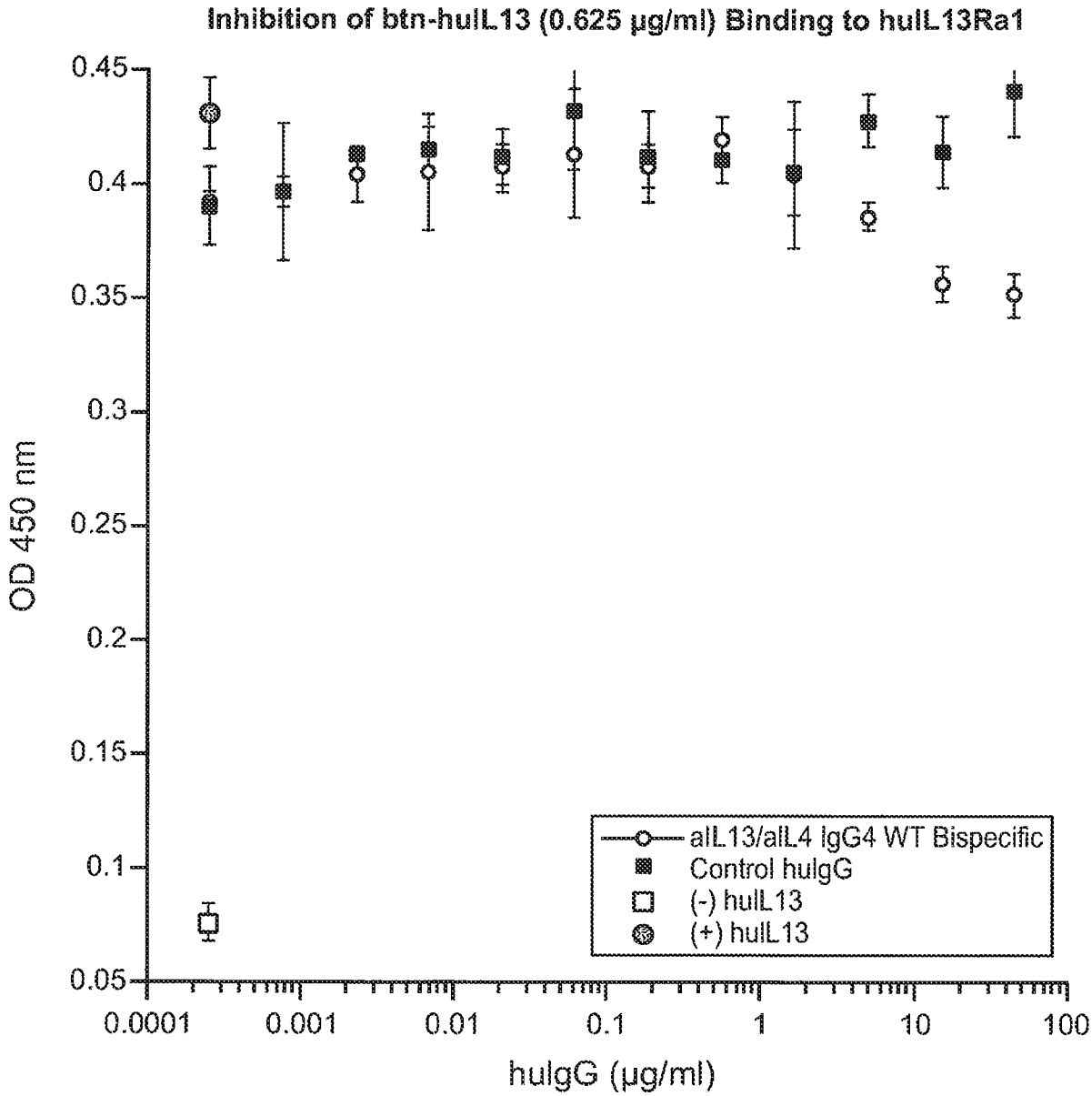
Inhibition of btn-hulL13 (0.625 µg/ml) Binding to hulL13Ra1
OD 450 nm
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0.0001
0.001
0.01
0.1
1
10
100
hulgG (µg/ml)
alL13/alL4 IgG4 WT Bispecific
Control hulgG
(-) hulL13
(+) hulL13

FIG. 16

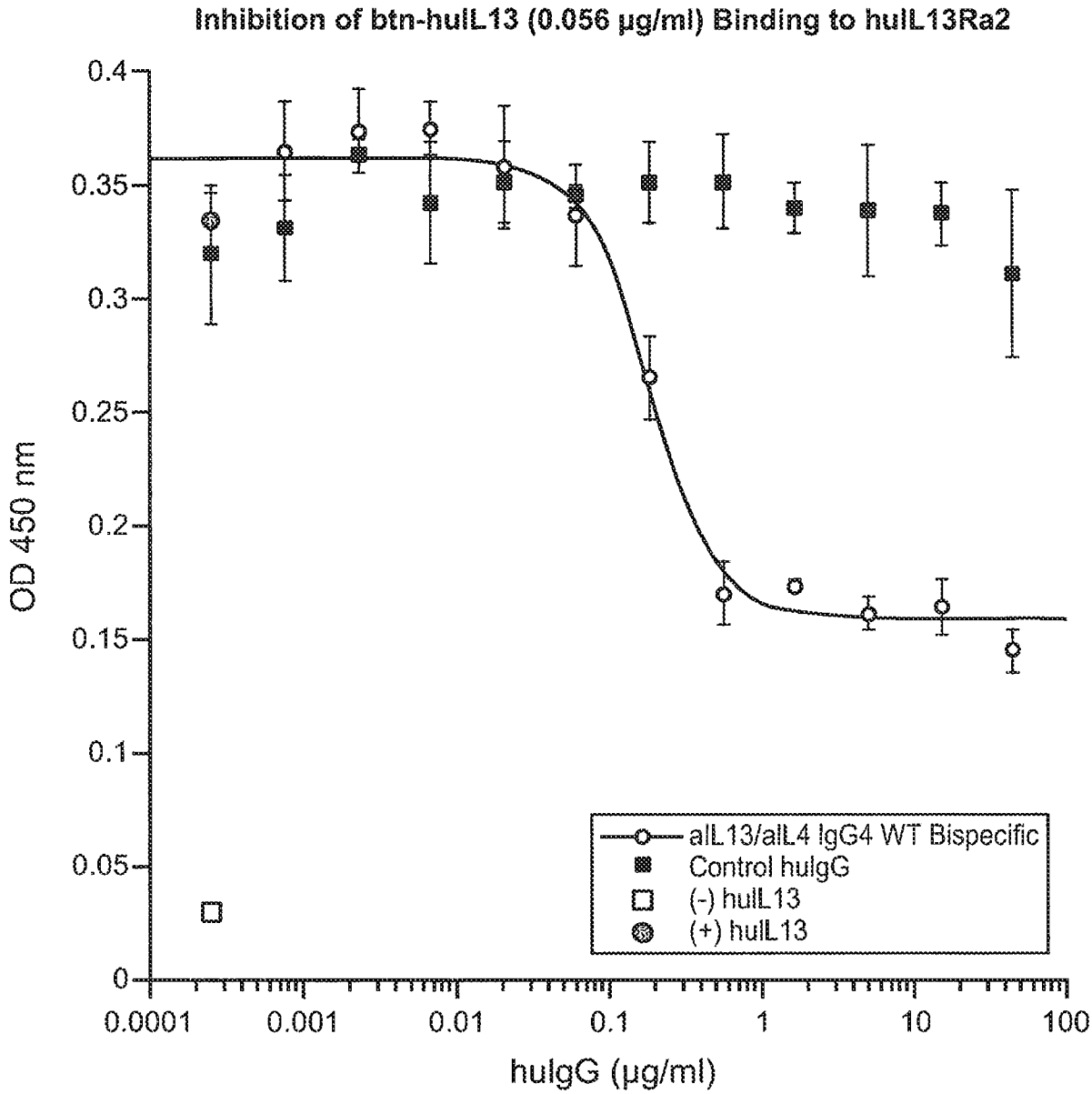
Inhibition of btn-hulL13 (0.056 µg/ml) Binding to hulL13Ra2
OD 450 nm
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0.0001
0.001
0.01
0.1
1
10
100
hulgG (µg/ml)
alL13/alL4 IgG4 WT Bispecific
Control hulgG
(-) hulL13
(+) hulL13

FIG. 17

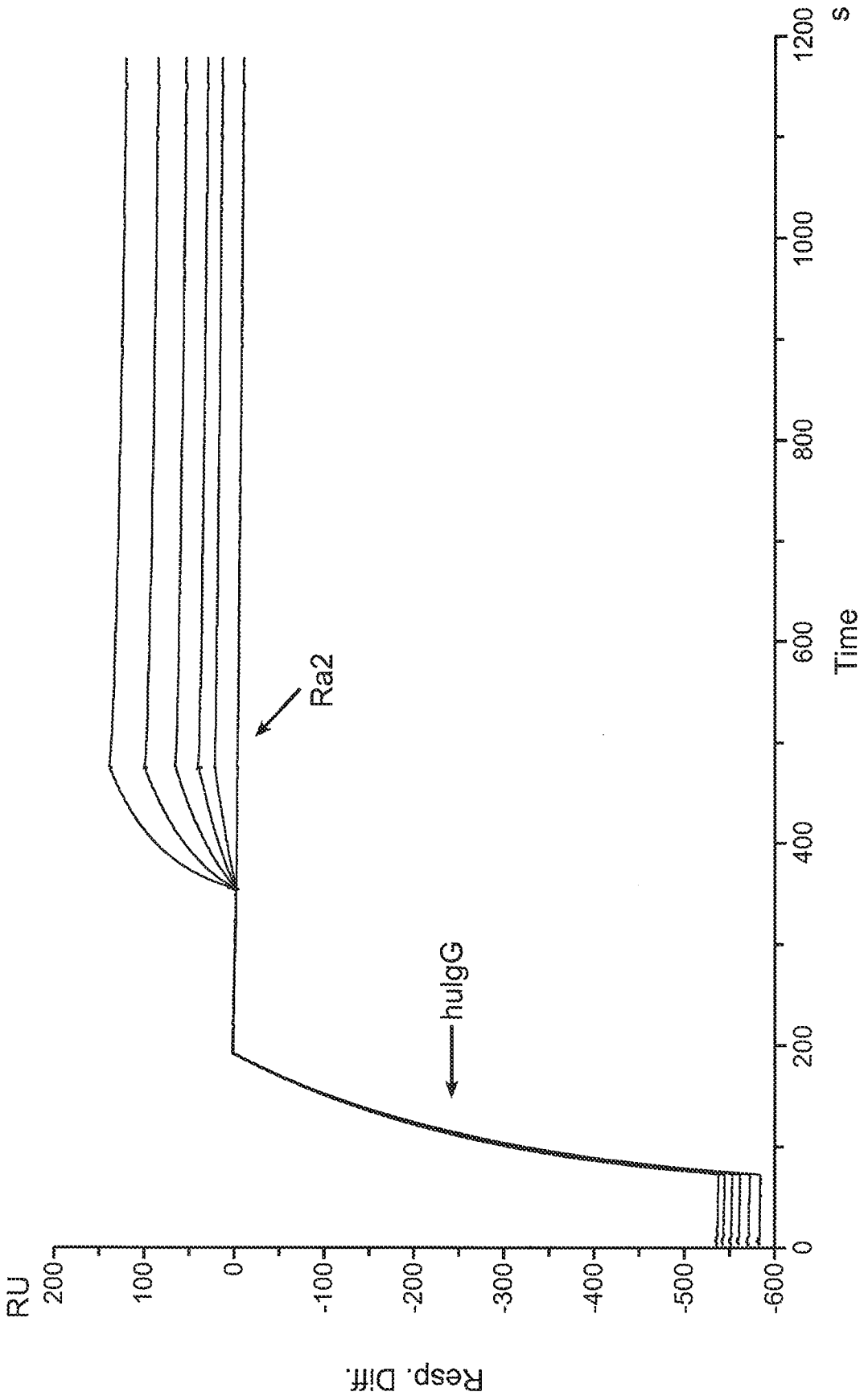
RU
200
100
0
-100
-200
-300
-400
-500
-600
Resp. Diff.
0
200
400
600
800
1000
1200
s
Time
Ra2
huIgG

FIG. 18

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Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

(88) Date of publication of the international search report:
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(54) Title: ANTI-IL-4 ANTIBODIES AND BISPECIFIC ANTIBODIES AND USES THEREOF

(57) Abstract: The invention provides anti-IL-4 antibodies and bispecific antibodies and methods of using the same.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 14/32998

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/00, A61K 39/395, C07K 16/00 (2014.01)
CPC - C07K 2317/31, A61K 38/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC- 424/136.1, 424/145.1, 530/388.23

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC- 530/387.3; CPC- C07K 2317/31, A61K 38/00, A61K 2039/505, C07K 16/468, C07K 16/30, C07K 2317/24, C07K 2317/92, C07K 2319/00, C07K 2316/96 (2014.01)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(PGPB,USPT,USOC,EPAB,JPAB); PatBase; Google/Scholar: multispecific, bifunctional, bispecific antibody, IL4, IL13, Genentech
GenCore 6.4.1: SEQ ID NO: 9, 10, 12-15,17,18, 38, 39

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- A	US 2010/0226923 A1 (RAO, et al.) 09 September 2010 (9.09.2010) claims 48-58; para [0312], [0318]-[0326], [0329], Table 5, 7	1 --- 2-4, 8, 19, 36-39, 42
X	KASAIAN, et al. An IL-4/IL-13 Dual Antagonist Reduces Lung Inflammation, Airway Hyperresponsiveness, and IgE Production in Mice. Am J Respir Cell Mol Biol ePub 28 February 2013, 49(1):37-46; Abstract, pg 38, col 1; pg 39, Figure 2 and its legend	1
X	US 2007/0104710 A1 (TOMLINSON, et al.) 10 May 2007 (10.05.2007) claims 9, 12, 13	1
A	US 2011/0182897 A1 (HULTBERG, et al.) 28 July 2011 (28.07.2011) SEQ ID NO 2574, amino acids 106-114	2-4, 8, 19, 36-39, 42
A	WO 2006/029459 A1 (BATORI, et al.) 23 March 2006 (23.03.2006) SEQ ID NO 4	4, 8, 19, 36-39, 42

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 August 2014 (27.08.2014)	19 SEP 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 14/32998

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 5-7, 9-18, 20-35, 40, 41, 43-68
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

(19)中华人民共和国国家知识产权局

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L·C·吴 Y·张
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(51)Int.Cl.
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C07K 16/00(2006.01)

权利要求书6页 说明书69页
序列表34页 附图25页

(54)发明名称

抗IL-4抗体和双特异性抗体及其用途

(57)摘要

本发明涉及抗IL-4抗体和双特异性抗体及其使用方法。

CN 105307676 A

1. 多特异性抗体，其包含抗原结合结构域，所述抗原结合结构域包含特异地结合 IL-4 的第一 VH/VL 单位和特异地结合 IL-13 的第二 VH/VL 单位，其中所述抗体：

a) 抑制 IL-4 与 IL-4 受体 α（IL-4Rα）的结合，

b) 在体外抑制 IL-4- 诱导的细胞增殖，和 / 或

b) 在体外抑制 IL-13- 诱导的细胞增殖。

2. 权利要求 1 的多特异性抗体，其中第一 VH/VL 单位包括包含 SEQ ID NO:14 的氨基酸序列的 HVR-H3、包含 SEQ ID NO:17 的氨基酸序列的 HVR-L3、和包含 SEQ ID NO:13 或 SEQ ID NO:18 的氨基酸序列的 HVR-H2。

3. 权利要求 1 或权利要求 2 的多特异性抗体，其中第一 VH/VL 单位包括包含 SEQ ID NO:12 的氨基酸序列的 HVR-H1、包含 SEQ ID NO:13 或 SEQ ID NO:18 的氨基酸序列的 HVR-H2、和包含 SEQ ID NO:14 的氨基酸序列的 HVR-H3。

4. 权利要求 1-3 之任一项的多特异性抗体，其中第一 VH/VL 单位包括包含 SEQ ID NO:15 的氨基酸序列的 HVR-L1、包含 SEQ ID NO:16 的氨基酸序列的 HVR-L2、和包含 SEQ ID NO:17 的氨基酸序列的 HVR-L3。

5. 权利要求 1-4 之任一项的多特异性抗体，其中第一 VH/VL 单位包含：(a) 与 SEQ ID NO:9 的氨基酸序列具有至少 95% 序列同一性的 VH 序列；(b) 与 SEQ ID NO:10 的氨基酸序列具有至少 95% 序列同一性的 VL 序列；或 (c)(a) 的 VH 序列和 (b) 的 VL 序列。

6. 权利要求 1-5 之任一项的多特异性抗体，其中第一 VH/VL 单位包含选自 SEQ ID NOs:1 和 3 至 9 的 VH 序列。

7. 权利要求 1-6 之任一项的多特异性抗体，其中第一 VH/VL 单位包含选自 SEQ ID NOs:2、10 和 11 的 VL 序列。

8. 权利要求 1 的多特异性抗体，其中第一 VH/VL 单位包含 SEQ ID NO:9 的 VH 序列和 SEQ ID NO:10 的 VL 序列。

9. 权利要求 1-8 之任一项的多特异性抗体，其中第二 VH/VL 单位包含：

(a) 包含 SEQ ID NO:23 的氨基酸序列的 HVR-H3、包含 SEQ ID NO:26 的氨基酸序列的 HVR-L3、和包含 SEQ ID NO:22 的氨基酸序列的 HVR-H2；或

(b) 包含 SEQ ID NO:52 的氨基酸序列的 HVR-H3、包含 SEQ ID NO:55 的氨基酸序列的 HVR-L3、和包含 SEQ ID NO:51 的氨基酸序列的 HVR-H2。

10. 权利要求 1-9 之任一项的多特异性抗体，其中第二 VH/VL 单位包含：

(a) 包含 SEQ ID NO:21 的氨基酸序列或 SEQ ID NO:60 的氨基酸序列的 HVR-H1、包含 SEQ ID NO:22 的氨基酸序列的 HVR-H2、和包含 SEQ ID NO:23 的氨基酸序列的 HVR-H3；或

(b) 包含 SEQ ID NO:50 的氨基酸序列的 HVR-H1、包含 SEQ ID NO:51 的氨基酸序列的 HVR-H2、和包含 SEQ ID NO:52 的氨基酸序列的 HVR-H3。

11. 权利要求 1-10 之任一项的多特异性抗体，其中第二 VH/VL 单位包含：

(a) 包含 SEQ ID NO:24 的氨基酸序列的 HVR-L1、包含 SEQ ID NO:25 的氨基酸序列的 HVR-L2、和包含 SEQ ID NO:26 的氨基酸序列的 HVR-L3；或

(b) 包含 SEQ ID NO:53 的氨基酸序列的 HVR-L1、包含 SEQ ID NO:54 的氨基酸序列的 HVR-L2、和包含 SEQ ID NO:55 的氨基酸序列的 HVR-L3。

12. 权利要求 1-11 之任一项的多特异性抗体，其中第二 VH/VL 单位包含：

(a) 与 SEQ ID NO:19 的氨基酸序列具有至少 95%序列同一性的 VH 序列；

(b) 与 SEQ ID NO:20 的氨基酸序列具有至少 95%序列同一性的 VL 序 列；

(c)(a) 的 VH 序列和 (b) 的 VL 序列；

(d) 与 SEQ ID NO:49 的氨基酸序列具有至少 95%序列同一性的 VH 序列；

(e) 与 SEQ ID NO:48 的氨基酸序列具有至少 95%序列同一性的 VL 序列；或

(f)(d) 的 VH 序列和 (e) 的 VL 序列。

13. 权利要求 1-12 之任一项的多特异性抗体，其中第二 VH/VL 单位单位包含选自 SEQ ID NOs:19、56 或 49 的 VH 序列。

14. 权利要求 1-13 之任一项的多特异性抗体，其中第二 VH/VL 单位单位包含选自 SEQ ID NOs:20、57 或 48 的 VL 序列。

15. 权利要求 1-14 之任一项的多特异性抗体，其中第二 VH/VL 单位单位包含选自 SEQ ID NOs:19 或 56 的 VH 序列和 SEQ ID NO:20 或 57 的 VL 序列；或 SEQ ID NOs:49 的 VH 序列和 SEQ ID NO:48 的 VL 序列。

16. 权利要求 1-15 之任一项的多特异性抗体，其中该抗体与包含 SEQ ID NOs:9 的 VH 序列和 SEQ ID NO:10 的 VL 序列的抗体竞争结合 IL-4。

17. 权利要求 1-16 之任一项的多特异性抗体，其中该抗体与包含 SEQ ID NOs:19 的 VH 序列和 SEQ ID NO:20 的 VL 序列的抗体，或与包含 SEQ ID NOs:49 的 VH 序列和 SEQ ID NO:48 的 VL 序列的抗体，竞争结合 IL-13。

18. 权利要求 1-17 之任一项的多特异性抗体，其中该抗体结合 SEQ ID NO:29 的氨基酸 77 至 89 中的、或 SEQ ID NO:29 的氨基酸 82 至 89 中的表位。

19. 多特异性抗体，其包含特异地结合 IL-4 的第一 VH/VL 单位和特异地结合 IL-13 的第二 VH/VL 单位，其中第一 VH/VL 单位包含 SEQ ID NOs:9 的 VH 序列和 SEQ ID NO:10 的 VL 序列，且第二 VH/VL 单位包含 SEQ ID NO:19 的 VH 序列和 SEQ ID NO:20 的 VL 序列。

20. 前述权利要求任一项的多特异性抗体，其中该抗体是 IgG 抗体。

21. 权利要求 20 的多特异性抗体，其中所述抗体是 IgG1 或 IgG4 抗体。

22. 权利要求 21 的多特异性抗体，其中所述抗体是 IgG4 抗体。

23. 前述权利要求任一项的多特异性抗体，其中该抗体包含第一重链恒定区和第二重链恒定区，其中第一重链恒定区包含杵突变，第二重链恒定区包含臼突变。

24. 权利要求 23 的多特异性抗体，其中第一重链恒定区与结合 IL-4 的 VH/VL 单位的重链可变区部分融合。

25. 权利要求 23 或 24 的多特异性抗体，其中第二重链恒定区与结合 IL-13 的 VH/VL 单位的重链可变区部分融合。

26. 权利要求 23 的多特异性抗体，其中第一重链恒定区与结合 IL-13 的 VH/VL 单位的重链可变区部分融合。

27. 权利要求 23 或 26 的多特异性抗体，其中第二重链恒定区与结合 IL-4 的 VH/VL 单位的重链可变区部分融合。

28. 权利要求 23 至 27 任一项的多特异性抗体，其中所述抗体是 IgG1 抗体，所述杵突变包含 T366W 突变。

29. 权利要求 23 至 28 任一项的多特异性抗体，其中所述抗体是 IgG1 抗体，其中所述臼

突变包含选自 T366S,L368A, 和 Y407V 的至少一个、至少两个、或三个突变。

30. 权利要求 23 至 27 任一项的多特异性抗体，其中所述抗体是 IgG4 抗体，所述杵突变包含 T366W 突变。

31. 权利要求 23 至 27 和 30 任一项的多特异性抗体，其中所述抗体是 IgG4 抗体，其中所述臼突变包含选自 T366S,L368A, 和 Y407V 突变的至少一个、至少两个、或三个突变。

32. 权利要求 23 的多特异性抗体，其中该抗体包含含有 SEQ ID NO:34 的序列的第一重链恒定区。

33. 权利要求 23 或 32 的多特异性抗体，其中该抗体包含含有 SEQ ID NO:35 的序列的第二重链恒定区。

34. 权利要求 23 的多特异性抗体，其中该抗体包含含有 SEQ ID NO:36 的序列的第一重链恒定区。

35. 权利要求 23 或 34 的多特异性抗体，其中该抗体包含含有 SEQ ID NO:37 的序列的第二重链恒定区。

36. 结合 IL-4 和 IL-13 的多特异性抗体，其中该抗体包含含有 SEQ ID NO:38 的序列的第一重链、含有 SEQ ID NO:39 的序列的第一轻链、含有 SEQ ID NO:40 的序列的第二重链、含有 SEQ ID NO:41 的序列的第二轻链。

37. 结合 IL-4 的分离抗体，其中所述抗体包含：

(a) 包含 SEQ ID NO:14 的氨基酸序列的 HVR-H3、包含 SEQ ID NO:17 的氨基酸序列的 HVR-L3、和包含 SEQ ID NO:13 或 SEQ ID NO:18 的氨基酸序列的 HVR-H2；或

(b) 包含 SEQ ID NO:12 的氨基酸序列的 HVR-H1、包含 SEQ ID NO:13 或 SEQ ID NO:18 的氨基酸序列的 HVR-H2、和包含 SEQ ID NO:14 的氨基酸序列的 HVR-H3；或

(c) 包含 SEQ ID NO:15 的氨基酸序列的 HVR-L1、包含 SEQ ID NO:16 的氨基酸序列的 HVR-L2、和包含 SEQ ID NO:17 的氨基酸序列的 HVR-L3；或

(d) 与 SEQ ID NO:9 的氨基酸序列具有至少 95%序列同一性的 VH 序列；或

(e) 与 SEQ ID NO:10 的氨基酸序列具有至少 95%序列同一性的 VL 序列。

38. 权利要求 37 的分离的抗体，其中该抗体包括包含 SEQ ID NO:12 的氨基酸序列的 HVR-H1、包含 SEQ ID NO:13 或 SEQ ID NO:18 的氨基酸序列的 HVR-H2、包含 SEQ ID NO:14 的氨基酸序列的 HVR-H3、包含 SEQ ID NO:15 的氨基酸序列的 HVR-L1、包含 SEQ ID NO:16 的氨基酸序列的 HVR-L2、包含 SEQ ID NO:17 的氨基酸序列的 HVR-L3。

39. 权利要求 37 或 38 的分离的抗体，其中该抗体包括与 SEQ ID NO:9 的氨基酸序列具有至少 95%序列同一性的 VH 序列和与 SEQ ID NO:10 的氨基酸序列具有至少 95%序列同一性的 VL 序列。

40. 权利要求 37-39 之任一项的分离的抗体，其中该抗体包含选自 SEQ ID NOs:1 和 3 至 9 的 VH 序列。

41. 权利要求 37-40 之任一项的分离的抗体，其中该抗体包含选自 SEQ ID NOs:2、10 和 11 的 VL 序列。

42. 分离的抗体，其中该抗体包含 SEQ ID NOs:9 的 VH 序列和 SEQ ID NO:10 的 VL 序列。

43. 分离的核酸，其编码：

(a) 权利要求 1 至 42 之任一项的抗体；

(b) 权利要求 1-34 之任一项的多特异性抗体的第一 VH/VL 单位；或

(c) 权利要求 1-34 之任一项的多特异性抗体的第二 VH/VL 单位。

44. 宿主细胞，其包含权利要求 43 的核酸。

45. 权利要求 44 的宿主细胞，其中所述宿主细胞是大肠杆菌细胞或 CHO 细胞。

46. 产生抗体的方法，包括培养权利要求 44 或权利要求 45 的宿主细胞。

47. 免疫缀合物，其包含权利要求 1 至 42 之任一项的抗体和细胞毒性剂。

48. 包含权利要求 1-42 之任一项的抗体和可药用载体的药物制剂。

49. 权利要求 1 至 42 之任一项的抗体用作药物。

50. 权利要求 1 至 42 之任一项的抗体用于治疗嗜酸性粒细胞病症、IL-13 介导的病症、IL-4 介导的病症、或呼吸病症。

51. 权利要求 50 的抗体，其中嗜酸性粒细胞病症选自：哮喘、重度哮喘、慢性哮喘、特应性哮喘、特应性皮炎、变态反应、变应性鼻炎、非变应性鼻炎、接触性皮炎、多形红斑、大疱皮肤病、银屑病、湿疹、类风湿性关节炎、幼年慢性关节炎、慢性嗜酸性肺炎、变应性支气管肺 曲霉病、腹腔疾病、丘斯综合征（特应性结节性动脉外膜炎）、嗜酸粒细胞增多肌痛综合征、嗜酸性粒细胞增多综合征、水肿反应，包括周期性血管性水肿、蠕虫感染、荨麻疹、盘尾丝虫皮炎、嗜酸性粒细胞相关胃肠道病症、嗜酸性粒细胞性食管炎、嗜酸性粒细胞性胃炎、嗜酸性粒细胞性胃肠炎、嗜酸性粒细胞性肠炎、嗜酸性粒细胞性结肠炎、溃疡性结肠炎、惠普尔病、鼻微息肉病、鼻息肉病、阿司匹林不耐受、阻塞性睡眠呼吸暂停、局限性回肠炎、硬皮病、心肌内膜纤维化、纤维化、炎性肠病、特发性间质性肺炎、嗜酸性肺炎、超敏性肺炎、杯状细胞化生、肺纤维化、特发性肺纤维化 (IPF)、硬化症继发的肺纤维化、慢性阻塞性肺疾病 (COPD)、肝纤维化、眼色素层炎、癌症、成胶质细胞瘤、霍奇金淋巴瘤、和非霍奇金淋巴瘤。

52. 权利要求 50 的抗体，其中 IL-13 介导的疾病选自：特应性皮炎、变应性鼻炎、哮喘、纤维化、炎性肠病、局限性回肠炎、肺炎性病症、肺纤维化、特发性肺纤维化 (IPF)、慢性阻塞性肺疾病 (COPD)、肝纤维化、癌症、成胶质细胞瘤、和非霍奇金淋巴瘤。

53. 权利要求 50 的抗体，其中 IL-4 介导的疾病选自：特应性皮炎、变应性鼻炎、哮喘、纤维化、炎性肠病、局限性回肠炎、肺炎性病症、肺纤维化、特发性肺纤维化 (IPF)、慢性阻塞性肺疾病 (COPD)、肝纤维化、癌症、成胶质细胞瘤、和非霍奇金淋巴瘤。

54. 权利要求 50 的抗体，其中呼吸病症选自：哮喘、变应性哮喘、非变应性哮喘、支气管炎、慢性支气管炎、慢性阻塞性肺疾病 (COPD)、肺气肿、香烟诱导的肺气肿、气道炎症、囊性纤维化、肺纤维化、变应性鼻炎、和支气管扩张。

55. 权利要求 1 至 42 之任一项的抗体在制备用于治疗嗜酸性粒细胞病症、IL-13 介导的病症、IL-4 介导的病症、或呼吸病症的药物中的用途。

56. 权利要求 55 的用途，其中嗜酸性粒细胞病症选自：哮喘、重度哮喘、重度哮喘、慢性哮喘、特应性哮喘、特应性皮炎、变态反应、变应性鼻炎、非变应性鼻炎、接触性皮炎、多形红斑、大疱皮肤病、银 屑病、湿疹、类风湿性关节炎、幼年慢性关节炎、慢性嗜酸性肺炎、变应性支气管肺曲霉病、腹腔疾病、丘斯综合征（特应性结节性动脉外膜炎）、嗜酸粒细胞增多肌痛综合征、嗜酸性粒细胞增多综合征、水肿反应，包括周期性血管性水肿、蠕虫感染、荨麻疹、盘尾丝虫皮炎、嗜酸性粒细胞相关胃肠道病症、嗜酸性粒细胞性食管炎、嗜酸性粒细胞

性胃炎、嗜酸性粒细胞性胃肠炎、嗜酸性粒细胞性肠炎、嗜酸性粒细胞性结肠炎、溃疡性结肠炎、惠普尔病、鼻微息肉病、鼻息肉病、阿司匹林不耐受、阻塞性睡眠呼吸暂停、局限性回肠炎、硬皮病、心肌内膜纤维化、纤维化、炎性肠病、特发性间质性肺炎、嗜酸性肺炎、超敏性肺炎、杯状细胞化生、肺纤维化、特发性肺纤维化 (IPF)、硬化症继发的肺纤维化、慢性阻塞性肺疾病 (COPD)、肝纤维化、眼色素层炎、癌症、成胶质细胞瘤、霍奇金淋巴瘤、和非霍奇金淋巴瘤。

57. 权利要求 55 的用途，其中 IL-13 介导的疾病选自：特应性皮炎、变应性鼻炎、哮喘、纤维化、炎性肠病、局限性回肠炎、肺炎性病症、肺纤维化、特发性肺纤维化 (IPF)、慢性阻塞性肺疾病 (COPD)、肝纤维化、癌症、成胶质细胞瘤、和非霍奇金淋巴瘤。

58. 权利要求 55 的用途，其中 IL-4 介导的疾病选自：特应性皮炎、变应性鼻炎、哮喘、纤维化、炎性肠病、局限性回肠炎、肺炎性病症、肺纤维化、特发性肺纤维化 (IPF)、慢性阻塞性肺疾病 (COPD)、肝纤维化、癌症、成胶质细胞瘤、和非霍奇金淋巴瘤。

59. 权利要求 55 的用途，其中呼吸病症选自：哮喘、变应性哮喘、非变应性哮喘、支气管炎、慢性支气管炎、慢性阻塞性肺疾病 (COPD)、肺气肿、香烟诱导的肺气肿、气道炎症、囊性纤维化、肺纤维化、变应性鼻炎、和支气管扩张。

60. 治疗患有嗜酸性粒细胞病症的个体的方法，包括向个体施用有效量的权利要求 1-42 之任一项的抗体。

61. 权利要求 60 的方法，其中嗜酸性粒细胞病症选自：哮喘、重度哮喘、慢性哮喘、特应性哮喘、特应性皮炎、变态反应、变应性鼻炎、非 变应性鼻炎、接触性皮炎、多形红斑、大疱皮肤病、银屑病、湿疹、类风湿性关节炎、幼年慢性关节炎、慢性嗜酸性肺炎、变应性支气管肺曲霉病、腹腔疾病、丘斯综合征（特应性结节性动脉外膜炎）、嗜酸粒细胞增多肌痛综合征、嗜酸性粒细胞增多综合征、水肿反应，包括周期性血管性水肿、蠕虫感染、荨麻疹、盘尾丝虫皮炎、嗜酸性粒细胞相关胃肠道病症、嗜酸性粒细胞性食管炎、嗜酸性粒细胞性胃炎、嗜酸性粒细胞性胃肠炎、嗜酸性粒细胞性肠炎、嗜酸性粒细胞性结肠炎、溃疡性结肠炎、惠普尔病、鼻微息肉病、鼻息肉病、阿司匹林不耐受、阻塞性睡眠呼吸暂停、局限性回肠炎、硬皮病、心肌内膜纤维化、纤维化、炎性肠病、特发性间质性肺炎、嗜酸性肺炎、超敏性肺炎、杯状细胞化生、肺纤维化、特发性肺纤维化 (IPF)、硬化症继发的肺纤维化、慢性阻塞性肺疾病 (COPD)、肝纤维化、眼色素层炎、癌症、成胶质细胞瘤、霍奇金淋巴瘤、和非霍奇金淋巴瘤

62. 权利要求 60 的方法，其中 IL-13 介导的疾病选自：特应性皮炎、变应性鼻炎、哮喘、纤维化、炎性肠病、局限性回肠炎、肺炎性病症、肺纤维化、特发性肺纤维化 (IPF)、慢性阻塞性肺疾病 (COPD)、肝纤维化、癌症、成胶质细胞瘤、和非霍奇金淋巴瘤。

63. 权利要求 60 的方法，其中 IL-4 介导的疾病选自：特应性皮炎、变应性鼻炎、哮喘、纤维化、炎性肠病、局限性回肠炎、肺炎性病症、肺纤维化、特发性肺纤维化 (IPF)、慢性阻塞性肺疾病 (COPD)、肝纤维化、癌症、成胶质细胞瘤、和非霍奇金淋巴瘤。

64. 权利要求 60 的方法，其中呼吸病症选自：哮喘、变应性哮喘、非变应性哮喘、支气管炎、慢性支气管炎、慢性阻塞性肺疾病 (COPD)、肺气肿、香烟诱导的肺气肿、气道炎症、囊性纤维化、肺纤维化、变应性鼻炎、和支气管扩张。

65. 权利要求 60-64 之任一项的方法，还包括向个体施用 TH2 途径抑制剂。

66. 权利要求 65 的方法，其中 TH2 途径抑制剂抑制选自以下的至少 一个靶标：ITK, B

TK, IL-9, IL-5, IL-13, IL-4, OX40L, TSLP, IL-25, IL-33, IgE, IL-9 受体 , IL-5 受体 , IL-4 受体 α , IL-13 受体 α 1, IL-13 受体 α 2, OX40, TSLP-R, IL-7R α , IL17RB, ST2, CCR3, CCR4, CRTH2, Fc ε RI, Fc ε RII/CD23, Flap, Syk 激酶 ;CCR4, TLR9, CCR3, IL5, IL3, 和 GM-CSF。

67. 根据权利要求 60-66 之任一项的方法，其中所述个体正患有中度至重度哮喘。

68. 根据权利要求 60-66 之任一项的方法，其中所述个体正患有特发性肺纤维化。

抗 IL-4 抗体和双特异性抗体及其用途

[0001] 相关申请的交叉引用

[0002] 本申请要求 2013 年 4 月 5 日提交的美国临时申请号 61/808,748 的优选权利益，其公开内容整体合并入本文作为参考。

[0003] 序列表

[0004] 本申请包括通过 EFS-Web 提交的序列表，该序列表在此整体引入作为参考。在 2014 年三月 12 日创建的所述 ASCII 副本命名为 2014.MAR.12P5609R1-WO_SL 并且大小是 75,442 比特。

技术领域

[0005] 本发明涉及抗 IL-4 抗体和双特异性抗体及其使用方法。

背景技术

[0006] 哮喘是一种复杂疾病，全球发病率日益增加。除了其他事件，嗜酸性粒细胞炎症已在哮喘患者的气道中报道。该疾病的病理生理学具有如下特征：变化的气流阻塞、气道炎症、粘液分泌过多和上皮下纤维化。临床上，患者可以呈现咳嗽、哮鸣和呼吸短促。虽然许多患者用目前可得的疗法足够治疗，但一些哮喘患者尽管使用目前疗法仍具有持续性疾病。

[0007] 许多研究已经提示在哮喘和变态反应的发病机理中牵涉 IL-4、IL-13 及其受体（参见，例如，Wills-Karp, 2004, Immunol. Rev. 202, 175 - 190 ;Brightling 等，2010, Clin. Exp. Allergy 40, 42 - 49 ;Finkelman 等，2010, J Immunol 184, 1663 - 1674 ;Maes 等，2012, Am. J. Respir. Cell Mol. Biol. 47, 261 - 270 ;Steinke and Borish, 2001, Respir. Res. 2, 66 - 70）。IL-4 结合两个受体，一个是 IL-4Rα 和共同 γ 链（γc）的异二聚体，另一是 IL-4 受体 α（IL-4Rα）和 IL-13 受体 α1（IL-13Rα1）的异二聚体。后一受体 IL-4Rα / IL-13Rα1 是与 IL-13 共享的受体，IL-13 也独特地结合由 IL-13 受体 α2（IL-13Rα2）组成的单链受体。IL-4, IL-13, 和 IL-4Rα 基因的多态性与哮喘和变态反应，包括特征例如 IgE 水平、特应性的流行性、和哮喘疾病的严重性，相关。此外，在哮喘和其它变态性疾病中 IL-4、IL-13 及其受体的表达增加。而且，在哮喘的临床前模型中，IL-4、IL-13 及其受体的中和或缺乏可以改善疾病。

[0008] 林林总总的用于治疗哮喘的药物在销售中或在开发中。哮喘治疗的众多靶标之一是 IL-13。IL-13 是由活化的 T 细胞、NKT 细胞、嗜碱性粒细胞、嗜酸性粒细胞和肥大细胞产生的多效性 TH2 细胞因子，并且它已被强烈提示在临床前模型中牵涉哮喘发病机理。IL-13 拮抗剂，包括抗 IL-13 抗体，之前已经有描述。参见，例如，国际专利申请公布号 WO 2005/062967。这些抗体也已经被开发用作人类治疗剂。近来，几项研究已经证实抗 IL-13 单克隆抗体在哮喘治疗中的临床活性（参见，例如，Corren 等，2011, N. Engl. J. Med. 365, 1088-1098 ;Gauvreau 等，2011, Am. J. Respir. Crit. Care Med. 183, 1007-1014 ; Ingram and Kraft, 2012, J. Allergy Clin. Immunol. 130, 829-42 ;Webb, 2011, Nat Biotechnol 29, 860-863）。其中，lebrikizumab，一种中和 IL-13 活性的人源化 IgG4 抗

体，在尽管进行了治疗（对于大多数，使用了吸入皮质类固醇和长效 β2- 肾上腺素能受体激动剂）但仍保持症状的哮喘患者中，改善了肺功能 (Corren 等，2011, N. Engl. J. Med. 365, 1088-1098)。此外，结合 IL-13 和 IL-4 的双特异性抗体已有描述。参见，例如，美国申请公布号 2010/0226923。

[0009] 然而，中度至重度哮喘患者仍然需要备选的治疗可选方案。因此，需要鉴定用于治疗哮喘的更佳疗法和用于理解如何治疗哮喘患者的改良方法。

[0010] 特发性肺纤维化 (Idiopathic pulmonary fibrosis(IPF)) 是一种限制性肺疾病，特征在于肺实质的进行性间质性纤维化，在美国影响大约 100,000 名患者 (Raghu 等，Am J Respir Crit Care Med 174:810-816(2006))。与 IPF 相关的该间质性纤维化导致肺功能的进行性丧失，导致大多数患者因呼吸衰竭而死亡。自诊断时起平均存活时间为 2-3 年 (RAGHU 等，AM J RESPIR CRIT CARE MED 183:788-824(2011))。IPF 的病因学和关键分子及病理生理学驱动因子未知。经证实在 IPF 患者中可以延长存活的唯一治疗是肺移植 (THABUT 等，ANNALS OF INTERNAL MEDICINE151:767-774(2009))。然而，肺移植与显著的发病率相关，并非所有 IPF 患者均适用于该治疗，并且相对缺乏合适的供体肺。尽管进行了许多努力，但迄今仍没有药物疗法被证实可以在随机化、安慰剂对照干预试验中在 IPF 患者中实质性地延长存活，尽管一些干预看起来可以在一些患者中减慢肺功能下降的速度 (RAGHU 等，AM J RESPIR CRIT CARE MED183:788-824(2011) ;RICHELDI 等，THE NEW ENGLAND J. OF MED. 365:1079-1087(2011))。

[0011] IL-4 和 IL-13 信号传导在体外可以从许多细胞类型诱导纤维发生反应 (fibrogenic responses)。已经证实，使用 IL-4 或 IL-13 处理成纤维细胞可以诱导胶原产生和分化为成肌纤维细胞 (myofibroblast) 表型 (BOROWSKI 等，J. BRITISH SOC. ALLERGY CLIN. IMMUNOL., 38:619-628(2008) ;HASHIMOTO 等，J. ALLERGY CLIN. IMMUNOL., 107:1001-1008(2001) ;MURRAY, 等，INT. J. BIOCHEM. CELL BIOL., 40:2174-2182(2008) ;SAITO 等，INTL. ARCHIVES ALLERGY IMMUNOL., 132:168-176(2003))。也已经提出，选择性活化 (alternatively activated) 的巨噬细胞，部分地基于其能够产生刺激成纤维细胞和成肌纤维细胞的生长因子例如 TGF β 和 PDGF，而是纤维发生进程的主要贡献者。IL-4 和 IL-13 是选择性活化的巨噬细胞表型的有力诱导者，并可以至少部分地通过其对这些细胞的活性而驱动纤维发生反应 (DOYLE 等，EUR. J. IMMUNOL., 24:1441-1445(1994) ;SONG 等，CELL. IMMUNOL., 204:19-28(2000) ; WYNN AND BARRON, SEMINARS LIVER DIS., 30:245-257(2010)。

[0012] IL-4 和 IL-13 也可以在体内在多种组织中驱动纤维发生反应。IL-4 或 IL-13 在小鼠肺中的转基因过表达足以诱导胶原蛋白基因表达和显著的上皮下纤维化 (Lee 等，J. Exper. Med., 194:890-821(2001) ;Ma 等 J. Clin. Invest., 116:1274-1283(2006) ;Zhu 等，J. Clin. Invest. 103:779-788(1999))。此外，许多研究表明 IL-4 和 IL-13 在临床前动物模型中作为纤维化驱动者的作用。IL-13 被靶向破坏的小鼠或用特异于 IL-13 的阻断性抗体处理的小鼠在博来霉素 - 和 FITC 诱导的肺纤维化模型中表现出减少的胞外基质沉积 (BELPERIO 等，AM. J. RESPIR. CELL MOL. BIOL., 27:419-427(2002) ;KOLODSICK 等，J. IMMUNOL., 172:4068-4076(2004) ;LIU 等，J. IMMUNOL., 173:3425-3431(2004))。类似地，已经证实在博来霉素诱导的肺纤维化模型中 IL-4 在维持纤维化反应中是重要的 (HUAUX

等，J. IMMUNOL.，170:2083-2092(2003)。

[0013] 大量研究得出结论，IL-4和/或IL-13的表达和活性在IPF患者中升高。已经发现，与正常对照相比，在来自IP患者的肺活检样品中IL-4，IL-13和IL-4/IL-13受体亚基的表达在mRNA和蛋白质两个水平上均增加(JAKUBZIAK等，J. CLIN. PATHOL.，57:477-486(2004))。值得注意的是，在该研究中，在IPF活检物中通过免疫组织化学发现IL-13Rα2——被IL-4或IL-13信号传导高度诱导的基因(DAVID等，ONCOGENE，22:2286-3394(2003))——在成纤维病灶中表达，提示在这些细胞中活跃的IL-4或IL-13信号传导。还发现，与正常对照相比，在IPF患者的支气管肺泡灌洗液中IL-4和IL-13升高。值得注意的是，这些样品中的IL-13水平与肺功能的关键量度——预计的FVC和DLCO百分数(PARK等，J. KOREAN MED. SCI.，24:614-620(2009))——负相关。

[0014] IPF患者仍然需要替代性治疗可选方案。因此，需要鉴定用于治疗IPF的更佳疗法和用于理解如何治疗IPF患者的改良方法。

[0015] 本文引用的所有参考文献，包括专利申请和出版物，为了任何目的整体引入此处作为参考。

[0016] 发明概述

[0017] 在一些实施方案中，提供多特异性抗体，其中该多特异性抗体包含抗原结合结构域，所述抗原结合结构域包含特异地结合IL-4的第一VH/VL单位和特异地结合IL-13的第二VH/VL单位。在一些实施方案中，多特异性抗体：

[0018] a)抑制IL-4与IL-4受体α(IL-4Rα)的结合，

[0019] b)在体外抑制IL-4-诱导的细胞增殖，和/或

[0020] b)在体外抑制IL-13-诱导的细胞增殖。

[0021] 在一些实施方案中，多特异性抗体的第一VH/VL单位包括包含SEQ ID NO:14的氨基酸序列的HVR-H3、包含SEQ ID NO:17的氨基酸序列的HVR-L3、和包含SEQ ID NO:13或SEQ ID NO:18的氨基酸序列的HVR-H2。在一些实施方案中，第一VH/VL单位包括包含SEQ ID NO:12的氨基酸序列的HVR-H1、包含SEQ ID NO:13或SEQ ID NO:18的氨基酸序列的HVR-H2、和包含SEQ ID NO:14的氨基酸序列的HVR-H3。在一些实施方案中，第一VH/VL单位包括包含SEQ ID NO:15的氨基酸序列的HVR-L1、包含SEQ ID NO:16的氨基酸序列的HVR-L2、和包含SEQ ID NO:17的氨基酸序列的HVR-L3。在一些实施方案中，第一VH/VL单位包括：(a)与SEQ ID NO:9的氨基酸序列具有至少95%序列同一性的VH序列；(b)与SEQ ID NO:10的氨基酸序列具有至少95%序列同一性的VL序列；或(c)(a)的VH序列和(b)的VL序列。在一些实施方案中，第一VH/VL单位包含选自SEQ ID NOs:1和3至9的VH序列。在一些实施方案中，第一VH/VL单位包含选自SEQ ID NOs:2、10和11的VL序列。在一些实施方案中，第一VH/VL单位包含SEQ ID NO:9的VH序列和SEQ ID NO:10的VL序列。

[0022] 在本文所述任何实施方案中，多特异性抗体的第二VH/VL单位可以包含：(a)包含SEQ ID NO:23的氨基酸序列的HVR-H3、包含SEQ ID NO:26的氨基酸序列的HVR-L3、和包含SEQ ID NO:22的氨基酸序列的HVR-H2；或(b)包含SEQ ID NO:52的氨基酸序列的HVR-H3、包含SEQ ID NO:55的氨基酸序列的HVR-L3、和包含SEQ ID NO:51的氨基酸序列的HVR-H2。在本文所述任何实施方案中，多特异性抗体的第二VH/VL单位可以包含：(a)包含SEQ ID NO:21的氨基酸序列或SEQ ID NO:60的氨基酸序列的HVR-H1、包含SEQ ID NO:22的氨基

酸序列的HVR-H2、和包含SEQ ID NO:23的氨基酸序列的HVR-H3；或(b)包含SEQ ID NO:50的氨基酸序列的HVR-H1、包含SEQ ID NO:51的氨基酸序列的HVR-H2、和包含SEQ ID NO:52的氨基酸序列的HVR-H3。在本文所述任何实施方案中，多特异性抗体的第二VH/VL单位可以包含：(a)包含SEQ ID NO:24的氨基酸序列的HVR-L1、包含SEQ ID NO:25的氨基酸序列的HVR-L2、和包含SEQ ID NO:26的氨基酸序列的HVR-L3；或(b)包含SEQ ID NO:53的氨基酸序列的HVR-L1、包含SEQ ID NO:54的氨基酸序列的HVR-L2、和包含SEQ ID NO:55的氨基酸序列的HVR-L3。在本文所述任何实施方案中，多特异性抗体的第二VH/VL单位可以包含：(a)与SEQ ID NO:19的氨基酸序列具有至少95%序列同一性的VH序列；(b)与SEQ ID NO:20的氨基酸序列具有至少95%序列同一性的VL序列；或(c)(a)的VH序列和(b)的VL序列；(d)与SEQ ID NO:49的氨基酸序列具有至少95%序列同一性的VH序列；(e)与SEQ ID NO:48的氨基酸序列具有至少95%序列同一性的VL序列；或(f)(d)的VH序列和(e)的VL序列。在本文所述任何实施方案中，多特异性抗体的第二VH/VL单位可以包含SEQ ID NO:19、56、或49的VH序列。在本文所述任何实施方案中，多特异性抗体的第二VH/VL单位可以包含SEQ ID NO:20,57,或48的VL序列。在本文所述任何实施方案中，多特异性抗体的第二VH/VL单位可以包含SEQ ID NO:19或56的VH序列和SEQ ID NO:20或57的VL序列；或SEQ ID NOs:49的VH序列和SEQ ID NO:48的VL序列。

[0023] 在一些实施方案中，多特异性抗体与包含SEQ ID NOs:9的VH序列和SEQ ID NO:10的VL序列的抗体竞争结合IL-4。在一些实施方案中，多特异性抗体与包含SEQ ID NOs:19的VH序列和SEQ ID NO:20的VL序列的抗体，或与包含SEQ ID NOs:49的VH序列和SEQ ID NO:48的VL序列的抗体，竞争结合IL-13。一些实施方案中，多特异性抗体结合SEQ ID NO:29的氨基酸77至89中的、或SEQ ID NO:29的氨基酸82至89中的表位。

[0024] 在一些实施方案中，提供多特异性抗体，其包含特异地结合IL-4的第一VH/VL单位和特异地结合IL-13的第二VH/VL单位，其中第一VH/VL单位包含SEQ ID NO:9的VH序列和SEQ ID NO:10的VL序列，且第二VH/VL单位包含SEQ ID NOs:19的VH序列和SEQ ID NO:20的VL序列。

[0025] 在本文所述任何实施方案中，多特异性抗体可以是IgG抗体。在本文所述任何实施方案中，多特异性抗体可以是IgG1或IgG4抗体。在本文所述任何实施方案中，多特异性抗体可以是IgG4抗体。

[0026] 在本文所述任何实施方案中，多特异性抗体可以包含第一重链恒定区和第二重链恒定区，其中第一重链恒定区包含杵(knob)突变，第二重链恒定区包含臼(hole)突变。一些实施方案中，第一重链恒定区与结合IL-4的VH/VL单位的重链可变区部分融合。一些实施方案中，第二重链恒定区与结合IL-13的VH/VL单位的重链可变区部分融合。一些实施方案中，第一重链恒定区与结合IL-13的VH/VL单位的重链可变区部分融合。一些实施方案中，第二重链恒定区与结合IL-4的VH/VL单位的重链可变区部分融合。

[0027] 一些实施方案中，多特异性抗体是包含杵突变的IgG1抗体，所述杵突变包含T366W突变。一些实施方案中，多特异性抗体是包含臼突变的IgG1抗体，所述臼突变包含选自T366S,L368A,和Y407V的至少一个、至少两个、或三个突变。一些实施方案中，多特异性抗体是包含杵突变的IgG4抗体，所述杵突变包含T366W突变。一些实施方案中，多特异性抗体是包含臼突变的IgG4抗体，所述臼突变包含选自T366S,L368A,和Y407V的至少一

个、至少两个、或三个突变。在一些实施方案中，多特异性抗体包含含有 SEQ ID NO:34 或 SEQ ID NO:36 的序列的第一重链恒定区。在一些实施方案中，多特异性抗体包含含有 SEQ ID NO:35 或 SEQ ID NO:37 的序列的第二重链恒定区。

[0028] 一些实施方案中，提供多特异性抗体，其中该抗体包含含有 SEQ ID NO:38 的序列的第一重链、含有 SEQ ID NO:39 的序列的第一轻链、含有 SEQ ID NO:40 的序列的第二重链、含有 SEQ ID NO:41 的序列的第二轻链。

[0029] 在一些实施方案中，提供与 IL-4 结合的分离的抗体。在一些实施方案中，抗体包含：(a) 包含 SEQ ID NO:14 的氨基酸序列的 HVR-H3、包含 SEQ ID NO:17 的氨基酸序列的 HVR-L3、和包含 SEQ ID NO:13 或 SEQ ID NO:18 的氨基酸序列的 HVR-H2；或 (b) 包含 SEQ ID NO:12 的氨基酸序列的 HVR-H1、包含 SEQ ID NO:13 或 SEQ ID NO:18 的氨基酸序列的 HVR-H2、和包含 SEQ ID NO:14 的氨基酸序列的 HVR-H3；或 (c) 包含 SEQ ID NO:15 的氨基酸序列的 HVR-L1、包含 SEQ ID NO:16 的氨基酸序列的 HVR-L2、和包含 SEQ ID NO:17 的氨基酸序列的 HVR-L3；或 (d) 与 SEQ ID NO:9 的氨基酸序列具有至少 95％序列同一性的 VH 序列；或 (e) 与 SEQ ID NO:10 的氨基酸序列具有至少 95％序列同一性的 VL 序列；在一些实施方案中，抗体包括包含 SEQ ID NO:12 的氨基酸序列的 HVR-H1、包含 SEQ ID NO:13 或 SEQ ID NO:18 的氨基酸序列的 HVR-H2、包含 SEQ ID NO:14 的氨基酸序列的 HVR-H3、包含 SEQ ID NO:15 的氨基酸序列的 HVR-L1、包含 SEQ ID NO:16 的氨基酸序列的 HVR-L2、和包含 SEQ ID NO:17 的氨基酸序列的 HVR-L3。在一些实施方案中，抗体包括与 SEQ ID NO:9 的氨基酸序列具有至少 95％序列同一性的 VH 序列和与 SEQ ID NO:10 的氨基酸序列具有至少 95％序列同一性的 VL 序列。在一些实施方案中，抗体包含选自 SEQ ID NOs:1 和 3 至 9 的 VH 序列。在一些实施方案中，抗体包含选自 SEQ ID NOs:2、10 和 11 的 VL 序列。

[0030] 在一些实施方案中，提供结合 IL-4 的分离的抗体，其中该抗体包含 SEQ ID NOs:9 的 VH 序列和 SEQ ID NO:10 的 VL 序列。

[0031] 在一些实施方案中，提供编码本文所述任何双特异性抗体或分离抗体的分离核酸。在一些实施方案中，提供编码本文所述任何多特异性抗体的第一 VH/VL 单位的分离核酸。在一些实施方案中，提供编码本文所述任何多特异性抗体的第二 VH/VL 单位的分离核酸。一些实施方案中，提供包含分离核酸的宿主细胞。一些实施方案中，宿主细胞是大肠杆菌 (E.coli) 细胞或 CHO 细胞。一些实施方案中，提供产生抗体的方法，包括培养宿主细胞。

[0032] 在一些实施方案中，提供免疫缀合物，其中所述免疫缀合物包含本文所述任何多特异性抗体或分离抗体和细胞毒性剂。

[0033] 在一些实施方案中，提供药物制剂，其包含本文所述任何多特异性抗体或分离抗体和可药用载体。

[0034] 一些实施方案中，提供本文所述抗体用作药物。一些实施方案中，提供本文所述抗体用于治疗嗜酸性粒细胞病症、IL-13 介导的病症、IL-4 介导的病症、或呼吸病症。一些实施方案中，提供本文所述抗体用于制备治疗嗜酸性粒细胞病症、IL-13 介导的病症、IL-4 介导的病症、或呼吸病症的药物的用途。一些实施方案中，提供在个体中治疗嗜酸性粒细胞病症、IL-13 介导的病症、IL-4 介导的病症、或呼吸病症的方法，包括向个体施用有效量的本文所述抗体。在一些此类实施方案中，方法还包括向个体施用 TH2 途径抑制剂。在一些实施方案中，TH2 途径抑制剂抑制选自以下的至少一个靶标：ITK, BTK, IL-9, IL-5, IL-1

3, IL-4, OX40L, TSLP, IL-25, IL-33, IgE, IL-9 受体, IL-5 受体, IL-4 受体 α, IL-13 受体 α 1, IL-13 受体 α 2, OX40, TSLP-R, IL-7R α, IL17RB, ST2, CCR3, CCR4, CRTH2, Fc ε RI, Fc ε R II/CD23, Flap, Syk 激酶 ;CCR4, TLR9, CCR3, IL5, IL3, 和 GM-CSF。在一些实施方案中，个体患有中度至重度哮喘。在一些实施方案中，个体患有特发性肺纤维化。

[0035] 在本文所述任何实施方案中，嗜酸性粒细胞病症可以选自 :哮喘 (asthma)、重度哮喘 (severe asthma)、慢性哮喘 (chronic asthma)、特应性哮喘 (atoptic asthma)、特应性皮炎 (atopic dermatitis)、变态反应 (allergy)、变应性鼻炎 (allergic rhinitis)、非变应性鼻炎 (non-allergic rhinitis)、接触性皮炎 (contact dermatitis)、多形红斑 (erythema multiform)、大疱皮肤病 (bullous skin disease)、银屑病 (psoriasis)、湿疹 (eczema)、类风湿性关节炎 (rheumatoid arthritis)、幼年慢性关节炎 (juvenile chronic arthritis)、慢性嗜酸性肺炎 (chronic eosinophilic pneumonia)、变应性支气管肺曲霉病 (allergic bronchopulmonary aspergillosis)、腹腔疾病 (coeliac disease)、丘斯综合征 (Churg-Strauss syndrome)(特应性结节性动脉外膜炎 (periarteritis nodosa plus atopy))、嗜酸粒细胞增多肌痛综合征 (eosinophilic myalgia syndrome)、嗜酸性粒细胞增多综合征 (hypereosinophilic syndrome)、水肿反应 (oedematous reactions), 包括周期性血管性水肿 (episodic angiodema)、蠕虫感染 (helminth infections)、荨麻疹 (urticaria)、盘尾丝虫皮炎 (onchocercal dermatitis)、嗜酸性粒细胞相关胃肠道病症、嗜酸性粒细胞性食管炎 (eosinophilic esophagitis)、嗜酸性粒细胞性胃炎 (eosinophilic gastritis)、嗜酸性粒细胞性胃肠炎 (eosinophilic gastroenteritis)、嗜酸性粒细胞性肠炎 (eosinophilic enteritis)、嗜酸性粒细胞性结肠炎 (eosinophilic colitis)、溃疡性结肠炎 (ulcerative colitis)、惠普尔病 (Whipple's disease)、鼻微息肉病 (nasal micropolyposis)、鼻息肉病 (nasal polyposis)、阿司匹林不耐受 (aspirin intolerance)、阻塞性睡眠呼吸暂停 (obstructive sleep apnea)、局限性回肠炎 (Crohn's disease)、硬皮病 (scleroderma)、心肌内膜纤维化 (endomyocardial fibrosis)、纤维化 (fibrosis)、炎性肠病 (inflammatory bowel disease)、特发性间质性肺炎 (idiopathic interstitial pneumonia)、嗜酸性肺炎 (eosinophilic pneumonia)、超敏性肺炎 (hypersensitivity pneumonitis)、杯状细胞化生 (goblet cell metaplasia)、肺纤维化 (pulmonary fibrosis)、特发性肺纤维化 (IPF)、硬化症继发的肺纤维化 (pulmonary fibrosis secondary to sclerosis)、慢性阻塞性肺疾病 (chronic obstructive pulmonary disease, COPD)、肝纤维化 (pulmonary fibrosis)、眼色素层炎 (uveitis)、癌症、成胶质细胞瘤 (glioblastoma)、霍奇金淋巴瘤 (Hodgkins lymphoma)、和非霍奇金淋巴瘤 (non-Hodgkins lymphoma)。一些实施方案中，IL-13 介导的疾病选自 :特应性皮炎、变应性鼻炎、哮喘、纤维化、炎性肠病、局限性回肠炎、肺炎性病症、肺纤维化、特发性肺纤维化 (IPF)、慢性阻塞性肺疾病 (COPD)、肝纤维化、癌症、成胶质细胞瘤、和非霍奇金淋巴瘤。一些实施方案中，IL-4 介导的疾病选自 :特应性皮炎、变应性鼻炎、哮喘、纤维化、炎性肠病、局限性回肠炎、肺炎性病症、肺纤维化、特发性肺纤维化 (IPF)、慢性阻塞性肺疾病 (COPD)、肝纤维化、癌症、成胶质细胞瘤、和非霍奇金淋巴瘤。在本文所述任何实施方案中，呼吸病症可以选自 :哮喘 (asthma), 变应性哮喘 (allergic asthma), 非变应性哮喘 (non-allergic asthma), 支气管炎 (bronchitis), 慢性支气管炎 (chronic bronchitis), 慢性阻塞性肺

疾病 (COPD), 肺气肿 (emphysema), 香烟诱导的肺气肿 (cigarette-induced emphysema), 气道炎症 (airway inflammation), 囊性纤维化 (cystic fibrosis), 肺纤维化 (pulmonary fibrosis), 变应性鼻炎 (allergic rhinitis), 和支气管扩张 (bronchiectasis)。

[0036] 附图简述

[0037] 图 1 显示, 抗体 19C11 是 IL-4 受体激活的有力拮抗剂, 见实施例 2 的描述。(A)19C11 阻断 IL-4 与固定化 IL-4Rα 的结合。19C11(实心圆), 对照 IgG(空心方块), 无 IgG(空心三角)。B)19C11 抗体抑制 IL-4- 诱导的 TF-1 细胞增殖。19C11(实心圆), 对照 IgG(空心方块), 无 IgG(空心三角), 无 IL-4 添加(实心三角)。

[0038] 图 2 显示, 在大肠杆菌中作为 IgG1- 同种型产生的抗 -IL-13. 杵和抗 -IL-4. 臼的 (A) 未还原的和 (B) 还原的样品的 Western 印迹, 见实施例 4 描述。片段名称为重链 (H) 和轻链 (L), 泳道标记物是 M(分子量标准) 和 C(对照, 无抗体表达质粒)。图 2 还显示免疫印迹, 比较抗 -IL-13. 杵 (C) 和抗 -IL-4. 臼 (D) 的不同同种型和突变, 见实施例 5 描述。上方小图显示非还原条件, 显示组装的半抗体 (HL); 而下方小图显示还原条件, 证实对于所有变体均合成了相似量的重链和轻链。

[0039] 图 3 显示对双特异性抗体的分析性表征, 见实施例 6 描述。(A) 组装的双特异性抗体的大小排阻层析。插入的小图显示相同图在高分子量区域上的放大视图。(B) 组装的双特异性抗体的非还原 CE-SDS PAGE 证实了铰链二硫键的形成和链间二硫键的完整性。主峰区域对应于具有形成的链间二硫键的完整抗体。少数次峰反映缺乏全部链间二硫键以稳定异源二聚体的完整抗体。(C) 还原性 CE-SDS 证实了存在轻链和重链的预期分布并显示了该物质的纯度。除了完整轻链和重链的主峰, 仅检测到痕量峰。

[0040] 图 4 显示, 完整 (A)IgG1-, (B)IgG4- 和 (C)$IgG4_{R409K}$- 同种型基的双特异性抗体的 ESI-TOF 质谱分析, 见实施例 6 描述。

[0041] 图 5 显示, 针对人 IL-4-(A), 人 IL-13-(B), 或人 IL-4/IL-13-(C) 诱导的增殖, 抗 -IL-4/IL-13IgG1 同种型和抗 -IL-4/IL-13IgG4- 同种型双特异性抗体的剂量依赖性抑制作用, 见实施例 8 描述。抗 -IL-4/IL-13IgG1- 同种型 (实心圆), 抗 -IL-4/IL-13IgG4 同种型 (空心三角)、无抗体添加 (空心方块), 无细胞因子和抗体添加 (实心方块)。

[0042] 图 6 显示, 针对食蟹猴 (cynomolgus monkey)IL-4-(A), 食蟹猴 IL-13-(B) 诱导的增殖, 抗 -IL-4/IL-13IgG1 同种型和抗 -IL-4/IL-13IgG4- 同种型双特异性抗体的剂量依赖性抑制作用, 见实施例 8 描述。抗 -IL-4/IL-13IgG1- 同种型 (实心圆), 抗 -IL-4/IL-13IgG4 同种型 (在 (A) 中实心圆, 在 (B) 中空心三角)、无抗体添加 (空心方块), 无细胞因子和抗体添加 (实心方块)。

[0043] 图 7 显示, 在食蟹猴中单剂静脉内或皮下施用后, 血清抗 -IL-4/IL-13IgG4(A) 和 IgG1(B) 双特异性抗体浓度的平均值 (±SD), 见实施例 9 描述。ELISA 的量化限 (LOQ) 是 0.078 μg/mL。使用 LOQ 以上的所有数据, 排除 LOQ 以下的所有数据。当 $n \leq 2$ 时, 未计算 SD。

[0044] 图 8 显示, 在向食蟹猴静脉内施用后, 抗 -IL-4/IL-13IgG4 和抗 -IL-4/IL-13IgG1 抗体的支气管肺泡灌洗 (BAL) 液浓度和上皮衬液 (epithelial lining fluid(ELF)) 浓度, 其中所述食蟹猴用 A. suum 提取物进行了攻击以引发类似暴露于变应原的哮喘患者的变应性炎症反应, 见实施例 10 描述。对于抗 -IL-4/IL-13, ELISA 的量化限 (LOQ) 是 0.078 μg/

mL。使用 LOQ 以上的所有数据，排除 LOQ 以下的所有数据。当 n ≤ 2 时，未计算 SD。

[0045] 图 9 显示 (A) 用于治疗变应性气道炎症和哮喘小鼠模型的研究设计，见实施例 11 描述。图 9 也显示，在各种处理后在变应性气道炎症和哮喘小鼠模型动物中，(B) 肺嗜酸性粒细胞数，(C) 支气管肺泡灌洗液嗜酸性粒细胞数，(D) 抗原特异性 IgE 水平，和 (E) 血清 TARC 水平，见实施例 11 描述。对于每个条形图，头 4 个柱从左向右为：对照处理、抗 -IL-4 抗体处理、抗 -IL-13 抗体处理、和抗 -IL-4/IL-13 双特异性抗体处理。第五和第六个柱，如果存在的话，是幼稚 (naive) 小鼠。

[0046] 图 10 显示，人 κ 1 轻链可变区共有序列 (SEQ ID NO:61)、mu19C11 抗体轻链可变区 (SEQ ID NO:2)、和 19C11-κ 1 嫁接轻链可变区 (SEQ ID NO:10) 的氨基酸序列，见实施例 3 描述。根据 Kabat 进行位置编号，从 mu19C11 嫁接到可变轻链 κ I 共有构架上的高变区被加框。

[0047] 图 11 显示，人 κ 3 轻链可变区共有序列 (SEQ ID NO:62)、mu19C11 抗体轻链可变区 (SEQ ID NO:2)、和 19C11-κ 3 嫁接轻链可变区 (SEQ ID NO:11) 的氨基酸序列，见实施例 3 描述。根据 Kabat 进行位置编号，从 mu19C11 嫁接到可变轻链 κ I 共有构架上的高变区被加框。

[0048] 图 12 显示，人 VH1 重链可变区共有序列 (SEQ ID NO:63)、mu19C11 抗体重链可变区 (SEQ ID NO:1)、和 19C11-VH1 嫁接物 (SEQ ID NO:3)、19C11-VH1.L(SEQ ID NO:4)、和 19C11-VH1.FFL(SEQ ID NO:5) 重链可变区的氨基酸序列，见实施例 3 描述。根据 Kabat 进行位置编号，从 mu19C11 嫁接到可变重链亚组 I 共有构架上的高变区和 vernier 位置被加框。

[0049] 图 13 显示，人 VH3 重链可变区共有序列 (SEQ ID NO:64)、mu19C11 抗体重链可变区 (SEQ ID NO:1)、和 19C11-VH3 嫁接物 (SEQ ID NO:6)、19C11-VH3.FLA(SEQ ID NO:7)、19C11-VH3.LA(SEQ ID NO:8) 和 19C11-VH3.LA.SV(SEQ ID NO:9) 重链可变区的氨基酸序列，见实施例 3 描述。根据 Kabat 进行位置编号，从 mu19C11 嫁接到可变重链亚组 I 共有构架上的高变区和 vernier 位置被加框。

[0050] 图 14 显示人源化抗体对 IL-4 的表面等离子体共振 (SPR) 亲合力测量表，见实施例 3 描述。

[0051] 图 15 显示，生物素化的人 IL-4 与人 IL-4R 的结合被递增浓度的抗 -IL-4/IL-13 双特异性抗体抑制的曲线图，见实施例 7 描述。

[0052] 图 16 显示，生物素化的人 IL-13 与人 IL-13Rα 1 的结合被递增浓度的抗 -IL-4/IL-13 双特异性抗体抑制的曲线图，见实施例 7 描述。

[0053] 图 17 显示，生物素化的人 IL-13 与人 IL-13Rα 2 的结合被递增浓度的抗 -IL-4/IL-13 双特异性抗体抑制的曲线图，见实施例 7 描述。

[0054] 图 18 显示，在抗 -IL-4/IL-13 双特异性抗体存在下 IL-13 与人 IL-13Rα 2 结合的 SPR 传感图 (sensograms)，见实施例 7 描述。显示的线条表示受体从 12.5nM 至 200nM 的二倍浓度系列。

[0055] 发明详述

[0056] 除非另有定义，本文使用的技术和科学术语具有本发明所属领域普通技术人员通常理解的含义。Singleton 等人，DICTIONARY OF MICROBIOLOGY AND MOLECULAR BIOLOGY

2ND ED., J. WILEY&SONS(NEW YORK, N. Y. 1994), 和 MARCH, ADVANCED ORGANIC CHEMISTRY REACTIONS, MECHANISMS AND STRUCTURE 4TH ED., JOHN WILEY&SONS(NEW YORK, N. Y. 1992), 为本领域技术人员提供有关本申请使用的许多术语的一般指导。

[0057] 一些定义

[0058] 为了解释本说明书的目的，将应用下述定义，并且在合适时，以单数使用的术语也涵盖复数形式，并且反之亦然。当下文给出的任何定义与引入本文作为参考的任何文献发生冲突时，以下文给出的定义为准。

[0059] 如本说明书和后附权利要求中使用的，除非上下文另有明确说明，单数形式“a”、“an”和“the”涵盖对复数形式的提及。因此，例如，提及“蛋白质”或“抗体”分别涵盖多个蛋白质或抗体；提及”细胞”包括细胞的混合物等。

[0060] 如本文使用的术语“生物样品”包括但不限于血液、血清、血浆、痰、支气管肺泡灌洗液、组织活检物（例如肺样品）、和鼻样品，包括鼻拭子或鼻息肉。

[0061] FE_{NO}测定法指测量 FE_{NO}（呼出气一氧化氮分数）水平的测定法。此水平可以使用例如手提式便携装置 NIOX MINO™. (Aerocrine, Solna, 瑞典），依照在 2005 年由 American Thoracic Society(ATS) 公开的指南，进行评估。FE_{NO}可以记为其他相似方式例如 FeNO 或 FENO，并且应当理解所有这些相似的变化形式具有相同含义。

[0062] 哮喘是一种复杂病症，具有如下特征：变化和复发的症状、可逆的气流阻塞（例如通过支气管扩张剂）和支气管高反应性，其可以与潜在的炎症相关或不相关。哮喘的例子包括，阿司匹林敏感性 / 恶化的哮喘、特应性哮喘、重度哮喘、轻度哮喘、中度至重度哮喘、未接受过皮质类固醇的哮喘 (corticosteroid naïve asthma)、慢性哮喘、皮质类固醇抵抗哮喘、皮质类固醇难治性哮喘、新近诊断且未治疗的哮喘、吸烟引起的哮喘、在皮质类固醇应用时得不到控制的哮喘、和如 J Allergy Clin Immunol(2010)126(5):926-938 中提及的其他哮喘。

[0063] “嗜酸性粒细胞病症”意指，与过量嗜酸性粒细胞数目相关的病症，其中由于身体局部或全身的嗜酸性粒细胞水平或活性，可以表现为非典型症状。与过量嗜酸性粒细胞数目或活性相关的病症包括但不限于，哮喘（包括阿司匹林敏感性哮喘、慢性哮喘和重度哮喘）；特应性哮喘；特应性皮炎；变态反应；变应性鼻炎（包括季节性变应性鼻炎）；非变应性鼻炎；接触性皮炎 (contact dermatitis)；多形红斑 (erythema multiform)；大疱皮肤病 (bullous skin diseases)；银屑病 (psoriasis)；湿疹 (eczema)；类风湿性关节炎 (rheumatoid arthritis)；幼年慢性关节炎 (juvenile chronic arthritis)；慢性嗜酸性肺炎 (chronic eosinophilic pneumonia)；变应性支气管肺曲霉病 (allergic bronchopulmonary aspergillosis)；腹腔疾病 (coeliac disease)；丘斯综合征 (Churg-Strauss syndrome)（特应性结节性动脉外膜炎 (periarteritis nodosa plus atopy)）；嗜酸粒细胞增多肌痛综合征 (eosinophilic myalgia syndrome)；嗜酸性粒细胞增多综合征 (hypereosinophilic syndrome)；水肿反应 (oedematous reactions)，包括周期性血管性水肿 (episodic angiodema)；蠕虫感染 (helminth infections)；荨麻疹 (urticaria)；盘尾丝虫皮炎 (onchocercal dermatitis)；嗜酸性粒细胞相关胃肠道病症 (EGID（包括但不限于，嗜酸性粒细胞性食管炎 (eosinophilic esophagitis)、嗜酸性粒细胞性胃炎 (eosinophilic gastritis)、嗜酸性粒细胞性胃肠炎 (eosinophilic

gastroenteritis)、嗜酸性粒细胞性肠炎(eosinophilic enteritis)、和嗜酸性粒细胞性结肠炎(eosinophilic colitis));溃疡性结肠炎(ulcerative colitis);惠普尔病(Whipple's disease);鼻微息肉病(nasal micropolyposis)和息肉病(polyposis);阿司匹林不耐受(aspirin intolerance);阻塞性睡眠呼吸暂停(obstructive sleep apnea);局限性回肠炎(Crohn's disease);硬皮病(scleroderma);心肌内膜纤维化(endomyocardial fibrosis);癌症(例如,成胶质细胞瘤(glioblastoma)(例如,多形性成胶质细胞瘤)、非霍奇金淋巴瘤(NHL)、霍奇金淋巴瘤(Hodgkins lymphoma));纤维化(fibrosis);炎性肠病(inflammatory bowel disease);特发性间质性肺炎(idiopathic interstitial pneumonia);嗜酸性肺炎(eosinophilic pneumonia);超敏性肺炎(hypersensitivity pneumonitis);杯状细胞化生(goblet cell metaplasia);肺纤维化(pulmonary fibrosis)(包括特发性肺纤维化(IPF)和硬化症继发的肺纤维化(pulmonary fibrosis secondary to sclerosis));慢性阻塞性肺疾病(chronic obstructive pulmonary disease,COPD);肝纤维化(hepatic fibrosis);和眼色素层炎(uveitis)。嗜酸性粒细胞衍生的分泌产物也已与以下关联:肿瘤中血管发生和结缔组织形成的促进、在病状例如慢性哮喘、局限性回肠炎、硬皮病和心肌内膜纤维化中可见的纤维化反应(MUNITZ A,LEVI-SCHAFFER F.ALLERGY 2004;59:268-75,ADAMKO等ALLERGY 2005;60:13-22,OLDHOFF,等ALLERGY 2005;60:693-6)。

[0064] IL-13介导的病症意指与过量IL-13水平或活性相关的病症,其中由于身体局部和/或全身的IL-13水平或活性,可以表现为非典型症状。IL-13介导的病症的例子包括:癌症(例如非霍奇金淋巴瘤、成胶质细胞瘤)、特应性皮炎、变应性鼻炎、哮喘、纤维化、炎性肠病、局限性回肠炎、肺炎性病症(包括肺纤维化例如IPF)、COPD、和肝纤维化。

[0065] IL-4介导的病症意指与过量IL-4水平或活性相关的病症,其中由于身体局部和/或全身的IL-4水平或活性,可以表现为非典型症状。IL-4介导的病症的例子包括:癌症(例如非霍奇金淋巴瘤、成胶质细胞瘤)、特应性皮炎、变应性鼻炎、哮喘、纤维化、炎性肠病、局限性回肠炎、肺炎性病症(包括肺纤维化例如IPF)、COPD、和肝纤维化。

[0066] 哮喘样症状包括选自下述的症状:呼吸短促、咳嗽(痰产生和/或痰质量和/或咳嗽频率的变化)、哮鸣、胸闷、支气管狭窄(bronchioconstriction)和归因于上述症状之一或这些症状的组合的夜间觉醒(JUNIPER ET AL(2000)AM.J.RESPIR.CRIT.CARE MED.,162(4),1330-1334.)。

[0067] 术语"呼吸病症"包括但不限于,哮喘(例如变应性和非变应性哮喘(例如,由于感染,例如呼吸道合胞病毒(RSV)感染,例如在幼儿中));支气管炎(例如慢性支气管炎);慢性阻塞性肺疾病(COPD)(例如肺气肿(例如香烟诱导的肺气肿);涉及气道炎症、嗜酸性粒细胞增多症、纤维化和过量粘液产生的状况,例如囊性纤维化、肺纤维化和变应性鼻炎。可以具有气道炎症、过量气道分泌物和气道阻塞的特征的疾病的例子包括,哮喘、慢性支气管炎、支气管扩张和囊性纤维化。

[0068] 恶化(通常被称为哮喘发作或急性哮喘)是下述状况的新的或进行性增加的事件:呼吸短促、咳嗽(痰产生和/或痰质量和/或咳嗽频率的变化)、哮鸣、胸闷、归于上述症状之一或这些症状的组合的夜间觉醒。恶化常特征在于:呼出气流(PEF或FEV1)的减少。然而,PEF变异性在恶化过程中通常不增加,但它可以增加从而导致自恶化的恢复、或

它可以在从恶化恢复的过程中增加。恶化的严重性可以从轻度到威胁生命，并且可以基于症状和肺功能两者进行评估。如本文描述的重度哮喘恶化包括导致下述之任一或组合的恶化：随后的住院哮喘治疗、高皮质类固醇使用（例如翻两番皮质类固醇日总剂量、或大于或等于500微克FP或等价物的日总剂量持续连续三天或更多天）、或经口/肠胃外皮质类固醇使用。

[0069] “TH2途径抑制剂”或“TH2抑制剂”是抑制TH2途径的活性剂。TH2途径抑制剂的例子包括选自下述的任何一种靶标的活性的抑制剂：ITK、BTK、IL-9（例如MEDI-528）、IL-5（例如美泊利单抗，CAS编号196078-29-2；resilizumab）、IL-13（例如IMA-026、IMA-638（也称为安芦珠单抗，INN编号910649-32-0；QAX-576；IL4/IL13阱）、tralokinumab（也称为CAT-354，CAS编号1044515-88-9）；AER-001、ABT-308（也称为人源化13C5.5抗体）、IL-4（例如AER-001、IL4/IL13阱）、OX40L、TSLP、IL-25、IL-33和IgE（例如XOLAIR、QGE-031；MEDI-4212）；和受体例如：IL-9受体、IL-5受体（例如MEDI-563（贝那利珠单抗，CAS编号1044511-01-4））、IL-4受体α（例如AMG-317、AIR-645）、IL-13受体α1（例如R-1671）和IL-13受体α2、OX40、TSLP-R、IL-7Rα（TSLP的共受体）、IL17RB（IL-25的受体）、ST2（IL-33的受体）、CCR3、CCR4、CRTH2（例如AMG-853、AP768、AP-761、MLN6095、ACT129968）、Fcε RI、Fcε RII/CD23（IgE的受体）、Flap（例如GSK2190915）、Syk激酶（R-343、PF3526299）；CCR4（AMG-761）、TLR9（QAX-935），和CCR3、IL5、IL3、GM-CSF的多细胞因子抑制剂（例如TPI ASM8）。上述靶标的抑制剂的例子公开于例如WO2008/086395；WO2006/085938；US 7,615,213；US 7,501,121；WO2006/085938；WO 2007/080174；US 7,807,788；WO2005007699；WO2007036745；WO2009/009775；WO2007/082068；WO2010/073119；WO2007/045477；WO2008/134724；US2009/0047277； 和WO2008/127,271中。

[0070] 术语“小分子”指具有50道尔顿 - 2500道尔顿的分子量的有机分子。

[0071] 术语“抗体”以最广泛含义使用且特别涵盖例如单克隆抗体、多克隆抗体、具有多表位特异性的抗体、单链抗体、多特异性抗体和抗体片段。此类抗体可以是嵌合、人源化、人和合成的。此类抗体和生成其的方法在下文更详细地描述。

[0072] 术语“多特异性抗体”以最广的含义使用，尤其涵盖包含具有多表位特异性（即，能够特异地结合一个生物分子上的两个或更多个不同表位或能够特异地结合两个或更多个不同生物分子上的表位）的抗原结合结构域的抗体。一些实施方案中，多特异性抗体（例如双特异性抗体）的抗原结合结构域包含两个VH/VL单位，其中第一VH/VL单位特异地结合第一表位，第二VH/VL单位特异地结合第二表位，其中每个VH/VL单位均包含重链可变结构域（VH）和轻链可变结构域（VL）。此类多特异性抗体包括，但不限于，全长抗体、具有两个或更多个VL和VH结构域的抗体、抗体片段例如Fab、Fv、dsFv、scFv、双抗体（diabody）、双特异性双抗体和三抗体（triabody）、已共价或非共价连接的抗体片段。还包含至少一部分重链恒定区和/或至少一部分轻链恒定区的VH/VL单位也可以称作“hemimer”或“半抗体”（half antibody）。根据一些实施方案，多特异性抗体是以5μM至0.001pM、3μM至0.001pM、1μM至0.001pM、0.5μM至0.001pM、或0.1μM至0.001pM的亲和力与每个表位结合的IgG抗体。一些实施方案中，hemimer包含足以允许与第二hemimer形成分子内二硫键的重链可变区部分。一些实施方案中，hemimer包含杵突变或臼突变，例如，以允许与

包含互补臼突变或杵突变的第二 hemimer 或半抗体异二聚体化。杵突变和臼突变将在下面进一步讨论。

[0073] “双特异性抗体”是包含能够特异地结合一个生物分子上的两个不同表位或能够特异地结合两个不同生物分子上的表位的抗原结合结构域的多特异性抗体。双特异性抗体在本文中也可以称作具有“双重特异性”或是“双重特异性的”。

[0074] 如此处提及的术语“杵入臼”(“Knob-into-hole”)或“KnH”技术是指这样的技术,即通过在两个多肽相互作用的界面上将突出物 (protuberance)(杵)引入一条多肽并将腔(臼 (hole))引入另一条多肽,从而引导两条多肽在体外或体内配对在一起。例如,已将 KnH 引入到抗体的 Fc:Fc 结合界面、C_L:C_{H1}界面或 VH/VL 界面(参见,例如,US 2011/0287009,US2007/0178552,WO 96/027011,WO 98/050431, 和 ZHU 等,1997,PROTEIN SCIENCE 6:781-788)。在一些实施方案中,在制备多特异性抗体的过程中 KnHs 驱动两条不同重链配对在一起。例如,在其 Fc 区中具有 KnH 的多特异性抗体还可以包含与各 Fc 区分别连接的单可变域,或还可以包含与相似或不同轻链可变域配对的不同重链可变域。KnH 技术也可用于配对两个不同受体胞外域或含有不同靶识别序列的任何其他多肽序列(例如,包括 affibody、肽体 (peptibody) 和其他 Fc 融合物)。

[0075] 如本文所用,术语“杵突变”指,将突出物(杵)引入多肽中的突变,其中所述突出物被引入到该多肽与另一条多肽相互作用的界面上。一些实施方案中,另一多肽具有臼突变。

[0076] 如本文所用,术语“臼突变”指,将腔(臼)引入多肽中的突变,其中所述腔被引入到该多肽与另一条多肽相互作用的界面上。一些实施方案中,另一多肽具有杵突变。

[0077] 术语“治疗剂”指用于治疗疾病的任何活性剂。治疗剂可以是,例如,多肽(一或多种)(例如抗体、免疫粘附素或肽体 (peptibody))、可以与蛋白质结合的适体或小分子、或可以与编码靶标的核酸分子结合的核酸分子(即,siRNA)、等等。

[0078] 术语“控制剂”或“防止剂”指用于控制哮喘炎症的任何治疗剂。控制剂的例子包括皮质类固醇、白三烯受体拮抗剂(例如抑制白三烯的合成或活性,例如孟鲁司特 (montelukast)、齐留通 (zileuton)、普仑司特 (pranlukast)、扎鲁司特 (zafirlukast))、LABAs、皮质类固醇 /LABA 联合组合物、茶碱(包括氨茶碱 (aminophylline))、色甘酸钠 (cromolyn sodium)、萘多罗米钠 (nedocromil sodium)、奥马珠单抗 (omalizumab)、LAMAs、MABA(例如双功能毒蕈碱拮抗剂 - β 2 激动剂)、5- 脂加氧酶活化蛋白质 (FLAP) 抑制剂、和酶 PDE-4 抑制剂(例如罗氟司特 (roflumilast))。“第二控制剂”典型地指与第一控制剂不同的控制剂。

[0079] 术语“省皮质类固醇的”或“CS”意指,在服用皮质类固醇治疗疾病的患者中,由于另一种治疗剂的施用,减少了用于治疗该疾病的皮质类固醇的频率和 / 或量、或消除了对皮质类固醇的使用。“CS 剂”指可以在服用皮质类固醇的患者中引起 CS 的治疗剂。

[0080] 术语“皮质类固醇”包括但不限于,氟替卡松 (fluticasone)(包括丙酸氟替卡松 (FP))、倍氯米松 (beclometasone)、布地奈德 (budesonide)、环索奈德 (ciclesonide)、莫米他松 (mometasone)、去氟肤轻松 (flunisolide)、倍他米松 (betamethasone) 和曲安西龙 (triamcinolone)。“可吸入皮质类固醇”意指适于通过吸入递送的皮质类固醇。示例性可吸入皮质类固醇是氟替卡松、二丙酸倍氯米松、布地奈德、糠酸莫米他松 (mometasone

furoate)、环索奈德、去氟肤轻松、醋酸曲安缩松(Triamcinolone Acetonide)、和目前可获得或将来可获得的任何其他皮质类固醇。可以吸入且与长效β2-激动剂组合的皮质类固醇的例子包括但不限于:布地奈德/福莫特罗(formoterol)和氟替卡松/沙美特罗(salmeterol)。

[0081] 皮质类固醇/LABA组合药物的例子包括糠酸氟替卡松/三氟甲磺酸维兰特罗(vilanterol trifenatate)和茚达特罗(indacaterol)/莫米他松。

[0082] 术语"LABA"意指长效β2-激动剂,所述激动剂包括例如沙美特罗(salmeterol)、福莫特罗(formoterol)、班布特罗(bambuterol)、舒喘灵(albuterol)、茚达特罗、阿福特罗(arformoterol)和克伦特罗(clenbuterol)。

[0083] 术语"LAMA"意指长效毒蕈碱拮抗剂,所述拮抗剂包括:噻托溴铵(tiotropium)。

[0084] LABA/LAMA组合的例子包括但不限于:奥达特罗噻托溴铵(Boehringer Ingelheim's)和茚达特罗glycopyrronium(Novartis)。

[0085] 术语"SABA"意指短效β-2激动剂,所述激动剂包括但不限于,沙丁胺醇(salbutamol)、左沙丁胺醇(levosalbutamol)、非诺特罗(fenoterol)、特布他林(terbutaline)、吡布特罗(pirbuterol)、丙卡特罗(procaterol)、比托特罗(bitolterol)、利米特罗(rimiterol)、卡布特罗(carbuterol)、妥洛特罗(tulobuterol)和瑞普特罗(reproterol)。

[0086] 白三烯受体拮抗剂(有时称为leukast)(LTRA)是抑制白三烯的药物。白三烯抑制剂的例子包括孟鲁司特、齐留通、普仑司特和扎鲁司特。

[0087] 术语"FEV1"指在用力呼气的第一秒内呼出的空气体积。它是气道阻塞的量度。诱导20%的FEV1下降所需的乙酰甲胆碱(methacholine)激发浓度(provocative concentration)(PC20)是气道高反应性的量度。FEV1可以以其他相似方式例如FEV_1表示,并且应当理解所有这些相似的变化形式具有相同含义。

[0088] 术语"FEV1的相对变化"=(在治疗第12周时的FEV1-在治疗开始前的FEV1)除以FEV1。

[0089] 如本文所用,"FVC"指"用力肺活量"(Forced Vital Capacity),指用于测量在完全吸气和最大呼出至残留体积之间肺空气体积变化的标准检查(相对于FEV1中一秒内排出的空气体积)。它是功能性肺容量(functional lung capacity)的量度。在限制性肺疾病,例如间质性肺疾病包括IPF、超敏性肺炎(hypersensitivity pneumonitis)、结节病(sarcoidosis)、和全身性硬化症(systemic sclerosis)的患者中,典型地由于肺实质的结疤,FVC减少。

[0090] 术语"轻度哮喘"指患者一般经历每周小于两次的症状或恶化、每月小于两次的夜间症状,并且在两次恶化之间是无症状的。轻度、间歇性哮喘常根据需要用下述进行治疗:吸入性支气管扩张剂(短效吸入β2-激动剂);已知触发剂的避免;每年流感疫苗接种;每6-10年肺炎球菌疫苗接种,以及在一些情况下,在暴露于鉴定的触发剂前,吸入性β2-激动剂、色甘酸或萘多罗米。如果患者对短效β2-激动剂具有增加的需要(例如对于急性恶化,在1天内使用短效β2-激动剂超过三到四次;或针对症状,每月使用超过一盒(canister)),则患者可能需要治疗的逐步增加。

[0091] 术语"中度哮喘"一般指这样的哮喘,其中患者经历每周超过两次的恶化,且恶

化影响睡眠和活动；患者具有每月超过两次的由哮喘导致的夜间觉醒；患者具有慢性哮喘症状，所述症状每天或每隔一天需要短效吸入 β2- 激动剂；以及患者的治疗前基线 PEF 或 FEV1 是预计的 60% - 80%，并且 PEF 变异性是 20 - 30%。

[0092] 术语“重度哮喘”一般指这样的哮喘，其中患者具有几乎连续的症状、频繁恶化、频繁的由哮喘导致的夜间觉醒、有限的活动、小于预计的 60% 的 PEF 或 FEV1 基线、和 20 - 30% 的 PEF 变异性。

[0093] 急救药品 (rescue medications) 的例子包括舒喘灵 (albuterol)、万托林 (ventolin) 及其他。

[0094] “抵抗”指在用治疗剂治疗后几乎未表现出或根本未表现出临床上显著改善的疾病。例如，需要用高剂量 ICS（例如，翻两番皮质类固醇总日剂量或大于或等于 500 微克日总剂量的 FP（或等价物））治疗至少连续三天或更多天、或全身皮质类固醇治疗两周试验以确立哮喘是否保持不受控制或 FEV1 不改善的哮喘，通常被视为重度难治性哮喘。

[0095] 如本文提供的治疗剂可以通过任何合适的方式进行施用，包括肠胃外、皮下、腹膜内、肺内和鼻内。肠胃外输注包括肌内、静脉内、动脉内、腹膜内或皮下施用。在一些实施方案中，治疗剂是吸入剂。根据一些实施方案，给药是通过注射例如静脉内或皮下注射给予。在一些实施方案中，治疗剂使用注射器（例如预装或非预装）或自动注射器进行施用。

[0096] 对于疾病的预防或治疗，治疗剂的合适剂量可以依赖待治疗疾病的类型、疾病的严重性和过程、治疗剂是施用用于预防还是治疗目的、先前治疗、患者的临床史和对治疗剂的应答、和主治医生的判断。治疗剂可以适宜地一次性地或经过一系列治疗而施用于患者。可以以与良好医疗实践相符的方式配制、定剂量和施用治疗剂组合物。在此考虑的因素包括待治疗的特定病症、待治疗的特定哺乳动物、个体患者的临床状况、病症原因、活性剂递送部位、施用方法、施用时间安排和医学从业者已知的其他因素。

[0097] “患者应答”或“应答”（及其语法变体）可以使用指示有益于患者的任何终点进行评价，包括但不限于 (1) 疾病进展的一定程度抑制，包括减慢和完全停滞，；(2) 疾病发作数和 / 或症状的减少；(3) 损伤大小的减少；(4) 疾病细胞向邻近周围器官和 / 或组织浸润的抑制（即减少、减慢或完全停止）；(5) 疾病扩散的抑制（即减少、减慢或完全停止）；(6) 自身免疫反应的降低，其可以，但不必，导致疾病损伤的消退或消除；(7) 与病症相关的一种或多种症状的一定程度缓解；(8) 在治疗后无疾病呈现的长度 (length of disease-free presentation) 的增加；和 / 或 (9) 在治疗后在给定时间点上减少的死亡率。

[0098] “亲和力”指在分子（例如抗体）的单个结合位点及其结合配偶体（例如抗原）之间的非共价相互作用的总和强度。除非另外指出，否则如本文所用，“结合亲和力”指反映结合对子的成员（例如，抗体和抗原）之间 1:1 相互作用的固有结合亲和力。分子 X 对于其配偶体 Y 的亲和力一般可以通过解离常数 (Kd) 表示。亲和力可以通过本领域已知的常见方法，包括本文描述的那些，进行测量。在本文中描述用于测量结合亲和力的特定举例说明性和示例性实施方案。

[0099] “亲和力成熟的”抗体指与不具有此类改变的亲本抗体相比较，在一个或多个高变区 (HVRs) 中具有一个或多个改变的抗体，此类改变导致抗体对于抗原的亲和力的改善。

[0100] 术语“抗 -IL-4 抗体”和“结合 IL-4 的抗体”指这样的抗体，所述抗体能够以足够的亲和力结合 IL-4 从而使抗体可以用作为靶定 IL-4 的诊断剂和 / 或治疗剂。在一些实施

方案中，例如通过放射免疫测定法（RIA）所测定，抗 IL-4 抗体对不相关的非 IL-4 蛋白质的结合程度低于抗体对 IL-4 结合的约 10%。在特定实施方案中，与 IL-4 结合的抗体具有≤ 1 μM、≤ 100nM、≤ 10nM、≤ 1nM、≤ 0.1nM、≤ 0.01nM 或≤ 0.001nM（例如 10-8M 或更少，例如 10-8M 至 10-13M，例如 10-9M 至 10-13M）的解离常数（Kd）。在某些实施方案中，抗 IL-4 抗体结合在来自不同物种的 IL-4 间保守的 IL-4 表位上。在一些实施方案中，抗 IL-4 抗体是多特异性抗体，例如双特异性抗体。

[0101] 术语“抗 -IL-13 抗体”和“结合 IL-13 的抗体”指这样的抗体，所述抗体能够以足够的亲和力结合 IL-13 从而抗体可以用作为靶定 IL-13 的诊断剂和 / 或治疗剂。在一些实施方案中，例如通过放射免疫测定法（RIA）所测定，抗 IL-13 抗体对不相关的非 IL-13 蛋白质的结合程度低于抗体对 IL-13 结合的约 10%。在特定实施方案中，与 IL-13 结合的抗体具有≤ 1 μM、≤ 100nM、≤ 10nM、≤ 1nM、≤ 0.1nM、≤ 0.01nM 或≤ 0.001nM（例如 10-8M 或更少，例如 10-8M 至 10-13M，例如 10-9M 至 10-13M）的解离常数（Kd）。在某些实施方案中，抗 IL-13 抗体结合在来自不同物种的 IL-13 间保守的 IL-13 表位上。在一些实施方案中，抗 IL-13 抗体是多特异性抗体，例如双特异性抗体。

[0102] 术语“抗体”在本文中以最广泛的含义使用，并且涵盖各种抗体结构，包括但不限于单克隆抗体、多克隆抗体、多特异性抗体（例如双特异性抗体）和抗体片段，只要它们显示出所需抗原结合活性即可。

[0103] “抗体片段”指非完整抗体的分子，其包含完整抗体中与完整抗体所结合的抗原结合的部分。抗体片段的例子包括但不限于 Fv、Fab、Fab’、Fab’-SH、F(ab’)2；双抗体；线性抗体；单链抗体分子（例如 scFv）；和由抗体片段形成的多特异性抗体。

[0104] 与参考抗体“结合相同表位的抗体”指，该抗体在竞争测定法中阻断参考抗体与其抗原的结合达 50%或更多，且反过来地，参考抗体在竞争测定法中阻断该抗体与其抗原的结合达 50%或更多。示例性竞争测定法在本文中提供。

[0105] 用于本文目的的“受体人构架”是包含衍生自人免疫球蛋白构架或人共有构架（如下文定义）的轻链可变结构域（VL）构架或重链可变结构域（VH）构架的氨基酸序列的构架。“衍生自”人免疫球蛋白构架或人共有构架的受体人构架可以包含与其相同的氨基酸序列，或它可以含有氨基酸序列变化。在一些实施方案中，氨基酸变化数目是 10 个或更少、9 个或更少、8 个或更少、7 个或更少、6 个或更少、5 个或更少、4 个或更少、3 个或更少、或 2 个或更少。在一些实施方案中，VL 受体人构架在序列上与 VL 人免疫球蛋白构架序列或人共有构架序列相同。

[0106] 术语“嵌合”抗体指这样的抗体，其中重和 / 或轻链的部分衍生自特定来源或物种，而重和 / 或轻链的剩余部分衍生自不同来源或物种。

[0107] 抗体的“类”指抗体重链具有的恒定结构域或恒定区的类型。存在五大类抗体：IgA、IgD、IgE、IgG 和 IgM，并且其中几个可以进一步分为亚类（同种型），例如 IgG1、IgG2、IgG3、IgG4、IgA1 和 IgA2。与不同免疫球蛋白类别相对应的重链恒定域分别称作 α、δ、ε、γ 和 μ。

[0108] 如本文使用的术语“细胞毒素剂”指抑制或阻止细胞功能和 / 或引起细胞死亡或破坏的物质。细胞毒素剂包括但不限于放射性同位素（例如 At^{211}、I^{131}、I^{125}、Y^{90}、Re^{186}、Re^{188}、Sm^{153}、Bi^{212}、P^{32}、Pb^{212}和 Lu 的放射性同位素）；化学治疗剂或药物（例如

氨甲蝶呤(methotrexate)、阿霉素(adriamicin)、长春花生物碱(vinca alkaloids)(长春新碱(vincristine)、长春碱(vinblastine)、依托泊苷(etoposide))、多柔比星(doxorubicin)、美法仑(melphalan)、丝裂霉素C(mitocycin C)、苯丁酸氮芥(chlorambucil)、柔红霉素(daunorubicin)或其他嵌入剂);生长抑制剂;酶及其片段例如溶核酶;抗生素;毒素例如小分子毒素或细菌、真菌、植物或动物源的酶活性毒素,包括其片段和/或变体;和下文公开的多种抗肿瘤或抗癌剂。

[0109] "效应子功能"指可归于抗体的Fc区的那些生物活性,其随着抗体同种型而改变。抗体效应子功能的例子包括:C1q结合和补体依赖性细胞毒性(CDC);Fc受体结合;抗体依赖性细胞介导的细胞毒性(ADCC);吞噬作用;细胞表面受体(例如B细胞受体)的下调;和B细胞活化。

[0110] 活性剂例如药物制剂的"有效量"指,在所需剂量和时间段,有效地实现期望的治疗或预防结果的量。

[0111] 术语"Fc区"在本文中用于定义免疫球蛋白重链的C端区域,其含有恒定区的至少部分。该术语包括天然序列Fc区和变体Fc区。在一些实施方案中,人IgG重链Fc区从Cys226或Pro230延伸到重链的羧基末端。然而,Fc区的C末端赖氨酸(Lys447)可以存在或不存在。除非本文另有说明,Fc区或恒定区中的氨基酸残基编号根据EU编号系统,也称为EU索引,见Kabat等人,Sequences of Proteins of Immunological Interest,第5版Public Health Service,National Institutes of Health,Bethesda,MD,1991中描述。

[0112] "构架"或"FR"指除了高变区(HVR)残基外的可变结构域残基。可变结构域的FR一般由四个FR结构域组成:FR1、FR2、FR3和FR4。相应地,HVR和FR序列一般以下述顺序在VH(或VL)中出现:FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4。

[0113] 术语"全长抗体"、"完整抗体"和"全抗体"在本文中可互换使用,指具有基本上类似于天然抗体结构的结构或具有含有如本文定义的Fc区的重链的抗体。

[0114] 术语"宿主细胞"、"宿主细胞系"和"宿主细胞培养物"可互换使用,并且指外源核酸已引入其内的细胞,包括此细胞的后代。宿主细胞包括"转化体"和"转化的细胞",其包括原代转化的细胞和由其衍生的后代,不考虑传代数目。后代可以在核酸内容上与亲本细胞不完全等同,而是可以含有突变。在本文中涵盖具有针对原始转化细胞所筛选或选择的相同功能或生物活性的突变体后代。

[0115] "人抗体"是具有氨基酸序列的抗体,所述氨基酸序列对应于通过人或人细胞产生的抗体或者衍生自如下非人来源的抗体的氨基酸序列,其中所述非人来源利用人抗体库或其他人抗体编码序列。人抗体的这个定义特别地排除包含非人抗原结合残基的人源化抗体。

[0116] "人共有构架"是代表在选择的人免疫球蛋白VL或VH构架序列中最常出现的氨基酸残基的构架。一般地,该选择的人免疫球蛋白VL或VH序列来自可变结构域序列亚组。一般地,该序列亚组是如Kabat等人,Sequences of Proteins of Immunological Interest,第五版,NIH Publication91-3242,Bethesda MD(1991),第1-3卷中的亚组。在一些实施方案中,对于VL,亚组是如Kabat等人,同上引文中的亚组κI。在一些实施方案中,对于VH,亚组是如Kabat等人,同上引文中的亚组III。

[0117] "人源化"抗体指包含来自非人HVRs的氨基酸残基和来自人FRs的氨基酸残基的

嵌合抗体。在特定实施方案中，人源化抗体将包含至少一个和一般两个可变结构域的基本上全部，其中 HVRs（例如 CDRs）的全部或基本上全部对应于非人抗体的，而 FRs 的全部或基本上全部对应于人抗体的。人源化抗体任选可以包含衍生自人抗体的抗体恒定区的至少部分。抗体例如非人抗体的“人源化形式”指已经历人源化的抗体。

[0118] 如本文使用的，术语“高变区”或“HVR”指抗体可变结构域中在序列上高变（“互补决定区”或“CDRs”）和/或形成结构上限定的环（“高变环”）和/或含有抗原接触残基（“抗原接触者”）的各个区域。一般而言，抗体包含六个 HVR；三个在 VH 中（H1、H2、H3），三个在 VL 中（L1、L2、L3）。在本文中，示例性 HVR 包括：

[0119] (a) 出现在氨基酸残基 26-32(L1)、50-52(L2)、91-96(L3)、26-32(H1)、53-55(H2)和 96-101(H3) 位的高变环 (CHOTHIA AND LESK, J. MOL. BIOL. 196:901-917(1987))；

[0120] (b) 出现在氨基酸残基 24-34(L1)、50-56(L2)、89-97(L3)、31-35b(H1)、50-65(H2) 和 95-102(H3) 位的高变环 (KABAT 等，SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST, 5TH ED. PUBLIC HEALTH SERVICE, NATIONAL INSTITUTES OF HEALTH, BETHESDA, MD(1991))；

[0121] (c) 出现在氨基酸残基 27c-36(L1)，46-55(L2)，89-96(L3)，30-35b(H1)，47-58(H2)，和 93-101(H3) 位的抗原接触者 (MACCALLUM 等 J. MOL. BIOL. 262:732-745(1996))；和

[0122] (d)(a)，(b)，和/或 (c) 的组合，包括 HVR 氨基酸残基 46-56(L2)，47-56(L2)，48-56(L2)，49-56(L2)，26-35(H1)，26-35b(H1)，49-65(H2)，93-102(H3)，和 94-102(H3)。

[0123] 在一些实施方案中，HVR 残基包含图 10 至 13 中或在本说明书其它地方鉴定的那些残基。

[0124] 除非另有所指，HVR 残基和可变域中的其他残基（如 FR 残基）在本文中根据 Kabat 等（见上引文）编号。

[0125] “免疫缀合物”是缀合至一种或多种异源分子，包括但不限于细胞毒素剂，的抗体。

[0126] “个体”或“受试者”是哺乳动物。哺乳动物包括但不限于驯养的动物（例如牛、绵羊、猫、犬和马）、灵长类动物（例如人和非人灵长类动物例如猴）、兔和啮齿类动物（例如小鼠和大鼠）。在特定实施方案中，个体或受试者是人。

[0127] “分离的”抗体是已与其天然环境中的组分分离的抗体。在一些实施方案中，抗体纯化至大于 95％或 99％纯度，如通过例如电泳（例如 SDS-PAGE、等电点聚焦 (IEF)、毛细管电泳）或色谱法（例如离子交换或反相 HPLC) 测定的。关于用于评价抗体纯度的方法的综述，参见例如 Flatman 等人，J. Chromatogr. B 848:79-87(2007)。

[0128] “分离的”核酸指已与其天然环境中的组分分离的核酸分子。分离的核酸包括包含在通常含有该核酸分子的细胞中的核酸分子，但是该核酸分子存在于染色体外或存在于与其天然染色体位置不同的染色体位置上。

[0129] “编码抗 IL-4 抗体的分离核酸”指编码抗体重和轻链（或其片段）的一个或多个核酸分子，包括在单个载体中或在分开载体中的此（一个或多个）核酸分子，以及存在于宿主细胞中的一个或多个位置上的此（一个或多个）核酸分子。

[0130] “编码抗 IL-13 抗体的分离核酸”指编码抗体重和轻链（或其片段）的一个或多个核酸分子，包括在单个载体中或在分开载体中的此（一个或多个）核酸分子，以及存在于宿主细胞中的一个或多个位置上的此（一个或多个）核酸分子。

[0131] 如本文使用的术语“单克隆抗体”指得自基本上同质的抗体群体的抗体，所谓基本上同质的抗体群体是指，除了一般以微小量存在的可能的变体抗体（例如含有天然突变或在单克隆抗体制品的生产过程中出现）外，群体中的各抗体是相同的和/或结合相同的表位。与一般包括针对不同决定簇（表位）的不同抗体的多克隆抗体制剂形成对比，单克隆抗体制剂的每个单克隆抗体都针对抗原上的一个决定簇。因此，修饰词“单克隆”是指抗体得自基本上同质的抗体群体的特征，并不应解释为要求通过任何特定方法生产该抗体。例如，单克隆抗体可以通过多种技术进行制备，包括但不限于杂交瘤法、重组DNA法、噬菌体展示法、和利用含有全部或部分人免疫球蛋白基因座的转基因动物的方法，这些方法和用于制备单克隆抗体的其他示例性方法在本文中描述。在一些实施方案中，单克隆抗体为多特异性抗体（例如双特异性）抗体。

[0132] “裸抗体”指不缀合至异源部分（例如细胞毒部分）或放射性标记的抗体。裸抗体可以存在于药物制剂中。

[0133] “天然抗体”指具有各种结构的天然存在的免疫球蛋白分子。例如，天然IgG抗体是约150,000道尔顿的异四聚体糖蛋白，由二硫键连接的两条相同轻链和两条相同重链组成。从N到C末端，每条重链具有可变区(VH)，也称为可变重链结构域或重链可变结构域，随后为三个恒定结构域(CH1、CH2和CH3)。类似地，从N到C末端，每条轻链具有可变区(VL)，也称为可变轻链结构域或轻链可变结构域，随后为恒定轻链(CL)结构域。抗体的轻链，基于其恒定结构域的氨基酸序列，可以分至称为kappa(κ)和lambda(λ)的两个类型之一。

[0134] 术语“包装说明书”用于指：习惯上包括在治疗产品的商业包装中的说明书，其含有与此治疗产品使用相关的如下信息：适应症、用法、剂量、施用、联合治疗、禁忌和/或警告。术语“包装说明书”也用于指习惯上包括在诊断产品的商业包装中的说明书，其含有关于如下的信息：预期用途、测试原理、试剂的准备和操作、样本的收集和准备、测定法和测定方案的校准、性能和精确度数据，例如测定法的灵敏度与特异性。

[0135] 相对于参考多肽序列而言，“氨基酸序列同一性百分比(%)”定义为，在比对参考序列和候选序列且在需要时引入空位以实现最大序列同一性百分比后，不将任何保守置换考虑为序列同一的部分，在候选序列中与参考多肽序列的氨基酸残基相同的氨基酸残基的百分比。用于确定氨基酸序列同一性百分比目的的比对可以以在本领域内的多种方法实现，例如使用可公开获得的计算机软件例如BLAST、BLAST-2、ALIGN或Megalign(DNASTAR)软件。技术人员可以确定用于比对序列的合适参数，包括为在待比较的序列的全长上实现最大对齐所需的任何算法。然而，对于本文的目的，%氨基酸序列同一性值使用序列比较计算机程序ALIGN-2生成。ALIGN-2序列比较计算机程序由Genentech,Inc.创作，并且源代码已与用户文档一起提交给美国版权局(U.S.Copyright Office)，Washington D.C.，20559，其注册为美国版权登记号TXU510087。ALIGN-2程序可从Genentech，Inc.，South San Francisco，California公开获得，或可以由源代码编译。ALIGN-2程序应编译以用于在UNIX操作系统（包括数字UNIX V4.0D）上使用。所有序列比较参数通过ALIGN-2程序设置并且不改变。

[0136] 在ALIGN-2用于氨基酸序列比较的情况下，给定氨基酸序列A与或相对于给定氨基酸序列B的%氨基酸序列同一性（其可以可替代地表达为，与或相对于给定氨基酸序列

B，具有或包含特定％氨基酸序列同一性的给定氨基酸序列 A）如下计算：

[0137] 100 乘以分数 X/Y，

[0138] 其中 X 是在 ALIGN-2 程序的 A 和 B 比对中由序列比对程序 ALIGN-2 评为相同匹配的氨基酸残基的数目，其中 Y 是 B 中的氨基酸残基的总数目。应当理解，当氨基酸序列 A 的长度不等于氨基酸序列 B 的长度时，A 相对于 B 的％氨基酸序列同一性不等于 B 相对于 A 的％氨基酸序列同一性。除非另有具体说明，本文使用的所有％氨基酸序列同一性值，如紧先前段落中所述，通过 ALIGN-2 计算机程序获得。

[0139] 术语“药物制剂”指这样的制品，该制品的形式使得其中含有的活性成分的生物活性是有效的，并且不含有对制剂将施用于的受试者具有无法接受的毒性的另外组分。

[0140] “药学可接受的载体”指，在药物制剂中除了活性成分外的对受试者无毒的成分。药学可接受的载体包括但不限于缓冲剂、赋形剂、稳定剂或防腐剂。

[0141] 除非另有说明，如本文使用的，术语“IL-4”指来自任何脊椎动物来源的任何天然 IL-4，所述脊椎动物来源包括哺乳动物例如灵长类动物（例如人）和啮齿类动物（例如小鼠和大鼠）。该术语包含“全长”、未加工 IL-4，以及由细胞中的加工而产生的任何形式的 IL-4。该术语还包含天然存在的 IL-4 变体，例如剪接变体或等位基因变体。示例性人 IL-4 的氨基酸序列显示在 SEQ ID NOS:27 和 28，以及 Swiss-Prot 登录号 P05112.2 中。示例性食蟹猴 IL-4 的氨基酸序列显示在 SEQ ID NO:33 中。

[0142] 除非另有说明，如本文使用的，术语“IL-13”指来自任何脊椎动物来源的任何天然 IL-13，所述脊椎动物来源包括哺乳动物例如灵长类动物（例如人）和啮齿类动物（例如小鼠和大鼠）。该术语包含“全长”、未加工 IL-13，以及由细胞中的加工而产生的任何形式的 IL-13。该术语还包含天然存在的 IL-13 变体，例如剪接变体或等位基因变体。示例性人 IL-13 的氨基酸序列显示在 SEQ ID NOS:29 和 30，以及 Swiss-Prot 登录号 P35225.2 中。示例性食蟹猴 IL-13 的氨基酸序列显示在 SEQ ID NO:32 中。

[0143] 如本文使用的，“治疗”（及其语法变体）指，尝试改变所治疗个体的自然过程的临床干预，其可以执行用于预防或在临床病理学的过程中执行。治疗的期望效应包括但不限于，防止疾病的出现或复发、减轻症状、减弱疾病的任何直接或间接病理后果、防止转移、降低疾病进展速率、改善或缓和疾病状态、和缓解或改善预后。在一些实施方案中，抗体用于延迟疾病的发展或减慢疾病的进展。

[0144] 术语“可变区”或“可变结构域”指参与抗体与抗原结合的抗体重或轻链的结构域。天然抗体的重链和轻链的可变结构域（分别为 VH 和 VL）一般具有相似结构，其中每个结构域包含四个保守构架区（FRs）和三个高变区（HVRs）。（参见，例如，Kindt 等 Kuby Immunology，6th ed.，W.H.Freeman and Co.，page 91(2007).）单个 VH 或 VL 结构域可能足以赋予抗原结合特异性。此外，结合特定抗原的抗体可以使用来自结合该抗原的抗体的 VH 或 VL 结构域分别筛选互补 VL 或 VH 结构域的文库，以分离。参见，例如，Portolano 等，J. Immunol. 150:880-887(1993)；Clarkson 等，Nature 352:624-628(1991).

[0145] 如本文使用的，术语“载体”指能够扩增与之连接的另一核酸的核酸分子。该术语包括作为自主复制核酸结构的载体以及掺入已引入其的宿主细胞的基因组内的载体。特定载体能够指导与之可操作地连接的核酸的表达。此类载体在本文中被称为表达载体”。

[0146] 组合物和方法

[0147] 在一些实施方案中，提供与 IL-4 结合的抗体。在一些实施方案中，提供与 IL-4 和 IL-13 结合的双特异性抗体。这些抗体可以用于例如诊断或治疗嗜酸性粒细胞病症，包括呼吸病症（例如哮喘和 IPF）、IL-4 介导的病症和 IL-13 介导的病症。

[0148] 示例性抗 IL-4 抗体

[0149] 在一些实施方案中，提供与 IL-4 结合的分离的抗体。在一些实施方案中，抗 IL-4 抗体包含选自以下的至少一个、两个、三个、四个、五个或六个 HVR：(a) 包含 SEQ ID NO:12 的氨基酸序列的 HVR-H1；(b) 包含 SEQ ID NO:13 或 SEQ ID NO:18 的氨基酸序列的 HVR-H2；(c) 包含 SEQ ID NO:14 的氨基酸序列的 HVR-H3；(d) 包含 SEQ ID NO:15 的氨基酸序列的 HVR-L1；(e) 包含 SEQ ID NO:16 的氨基酸序列的 HVR-L2；和 (f) 包含 SEQ ID NO:17 的氨基酸序列的 HVR-L3。

[0150] 在一些实施方案中，抗体包含选自以下的至少一个、至少两个、或所有三个 VH HVR 序列：(a) 包含 SEQ ID NO:12 的氨基酸序列的 HVR-H1；(b) 包含 SEQ ID NO:13 或 SEQ ID NO:18 的氨基酸序列的 HVR-H2；和 (c) 包含 SEQ ID NO:14 的氨基酸序列的 HVR-H3。一些实施方案中，抗体包含含有 SEQ ID NO:14 的氨基酸序列的 HVR-H3。一些实施方案中，抗体包含含有 SEQ ID NO:14 的氨基酸序列的 HVR-H3 和含有 SEQ ID NO:17 的氨基酸序列的 HVR-L3。在一些实施方案中，抗体包含含有 SEQ ID NO:14 的氨基酸序列的 HVR-H3、含有 SEQ ID NO:17 的氨基酸序列的 HVR-L3、和包含 SEQ ID NO:13 或 SEQ ID NO:18 的氨基酸序列的 HVR-H2。一些实施方案中，抗体包含 (a) 含有 SEQ ID NO:12 的氨基酸序列的 HVR-H1；(b) 包含 SEQ ID NO:13 或 SEQ ID NO:18 的氨基酸序列的 HVR-H2；和 (c) 包含 SEQ ID NO:14 的氨基酸序列的 HVR-H3。

[0151] 在一些实施方案中，抗体包含选自以下的至少一个、至少两个、或所有三个 VL HVR 序列：(a) 包含 SEQ ID NO:15 的氨基酸序列的 HVR-L1；(b) 包含 SEQ ID NO:16 的氨基酸序列的 HVR-L2；和 (e) 包含 SEQ ID NO:17 的氨基酸序列的 HVR-L3。一些实施方案中，抗体包含 (a) 含有 SEQ ID NO:15 的氨基酸序列的 HVR-L1；(b) 包含 SEQ ID NO:16 的氨基酸序列的 HVR-L2；和 (e) 包含 SEQ ID NO:17 的氨基酸序列的 HVR-L3。

[0152] 在一些实施方案中，抗体包含 (a) 包含选自以下的至少一个、至少两个、或所有三个 VH HVR 序列的 VH 结构域：(i) 包含 SEQ ID NO:12 的氨基酸序列的 HVR-H1、(ii) 包含 SEQ ID NO:13 或 SEQ ID NO:18 的氨基酸序列的 HVR-H2、和 (iii) 包含 SEQ ID NO:14 的氨基酸序列的 HVR-H3；和 (b) 包含选自以下的至少一个、至少两个、或所有三个 VL HVR 序列的 VL 结构域：(i) 包含 SEQ ID NO:15 的氨基酸序列的 HVR-L1、(ii) 包含 SEQ ID NO:16 的氨基酸序列的 HVR-L2、和 (iii) 包含 SEQ ID NO:17 的氨基酸序列的 HVR-L3。

[0153] 一些实施方案中，提供抗体，所述抗体包含 (a) 含有 SEQ ID NO:12 的氨基酸序列的 HVR-H1；(b) 包含 SEQ ID NO:13 或 SEQ ID NO:18 的氨基酸序列的 HVR-H2；(c) 包含 SEQ ID NO:14 的氨基酸序列的 HVR-H3；(d) 包含 SEQ ID NO:15 的氨基酸序列的 HVR-L1；(e) 包含 SEQ ID NO:16 的氨基酸序列的 HVR-L2；和 (f) 包含选自 SEQ ID NO:17 的氨基酸序列的 HVR-L3。一些实施方案中，提供抗体，所述抗体包含 (a) 含有 SEQ ID NO:12 的氨基酸序列的 HVR-H1；(b) 包含 SEQ ID NO:13 的氨基酸序列的 HVR-H2；(c) 包含 SEQ ID NO:14 的氨基酸序列的 HVR-H3；(d) 包含 SEQ ID NO:15 的氨基酸序列的 HVR-L1；(e) 包含 SEQ ID NO:16 的氨基酸序列的 HVR-L2；和 (f) 包含选自 SEQ ID NO:17 的氨基酸序列的 HVR-L3。

[0154] 在任何上述实施方案中，抗 IL-4 抗体是人源化的。在一些实施方案中，抗 IL-4 抗体包含如任何上述实施方案中的 HVRs，并且进一步包含受体人构架，例如人免疫球蛋白构架或人共有构架。在一些实施方案中，抗 IL-4 抗体包含如任何上述实施方案中的 HVRs，并且进一步包含含有 SEQ ID NOS:3 至 9 之任一的 FR1, FR2, FR3, 和 FR4 的 VH。在一些实施方案中，抗 IL-4 抗体包含如任何上述实施方案中的 HVRs，并且进一步包含含有 SEQ ID NO:9 的 FR1, FR2, FR3, 和 FR4 的 VH。在一些实施方案中，抗 IL-4 抗体包含如任何上述实施方案中的 HVRs，并且进一步包含含有 SEQ ID NOS:10 至 11 之任一的 FR1, FR2, FR3, 和 FR4 的 VL。在一些实施方案中，抗 IL-4 抗体包含如任何上述实施方案中的 HVRs，并且进一步包含含有 SEQ ID NO:10 的 FR1, FR2, FR3, 和 FR4 的 VL。

[0155] 在一些实施方案中，抗 IL-4 抗体包含与 SEQ ID NOS:1 和 3 至 9 之任一的氨基酸序列具有至少 90%、91%、92%、93%、94%、95%、96%、97%、98%、99% 或 100% 序列同一性的重链可变结构域 (VH) 序列。在一些实施方案中，具有至少 90%、91%、92%、93%、94%、95%、96%、97%、98% 或 99% 同一性的 VH 序列，相对于参考序列，含有置换（例如保守置换）、插入或缺失，但包含该序列的抗 IL-4 抗体保留与 IL-4 结合的能力。在一些实施方案中，总共 1 - 10 个氨基酸已在 SEQ ID NO:9 中被置换、插入和 / 或缺失。在特定实施方案中，置换、插入或缺失发生在 HVRs 外的区域中（即在 FRs 中）。任选地，抗 IL-4 抗体包含 SEQ ID NO:9 中的 VH 序列，包括该序列的翻译后修饰。在一个特定实施方案中，VH 包含选自以下的一个、两个或三个 HVR：(a) 包含 SEQ ID NO:12 的氨基酸序列的 HVR-H1、(b) 包含 SEQ ID NO:13 或 SEQ ID NO:18 的氨基酸序列的 HVR-H2、和 (c) 包含 SEQ ID NO:14 的氨基酸序列的 HVR-H3。

[0156] 在一些实施方案中，提供了抗 IL-4 抗体，其中所述抗体包含与 SEQ ID NO:2, 10, 和 11 之任一的氨基酸序列具有至少 90%、91%、92%、93%、94%、95%、96%、97%、98%、99% 或 100% 序列同一性的轻链可变结构域 (VL)。在特定实施方案中，具有至少 90%、91%、92%、93%、94%、95%、96%、97%、98% 或 99% 同一性的 VL 序列，相对于参考序列，含有置换（例如保守置换）、插入或缺失，但包含该序列的抗 IL-4 抗体保留与 IL-4 结合的能力。在一些实施方案中，总共 1 - 10 个氨基酸已在 SEQ ID NO:10 中被置换、插入和 / 或缺失。在特定实施方案中，置换、插入或缺失发生在 HVRs 外的区域中（即在 FRs 中）。任选地，抗 IL-4 抗体包含 SEQ ID NO:10 中的 VL 序列，包括该序列的翻译后修饰。在一个特定实施方案中，VL 包含选自以下的一个、两个或三个 HVR：(a) 包含 SEQ ID NO:15 的氨基酸序列的 HVR-L1、(b) 包含 SEQ ID NO:16 的氨基酸序列的 HVR-L2、和 (c) 包含 SEQ ID NO:17 的氨基酸序列的 HVR-L3。

[0157] 在一些实施方案中，提供了抗 IL-4 抗体，其中所述抗体包含如上文提供的任何实施方案中的 VH 和如上文提供的任何实施方案中的 VL。在一些实施方案中，抗体包含分别在 SEQ ID NO:9 和 SEQ ID NO:10 中的 VH 和 VL 序列，包括这些序列的翻译后修饰。

[0158] 在一些实施方案中，提供抗体，所述抗体与包含 SEQ ID NOs:9 的 VH 序列和 SEQ ID NO:10 的 VL 序列的抗 IL-4 抗体竞争结合 IL-4。在一些实施方案中，本发明提供与本文提供的抗 IL-4 抗体结合相同表位的抗体。例如，在特定实施方案中，提供了与抗 IL-4 抗体结合相同表位的抗体，其中所述抗 IL-4 抗体包含 SEQ ID NO:9 的 VH 序列和 SEQ ID NO:10 的 VL 序列。

[0159] 在一些实施方案中，根据任何上述实施方案的抗 IL-4 抗体可以是单克隆抗体，包括嵌合、人源化或人抗体。在一些实施方案中，抗 IL-4 抗体是抗体片段，例如 Fv、Fab、Fab'、scFv、双抗体或 F(ab')2 片段。在一些实施方案中，抗体是全长抗体，例如完整 IgG1 或 IgG4 抗体或如本文定义的其他抗体类或同种型。

[0160] 在一些实施方案中，根据任何上述实施方案的抗 IL-4 抗体可以并入单独或组合的如下文章节 1-7 中所述的任何特征。

[0161] 示例性抗 IL-13 抗体

[0162] 在一些实施方案中，提供与 IL-13 结合的分离的抗体。在一些实施方案中，抗 IL-13 抗体包含选自以下的至少一个、两个、三个、四个、五个或六个 HVR：(a) 包含 SEQ ID NO:21 或 SEQ ID NO:60 的氨基酸序列的 HVR-H1；(b) 包含 SEQ ID NO:22 的氨基酸序列的 HVR-H2；(c) 包含 SEQ ID NO:23 的氨基酸序列的 HVR-H3；(d) 包含 SEQ ID NO:24 的氨基酸序列的 HVR-L1；(e) 包含 SEQ ID NO:25 的氨基酸序列的 HVR-L2；和 (f) 包含 SEQ ID NO:26 的氨基酸序列的 HVR-L3。

[0163] 在一些实施方案中，提供抗体，所述抗体包含选自以下的至少一个、至少两个、或所有三个 VH HVR 序列：(a) 包含 SEQ ID NO:21 或 SEQ ID NO:60 的氨基酸序列的 HVR-H1；(b) 包含 SEQ ID NO:22 的氨基酸序列的 HVR-H2；和 (c) 包含 SEQ ID NO:23 的氨基酸序列的 HVR-H3。一些实施方案中，抗体包含含有 SEQ ID NO:23 的氨基酸序列的 HVR-H3。一些实施方案中，抗体包含含有 SEQ ID NO:23 的氨基酸序列的 HVR-H3 和含有 SEQ ID NO:26 的氨基酸序列的 HVR-L3。在一些实施方案中，抗体包括包含 SEQ ID NO:23 的氨基酸序列的 HVR-H3、包含 SEQ ID NO:26 的氨基酸序列的 HVR-L3、和包含 SEQ ID NO:22 的氨基酸序列的 HVR-H2。一些实施方案中，抗体包含 (a) 含有 SEQ ID NO:21 或 SEQ ID NO:60 的氨基酸序列的 HVR-H1；(b) 包含 SEQ ID NO:22 的氨基酸序列的 HVR-H2；和 (c) 包含 SEQ ID NO:23 的氨基酸序列的 HVR-H3。

[0164] 在一些实施方案中，提供抗体，所述抗体包含选自以下的至少一个、至少两个、或所有三个 VL HVR 序列：(a) 包含 SEQ ID NO:24 的氨基酸序列的 HVR-L1；(b) 包含 SEQ ID NO:25 的氨基酸序列的 HVR-L2；和 (e) 包含 SEQ ID NO:26 的氨基酸序列的 HVR-L3。一些实施方案中，抗体包含 (a) 含有 SEQ ID NO:24 的氨基酸序列的 HVR-L1；(b) 包含 SEQ ID NO:25 的氨基酸序列的 HVR-L2；和 (e) 包含 SEQ ID NO:26 的氨基酸序列的 HVR-L3。

[0165] 在一些实施方案中，抗体包含：(a) 包含选自以下的至少一个、至少两个、或所有三个 VH HVR 序列的 VH 结构域：(i) 包含 SEQ ID NO:21 或 SEQ ID NO:60 的氨基酸序列的 HVR-H1、(ii) 包含 SEQ ID NO:22 的氨基酸序列的 HVR-H2、和 (iii) 包含 SEQ ID NO:23 的氨基酸序列的 HVR-H3；和 (b) 包含选自以下的至少一个、至少两个、或所有三个 VL HVR 序列的 VL 结构域：(i) 包含 SEQ ID NO:24 的氨基酸序列的 HVR-L1、(ii) 包含 SEQ ID NO:25 的氨基酸序列的 HVR-L2、和 (iii) 包含 SEQ ID NO:26 的氨基酸序列的 HVR-L3。

[0166] 一些实施方案中，提供抗体，所述抗体包含 (a) 含有 SEQ ID NO:21 或 SEQ ID NO:60 的氨基酸序列的 HVR-H1；(b) 包含 SEQ ID NO:22 的氨基酸序列的 HVR-H2；(c) 包含 SEQ ID NO:23 的氨基酸序列的 HVR-H3；(d) 包含 SEQ ID NO:24 的氨基酸序列的 HVR-L1；(e) 包含 SEQ ID NO:25 的氨基酸序列的 HVR-L2；和 (f) 包含选自 SEQ ID NO:26 的氨基酸序列的 HVR-L3。

[0167] 在任何上述实施方案中，抗 IL-13 抗体是人源化的。在一些实施方案中，抗 IL-13 抗体包含如任何上述实施方案中的 HVRs，并且进一步包含受体人构架，例如人免疫球蛋白构架或人共有构架。在一些实施方案中，抗 IL-13 抗体包含如任何上述实施方案中的 HVRs，并且进一步包含含有 SEQ ID NO:19 的 FR1, FR2, FR3, 和 / 或 FR4 序列的 VH。在一些实施方案中，抗 IL-13 抗体包含如任何上述实施方案中的 HVRs，并且进一步包含含有 SEQ ID NO:20 的 FR1, FR2, FR3, 和 / 或 FR4 序列的 VL。在一些实施方案中，抗 IL-13 抗体包含如任何上述实施方案中的 HVRs，并且进一步包含含有 SEQ ID NO:56 的 FR1, FR2, FR3, 和 / 或 FR4 序列的 VH。在一些实施方案中，抗 IL-13 抗体包含如任何上述实施方案中的 HVRs，并且进一步包含含有 SEQ ID NO:57 的 FR1, FR2, FR3, 和 / 或 FR4 序列的 VL。

[0168] 在一些实施方案中，抗 IL-13 抗体包含与 SEQ ID NO:19 的氨基酸序列具有至少 90%、91%、92%、93%、94%、95%、96%、97%、98%、99% 或 100% 序列同一性的重链可变结构域 (VH) 序列。在一些实施方案中，具有至少 90%、91%、92%、93%、94%、95%、96%、97%、98% 或 99% 同一性的 VH 序列，相对于参考序列，含有置换 (例如保守置换)、插入或缺失，但包含该序列的抗 IL-13 抗体保留与 IL-13 结合的能力。在一些实施方案中，总共 1 - 10 个氨基酸已在 SEQ ID NO:19 中被置换、插入和 / 或缺失。在特定实施方案中，置换、插入或缺失发生在 HVRs 外的区域中 (即在 FRs 中)。在一些实施方案中，抗 IL-13 抗体包含 SEQ ID NO:19 中的 VH 序列，包括该序列的翻译后修饰。在一些实施方案中，抗 IL-13 抗体包含 SEQ ID NO:56 中的 VH 序列，包括该序列的翻译后修饰。在一些实施方案中，VH 包含选自以下的一个、两个或三个 HVR :(a) 包含 SEQ ID NO:21 或 SEQ ID NO:60 的氨基酸序列的 HVR-H1、(b) 包含 SEQ ID NO:22 的氨基酸序列的 HVR-H2、和 (c) 包含 SEQ ID NO:23 的氨基酸序列的 HVR-H3。

[0169] 在一些实施方案中，提供了抗 IL-13 抗体，其中所述抗体包含与 SEQ ID NO:20 的氨基酸序列具有至少 90%、91%、92%、93%、94%、95%、96%、97%、98%、99% 或 100% 序列同一性的轻链可变结构域 (VL)。在特定实施方案中，具有至少 90%、91%、92%、93%、94%、95%、96%、97%、98% 或 99% 同一性的 VL 序列，相对于参考序列，含有置换 (例如保守置换)、插入或缺失，但包含该序列的抗 IL-13 抗体保留与 IL-13 结合的能力。在一些实施方案中，总共 1 - 10 个氨基酸已在 SEQ ID NO:20 中被置换、插入和 / 或缺失。在特定实施方案中，置换、插入或缺失发生在 HVRs 外的区域中 (即在 FRs 中)。在一些实施方案中，抗 IL-13 抗体包含 SEQ ID NO:20 中的 VL 序列，包括该序列的翻译后修饰。在一些实施方案中，抗 IL-13 抗体包含 SEQ ID NO:57 中的 VL 序列，包括该序列的翻译后修饰。在一些实施方案中，VL 包含选自以下的一个、两个或三个 HVR :(a) 包含 SEQ ID NO:24 的氨基酸序列的 HVR-L1、(b) 包含 SEQ ID NO:25 的氨基酸序列的 HVR-L2、和 (c) 包含 SEQ ID NO:26 的氨基酸序列的 HVR-L3。

[0170] 在一些实施方案中，提供了抗 IL-13 抗体，其中所述抗体包含如上文提供的任何实施方案中的 VH 和如上文提供的任何实施方案中的 VL。在一些实施方案中，抗体包含在 SEQ ID NO:19 或 SEQ ID NO:56 中的 VH 和在 SEQ ID NO:20 或 SEQ ID NO:57 中 VL 序列，包括这些序列的翻译后修饰。

[0171] 在一些实施方案中，提供抗体，所述抗体与包含 SEQ ID NOs:19 的 VH 序列和 SEQ ID NO:20 的 VL 序列的抗 IL-13 抗体竞争结合 IL-13。在一些实施方案中，本发明提供与本文提

供的抗 IL-13 抗体结合相同的表位的抗体。参见，例如，Ultsch, M. 等，Structural Basis of Signaling Blockade by Anti-IL-13Antibody Lebrikizumab, J. Mol. Biol. (2013), dx. doi. org/10. 1016/j. jmb. 2013. 01. 024. 在一些实施方案中，本发明提供与本文提供的抗 IL-13 抗体结合相同的表位的抗体。例如，在特定实施方案中，提供与抗 IL-13 抗体结合相同的表位的抗体，其中所述抗 IL-13 抗体包含 SEQ ID NO:19 的 VH 序列和 SEQ ID NO:20 的 VL 序列。一些实施方案中，提供抗体，所述抗体结合人前体 IL-13(SEQ ID NO:29) 的氨基酸 63 至 74 中的、或成熟形式的人 IL-13(SEQ ID NO:30) 的氨基酸 45 至 56 中的表位，其中所述氨基酸是 YCAALESLINVS(SEQ ID NO:43)。一些实施方案中，提供抗体，所述抗体结合人前体 IL-13(SEQ ID NO:29) 的氨基酸 68 至 75 中的、或成熟形式的人 IL-13(SEQ ID NO:30) 的氨基酸 50 至 57 中的表位，其中所述氨基酸是 ESLINVSG(SEQ ID NO:42)。

[0172] 另一示例性抗 IL-13 抗体是 11H4 及其人源化形式，包括 hu11H4v6。Mu11H4 包含分别含有 SEQ ID NOs:45 和 44 的氨基酸序列的重链和轻链可变区。人源化 hu11H4v6 包含分别含有 SEQ ID NOs:49 和 48 的氨基酸序列的重链可变区和轻链可变区。人源化 hu11H4v6 包含分别含有 SEQ ID NOs:47 和 46 的氨基酸序列的重链和轻链。

[0173] 在一些实施方案中，抗 IL-13 抗体包含选自以下的至少一个、两个、三个、四个、五个或六个 HVR:(a) 包含 SEQ ID NO:50 的氨基酸序列的 HVR-H1 ;(b) 包含 SEQ ID NO:51 的氨基酸序列的 HVR-H2 ;(c) 包含 SEQ ID NO:52 的氨基酸序列的 HVR-H3 ;(d) 包含 SEQ ID NO:53 的氨基酸序列的 HVR-L1 ;(e) 包含 SEQ ID NO:54 的氨基酸序列的 HVR-L2 ;和 (f) 包含 SEQ ID NO:55 的氨基酸序列的 HVR-L3。

[0174] 在一些实施方案中，提供抗体，所述抗体包含选自以下的至少一个、至少两个、或所有三个 VH HVR 序列 :(a) 包含 SEQ ID NO:50 的氨基酸序列的 HVR-H1 ;(b) 包含 SEQ ID NO:51 的氨基酸序列的 HVR-H2 ;和 (c) 包含 SEQ ID NO:52 的氨基酸序列的 HVR-H3。一些实施方案中，抗体包含含有 SEQ ID NO:52 的氨基酸序列的 HVR-H3。一些实施方案中，抗体包含含有 SEQ ID NO:52 的氨基酸序列的 HVR-H3 和含有 SEQ ID NO:55 的氨基酸序列的 HVR-L3。在一些实施方案中，抗体包括包含 SEQ ID NO:52 的氨基酸序列的 HVR-H3、包含 SEQ ID NO:55 的氨基酸序列的 HVR-L3、和包含 SEQ ID NO:51 的氨基酸序列的 HVR-H2。一些实施方案中，抗体包含 (a) 含有 SEQ ID NO:50 的氨基酸序列的 HVR-H1 ;(b) 包含 SEQ ID NO:51 的氨基酸序列的 HVR-H2 ;和 (c) 包含 SEQ ID NO:52 的氨基酸序列的 HVR-H3。

[0175] 在一些实施方案中，提供抗体，所述抗体包含选自以下的至少一个、至少两个、或所有三个 VL HVR 序列 :(a) 包含 SEQ ID NO:53 的氨基酸序列的 HVR-L1 ;(b) 包含 SEQ ID NO:54 的氨基酸序列的 HVR-L2 ;和 (e) 包含 SEQ ID NO:55 的氨基酸序列的 HVR-L3。一些实施方案中，抗体包含 (a) 含有 SEQ ID NO:53 的氨基酸序列的 HVR-L1 ;(b) 包含 SEQ ID NO:54 的氨基酸序列的 HVR-L2 ;和 (e) 包含 SEQ ID NO:55 的氨基酸序列的 HVR-L3。

[0176] 在一些实施方案中，抗体包含 :(a) 包含选自以下的至少一个、至少两个、或所有三个 VH HVR 序列的 VH 结构域 :(i) 包含 SEQ ID NO:50 的氨基酸序列的 HVR-H1、(ii) 包含 SEQ ID NO:51 的氨基酸序列的 HVR-H2、和 (iii) 包含选自 SEQ ID NO:52 的氨基酸序列的 HVR-H3 ;和 (b) 包含选自以下的至少一个、至少两个、或所有三个 VL HVR 序列的 VL 结构域 :(i) 包含 SEQ ID NO:53 的氨基酸序列的 HVR-L1、(ii) 包含 SEQ ID NO:54 的氨基酸序列的 HVR-L2、和 (iii) 包含 SEQ ID NO:55 的氨基酸序列的 HVR-L3。

[0177] 一些实施方案中，提供抗体，所述抗体包含：(a) 包含 SEQ ID NO:50 的氨基酸序列的 HVR-H1；(b) 包含 SEQ ID NO:51 的氨基酸序列的 HVR-H2；(c) 包含 SEQ ID NO:52 的氨基酸序列的 HVR-H3；(d) 包含 SEQ ID NO:53 的氨基酸序列的 HVR-L1；(e) 包含 SEQ ID NO:54 的氨基酸序列的 HVR-L2；和 (f) 包含选自 SEQ ID NO:55 的氨基酸序列的 HVR-L3。

[0178] 在任何上述实施方案中，抗 IL-13 抗体是人源化的。在一些实施方案中，抗 IL-13 抗体包含如任何上述实施方案中的 HVRs，并且进一步包含受体人构架，例如人免疫球蛋白构架或人共有构架。在一些实施方案中，抗 IL-13 抗体包含如任何上述实施方案中的 HVRs，并且进一步包含含有 SEQ ID NO:49 的 FR1, FR2, FR3, 和 / 或 FR4 序列的 VH。在一些实施方案中，抗 IL-13 抗体包含如任何上述实施方案中的 HVRs，并且进一步包含含有 SEQ ID NO:48 的 FR1, FR2, FR3, 和 / 或 FR4 序列的 VL。

[0179] 在一些实施方案中，抗 IL-13 抗体包含与 SEQ ID NO:49 的氨基酸序列具有至少 90%、91%、92%、93%、94%、95%、96%、97%、98%、99% 或 100% 序列同一性的重链可变结构域 (VH) 序列。在一些实施方案中，具有至少 90%、91%、92%、93%、94%、95%、96%、97%、98% 或 99% 同一性的 VH 序列，相对于参考序列，含有置换 (例如保守置换)、插入或缺失，但包含该序列的抗 IL-13 抗体保留与 IL-13 结合的能力。在一些实施方案中，总共 1 - 10 个氨基酸已在 SEQ ID NO:49 中被置换、插入和 / 或缺失。在特定实施方案中，置换、插入或缺失发生在 HVRs 外的区域中 (即在 FRs 中)。任选地，抗 IL-13 抗体包含 SEQ ID NO:49 中的 VH 序列，包括该序列的翻译后修饰。在一个特定实施方案中，VH 包含选自以下的一个、两个或三个 HVR：(a) 包含 SEQ ID NO:50 的氨基酸序列的 HVR-H1、(b) 包含 SEQ ID NO:51 的氨基酸序列的 HVR-H2、和 (c) 包含 SEQ ID NO:52 的氨基酸序列的 HVR-H3。

[0180] 在一些实施方案中，提供了抗 IL-13 抗体，其中所述抗体包含与 SEQ ID NO:48 的氨基酸序列具有至少 90%、91%、92%、93%、94%、95%、96%、97%、98%、99% 或 100% 序列同一性的轻链可变结构域 (VL)。在特定实施方案中，具有至少 90%、91%、92%、93%、94%、95%、96%、97%、98% 或 99% 同一性的 VL 序列，相对于参考序列，含有置换 (例如保守置换)、插入或缺失，但包含该序列的抗 IL-13 抗体保留与 IL-13 结合的能力。在一些实施方案中，总共 1 - 10 个氨基酸已在 SEQ ID NO:48 中被置换、插入和 / 或缺失。在特定实施方案中，置换、插入或缺失发生在 HVRs 外的区域中 (即在 FRs 中)。任选地，抗 IL-13 抗体包含 SEQ ID NO:48 中的 VL 序列，包括该序列的翻译后修饰。在一个特定实施方案中，VL 包含选自以下的一个、两个或三个 HVR：(a) 包含 SEQ ID NO:53 的氨基酸序列的 HVR-L1、(b) 包含 SEQ ID NO:54 的氨基酸序列的 HVR-L2、和 (c) 包含 SEQ ID NO:55 的氨基酸序列的 HVR-L3。

[0181] 在一些实施方案中，提供了抗 IL-13 抗体，其中所述抗体包含如上文提供的任何实施方案中的 VH 和如上文提供的任何实施方案中的 VL。在一些实施方案中，抗体包含分别在 SEQ ID NO:49 和 SEQ ID NO:48 中的 VH 和 VL 序列，包括这些序列的翻译后修饰。

[0182] 在一些实施方案中，提供抗体，所述抗体与包含 SEQ ID NOs:49 的 VH 序列和 SEQ ID NO:48 的 VL 序列的抗 IL-13 抗体竞争结合 IL-13。在一些实施方案中，本发明提供与本文提供的抗 IL-13 抗体结合相同的表位的抗体。参见，例如，Ultsch, M. 等，Structural Basis of Signaling Blockade by Anti-IL-13Antibody Lebrikizumab, J. Mol. Biol. (2013), dx. doi. org/10. 1016/j. jmb. 2013. 01. 053. 在一些实施方案中，本发明提供与本文提供的抗

IL-13 抗体结合相同的表位的抗体。例如，在特定实施方案中，提供了与抗 IL-13 抗体结合相同的表位的抗体，其中所述抗 IL-13 抗体包含 SEQ ID NO:49 的 VH 序列和 SEQ ID NO:48 的 VL 序列。

[0183] 在一些实施方案中，根据任何上述实施方案的抗 IL-13 抗体可以是单克隆抗体，包括嵌合、人源化或人抗体。在一些实施方案中，抗 IL-13 抗体是抗体片段，例如 Fv、Fab、Fab'、scFv、双抗体或 F(ab')2 片段。在一些实施方案中，抗体是全长抗体，例如完整 IgG1 或 IgG4 抗体或如本文定义的其他抗体类或同种型。

[0184] 在一些实施方案中，根据任何上述实施方案的抗 IL-13 抗体可以并入单独或组合的如下文章节 1-7 中所述的任何特征。

[0185] 示例性抗 IL-4/IL-13 双特异性抗体

[0186] 在一些实施方案中，提供包含特异地结合 IL-4 和 IL-13 的抗原结合结构域的多特异性抗体（例如双特异性抗体）。一些实施方案中，抗原结合结构域不特异结合其它靶标。结合 IL-4 和 IL-13 的多特异性抗体可以包含根据本文中针对抗 IL4 抗体描述的任何实施方案的第一组可变区（VH 和 VL；也称作 VH/VL 单位）和根据本文中针对抗 IL13 抗体描述的任何实施方案的第二组可变区（VH 和 VL；也称作 VH/VL 单位）。

[0187] 一些实施方案中，多特异性抗体包含特异地结合 IL-4 和 IL-13 的抗原结合结构域，其中该抗体包含第一 VH/VL 单位，所述第一 VH/VL 单位包含含有 SEQ ID NO:9 的氨基酸序列的 VH（重链可变结构域）。一些实施方案中，多特异性抗体包含特异地结合 IL-4 和 IL-13 的抗原结合结构域，其中该抗体包含第一 VH/VL 单位，所述第一 VH/VL 单位包含含有 SEQ ID NO:10 的氨基酸序列的 VL（轻链可变结构域）。一些实施方案中，多特异性抗体包含特异地结合 IL-4 和 IL-13 的抗原结合结构域，其中该抗体包含第一 VH/VL 单位，所述第一 VH/VL 单位包含含有 SEQ ID NO:9 的氨基酸序列的 VH 和含有 SEQ ID NO:10 的氨基酸序列的 VL。一些实施方案中，多特异性抗体包含特异地结合 IL-4 和 IL-13 的抗原结合结构域，其中该抗体包含第一 VH/VL 单位，所述第一 VH/VL 单位与包含含有 SEQ ID NO:9 的氨基酸序列的 VH 和含有 SEQ ID NO:10 的氨基酸序列的 VL 的抗体竞争结合 IL-4。

[0188] 一些实施方案中，多特异性抗体包含特异地结合 IL-4 和 IL-13 的抗原结合结构域，其中该抗体包含第二 VH/VL 单位，所述第二 VH/VL 单位包含含有 SEQ ID NO:19 或 SEQ ID NO:56 的氨基酸序列的 VH（重链可变结构域）。一些实施方案中，多特异性抗体包含特异地结合 IL-4 和 IL-13 的抗原结合结构域，其中该抗体包含第二 VH/VL 单位，所述第二 VH/VL 单位包含含有 SEQ ID NO:20 或 SEQ ID NO:57 的氨基酸序列的 VL（轻链可变结构域）。一些实施方案中，多特异性抗体包含特异地结合 IL-4 和 IL-13 的抗原结合结构域，其中该抗体包含第二 VH/VL 单位，所述第二 VH/VL 单位包含含有 SEQ ID NO:19 或 SEQ ID NO:56 的氨基酸序列的 VH 和含有 SEQ ID NO:20 或 SEQ ID NO:57 的氨基酸序列的 VL。一些实施方案中，多特异性抗体包含特异地结合 IL-4 和 IL-13 的抗原结合结构域，其中该抗体包含第二 VH/VL 单位，所述第二 VH/VL 单位与包含含有 SEQ ID NO:19 的氨基酸序列的 VH 和含有 SEQ ID NO:20 的氨基酸序列的 VL 的抗体竞争结合 IL-13。一些实施方案中，多特异性抗体包含特异地结合 IL-4 和 IL-13 的抗原结合结构域，其中该抗体包含第二 VH/VL 单位，所述第二 VH/VL 单位结合由 SEQ ID NO:29 的氨基酸 82 至 89 组成的 IL-13 表位。一些实施方案中，多特异性抗体包含特异地结合 IL-4 和 IL-13 的抗原结合结构域，其中该抗体包含第

二 VH/VL 单位，所述第二 VH/VL 单位结合由 SEQ ID NO:29 的氨基酸 77 至 89 组成的 IL-13 表位。

[0189] 一些实施方案中，多特异性抗体包含特异地结合 IL-4 和 IL-13 的抗原结合结构域，其中该抗体包含第二 VH/VL 单位，所述第二 VH/VL 单位包含含有 SEQ ID NO:49 的氨基酸序列的 VH（重链可变结构域）。一些实施方案中，多特异性抗体包含特异地结合 IL-4 和 IL-13 的抗原结合结构域，其中该抗体包含第二 VH/VL 单位，所述第二 VH/VL 单位包含含有 SEQ ID NO:48 的氨基酸序列的 VL（轻链可变结构域）。一些实施方案中，多特异性抗体包含特异地结合 IL-4 和 IL-13 的抗原结合结构域，其中该抗体包含第二 VH/VL 单位，所述第二 VH/VL 单位包含含有 SEQ ID NO:49 的氨基酸序列的 VH 和含有 SEQ ID NO:48 的氨基酸序列的 VL。一些实施方案中，多特异性抗体包含特异地结合 IL-4 和 IL-13 的抗原结合结构域，其中该抗体包含第二 VH/VL 单位，所述第二 VH/VL 单位与包含含有 SEQ ID NO:49 的氨基酸序列的 VH 和含有 SEQ ID NO:48 的氨基酸序列的 VL 的抗体竞争结合 IL-13。

[0190] 一些实施方案中，多特异性抗体包含特异地结合 IL-4 和 IL-13 的抗原结合结构域，其中该抗体包含第一 VH/VL 单位，所述第一 VH/VL 单位包含含有 SEQ ID NO:9 的氨基酸序列的第一 VH 和含有 SEQ ID NO:10 的氨基酸序列的第一 VL；且该抗体包含第二 VH/VL 单位，所述第二 VH/VL 单位包含包含 SEQ ID NO:19 或 SEQ ID NO:56 的氨基酸序列的第二 VH 和含有 SEQ ID NO:20 或 SEQ ID NO:57 的氨基酸序列的第二 VL。

[0191] 一些实施方案中，多特异性抗体包含特异地结合 IL-4 和 IL-13 的抗原结合结构域，其中该抗体包含第一 VH/VL 单位，所述第一 VH/VL 单位包含含有 SEQ ID NO:9 的氨基酸序列的第一 VH 和含有 SEQ ID NO:10 的氨基酸序列的第一 VL；且包含第二 VH/VL 单位，所述第二 VH/VL 单位包含含有 SEQ ID NO:49 的氨基酸序列的第二 VH 和含有 SEQ ID NO:48 的氨基酸序列的第二 VL。

[0192] 一些实施方案中，多特异性抗体包含特异地结合 IL-4 和 IL-13 的抗原结合结构域，其中该抗体包含第一 VH/VL 单位，所述第一 VH/VL 单位包含与 SEQ ID NO:9 的氨基酸序列具有至少 90%，91%，92%，93%，94%，95%，96%，97%，98%，99%，或 100%序列同一性的 VH、和与 SEQ ID NO:10 的氨基酸序列具有至少 90%，91%，92%，93%，94%，95%，96%，97%，98%，99%，或 100%序列同一性的 VL。一些实施方案中，多特异性抗体包含特异地结合 IL-4 和 IL-13 的抗原结合结构域，其中该抗体包含第二 VH/VL 单位，所述第二 VH/VL 单位包含与 SEQ ID NO:19 的氨基酸序列具有至少 90%，91%，92%，93%，94%，95%，96%，97%，98%，99%，或 100%序列同一性的 VH、和与 SEQ ID NO:20 的氨基酸序列具有至少 90%，91%，92%，93%，94%，95%，96%，97%，98%，99%，或 100%序列同一性的 VL。一些实施方案中，多特异性抗体包含特异地结合 IL-4 和 IL-13 的抗原结合结构域，其中该抗体包含第二 VH/VL 单位，所述第二 VH/VL 单位包含与 SEQ ID NO:49 的氨基酸序列具有至少 90%，91%，92%，93%，94%，95%，96%，97%，98%，99%，或 100%序列同一性的 VH、和与 SEQ ID NO:48 的氨基酸序列具有至少 90%，91%，92%，93%，94%，95%，96%，97%，98%，99%，或 100%序列同一性的 VL。在一些实施方案中，总共 1 - 10 个氨基酸已在上述序列中被置换、插入和 / 或缺失。在特定实施方案中，置换、插入或缺失发生在 HVRs 外的区域中（即在 FRs 中）。

[0193] 一些实施方案中，多特异性抗体包含特异地结合 IL-4 和 IL-13 的抗原结合结构

域，其中该抗体包含第一 VH/VL 单位，所述第一 VH/VL 单位包含与 SEQ ID NO:9 的氨基酸序列具有至少 90%，91%，92%，93%，94%，95%，96%，97%，98%，99%，或 100%序列同一性的第一 VH、和与 SEQ ID NO:10 的氨基酸序列具有至少 90%，91%，92%，93%，94%，95%，96%，97%，98%，99%，或 100%序列同一性的第一 VL；和第二 VH/VL 单位，所述第二 VH/VL 单位包含与 SEQ ID NO:19 的氨基酸序列具有至少 90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或 100%序列同一性的第二 VH、和与 SEQ ID NO:20 的氨基酸序列具有至少 90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或 100%序列同一性的第二 VL。在一些实施方案中，总共 1 - 10 个氨基酸已在上述序列中被置换、插入和 / 或缺失。在特定实施方案中，置换、插入或缺失发生在 HVRs 外的区域中（即在 FRs 中）。

[0194] 一些实施方案中，多特异性抗体包含特异地结合 IL-4 和 IL-13 的抗原结合结构域，其中该抗体包含第一 VH/VL 单位，所述第一 VH/VL 单位包含与 SEQ ID NO:9 的氨基酸序列具有至少 90%，91%，92%，93%，94%，95%，96%，97%，98%，99%，或 100%序列同一性的第一 VH、和与 SEQ ID NO:10 的氨基酸序列具有至少 90%，91%，92%，93%，94%，95%，96%，97%，98%，99%，或 100%序列同一性的第一 VL；和第二 VH/VL 单位，所述第二 VH/VL 单位包含与 SEQ ID NO:49 的氨基酸序列具有至少 90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或 100%序列同一性的第二 VH、和与 SEQ ID NO:48 的氨基酸序列具有至少 90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或 100%序列同一性的第二 VL。在一些实施方案中，总共 1 - 10 个氨基酸已在上述序列中被置换、插入和 / 或缺失。在特定实施方案中，置换、插入或缺失发生在 HVRs 外的区域中（即在 FRs 中）。

[0195] 一些实施方案中，多特异性抗体包含特异地结合 IL-4 和 IL-13 的抗原结合结构域，其中该抗体包含第一 VH/VL 单位，所述第一 VH/VL 单位包含选自以下的至少一个、两个、三个、四个、五个或六个 HVR：(a) 包含 SEQ ID NO:12 的氨基酸序列的 HVR-H1；(b) 包含 SEQ ID NO:13 或 SEQ ID NO:18 的氨基酸序列的 HVR-H2；(c) 包含 SEQ ID NO:14 的氨基酸序列的 HVR-H3；(d) 包含 SEQ ID NO:15 的氨基酸序列的 HVR-L1；(e) 包含 SEQ ID NO:16 的氨基酸序列的 HVR-L2；和 (f) 包含 SEQ ID NO:17 的氨基酸序列的 HVR-L3。一些实施方案中，多特异性抗体包含特异地结合 IL-4 和 IL-13 的抗原结合结构域，其中该抗体包含第二 VH/VL 单位，所述第二 VH/VL 单位包含选自以下的至少一个、两个、三个、四个、五个或六个 HVR：(a) 包含 SEQ ID NO:21 或 SEQ ID NO:60 的氨基酸序列的 HVR-H1；(b) 包含 SEQ ID NO:22 的氨基酸序列的 HVR-H2；(c) 包含 SEQ ID NO:23 的氨基酸序列的 HVR-H3；(d) 包含 SEQ ID NO:24 的氨基酸序列的 HVR-L1；(e) 包含 SEQ ID NO:25 的氨基酸序列的 HVR-L2；和 (f) 包含 SEQ ID NO:26 的氨基酸序列的 HVR-L3。一些实施方案中，多特异性抗体包含特异地结合 IL-4 和 IL-13 的抗原结合结构域，其中该抗体包含第二 VH/VL 单位，所述第二 VH/VL 单位包含选自以下的至少一个、两个、三个、四个、五个或六个 HVR：(a) 包含 SEQ ID NO:50 的氨基酸序列的 HVR-H1；(b) 包含 SEQ ID NO:51 的氨基酸序列的 HVR-H2；(c) 包含 SEQ ID NO:52 的氨基酸序列的 HVR-H3；(d) 包含 SEQ ID NO:53 的氨基酸序列的 HVR-L1；(e) 包含 SEQ ID NO:54 的氨基酸序列的 HVR-L2；和 (f) 包含 SEQ ID NO:55 的氨基酸序列的 HVR-L3。

[0196] 一些实施方案中，多特异性抗体包含特异地结合 IL-4 和 IL-13 的抗原结合结构域，其中该抗体包含第一 VH/VL 单位，所述第一 VH/VL 单位包含选自以下的至少一个、两个、三个、四个、五个或六个 HVR：(a) 包含 SEQ ID NO:12 的氨基酸序列的 HVR-H1；(b) 包含 SEQ

ID NO:13 或 SEQ ID NO:18 的氨基酸序列的 HVR-H2；(c) 包含 SEQ ID NO:14 的氨基酸序列的 HVR-H3；(d) 包含 SEQ ID NO:15 的氨基酸序列的 HVR-L1；(e) 包含 SEQ ID NO:16 的氨基酸序列的 HVR-L2；和 (f) 包含 SEQ ID NO:17 的氨基酸序列的 HVR-L3；和第二 VH/VL 单位，所述第二 VH/VL 单位包含选自以下的至少一个、两个、三个、四个、五个或六个 HVR：(a) 包含 SEQ ID NO:21 或 SEQ ID NO:60 的氨基酸序列的 HVR-H1；(b) 包含 SEQ ID NO:22 的氨基酸序列的 HVR-H2；(c) 包含 SEQ ID NO:23 的氨基酸序列的 HVR-H3；(d) 包含 SEQ ID NO:24 的氨基酸序列的 HVR-L1；(e) 包含 SEQ ID NO:25 的氨基酸序列的 HVR-L2；和 (f) 包含 SEQ ID NO:26 的氨基酸序列的 HVR-L3。

[0197] 一些实施方案中，多特异性抗体包含特异地结合 IL-4 和 IL-13 的抗原结合结构域，其中该抗体包含第一 VH/VL 单位，所述第一 VH/VL 单位包含选自以下的至少一个、两个、三个、四个、五个或六个 HVR：(a) 包含 SEQ ID NO:12 的氨基酸序列的 HVR-H1；(b) 包含 SEQ ID NO:13 或 SEQ ID NO:18 的氨基酸序列的 HVR-H2；(c) 包含 SEQ ID NO:14 的氨基酸序列的 HVR-H3；(d) 包含 SEQ ID NO:15 的氨基酸序列的 HVR-L1；(e) 包含 SEQ ID NO:16 的氨基酸序列的 HVR-L2；和 (f) 包含 SEQ ID NO:17 的氨基酸序列的 HVR-L3；和第二 VH/VL 单位，所述第二 VH/VL 单位包含选自以下的至少一个、两个、三个、四个、五个或六个 HVR：(a) 包含 SEQ ID NO:50 的氨基酸序列的 HVR-H1；(b) 包含 SEQ ID NO:51 的氨基酸序列的 HVR-H2；(c) 包含 SEQ ID NO:52 的氨基酸序列的 HVR-H3；(d) 包含 SEQ ID NO:53 的氨基酸序列的 HVR-L1；(e) 包含 SEQ ID NO:54 的氨基酸序列的 HVR-L2；和 (f) 包含 SEQ ID NO:55 的氨基酸序列的 HVR-L3。

[0198] 一些实施方案中，多特异性抗体包含特异地结合 IL-4 和 IL-13 的抗原结合结构域，其中该抗体包含第一 VH/VL 单位，所述第一 VH/VL 单位包含选自以下的至少一个、至少两个、或所有三个 VH HVR 序列：(a) 包含 SEQ ID NO:12 的氨基酸序列的 HVR-H1；(b) 包含 SEQ ID NO:13 或 SEQ ID NO:18 的氨基酸序列的 HVR-H2；(c) 包含 SEQ ID NO:14 的氨基酸序列的 HVR-H3。；一些实施方案中，多特异性抗体包含特异地结合 IL-4 和 IL-13 的抗原结合结构域，其中该抗体包含第二 VH/VL 单位，所述第二 VH/VL 单位包含选自以下的至少一个、至少两个、或所有三个 VH HVR 序列：(a) 含有 SEQ ID NO:21 或 SEQ ID NO:60 的氨基酸序列的 HVR-H1；(b) 包含 SEQ ID NO:22 的氨基酸序列的 HVR-H2；(c) 包含 SEQ ID NO:23 的氨基酸序列的 HVR-H3。一些实施方案中，多特异性抗体包含特异地结合 IL-4 和 IL-13 的抗原结合结构域，其中该抗体包含第二 VH/VL 单位，所述第二 VH/VL 单位包含选自以下的至少一个、至少两个、或所有三个 VH HVR 序列：(a) 含有 SEQ ID NO:50 的氨基酸序列的 HVR-H1；(b) 包含 SEQ ID NO:51 的氨基酸序列的 HVR-H2；(c) 包含 SEQ ID NO:52 的氨基酸序列的 HVR-H3。

[0199] 一些实施方案中，多特异性抗体包含特异地结合 IL-4 和 IL-13 的抗原结合结构域，其中该抗体包含第一 VH/VL 单位，所述第一 VH/VL 单位包含选自以下的至少一个、至少两个、或所有三个 VH HVR 序列：(a) 含有 SEQ ID NO:12 的氨基酸序列的 HVR-H1；(b) 包含 SEQ ID NO:13 或 SEQ ID NO:18 的氨基酸序列的 HVR-H2；(c) 包含 SEQ ID NO:14 的氨基酸序列的 HVR-H3；和第二 VH/VL 单位，所述第二 VH/VL 单位包含选自以下的至少一个、至少两个、或所有三个 VH HVR 序列：(a) 含有 SEQ ID NO:21 或 SEQ ID NO:60 的氨基酸序列的 HVR-H1；(b) 包含 SEQ ID NO:22 的氨基酸序列的 HVR-H2；(c) 包含 SEQ ID NO:23 的氨基酸

序列的HVR-H3；一些实施方案中，多特异性抗体包含特异地结合IL-4和IL-13的抗原结合结构域，其中该抗体包含第一VH/VL单位，所述第一VH/VL单位包含选自以下的至少一个、至少两个、或所有三个VH HVR序列：(a)含有SEQ ID NO:12的氨基酸序列的HVR-H1；(b)包含SEQ ID NO:13或SEQ ID NO:18的氨基酸序列的HVR-H2；(c)包含SEQ ID NO:14的氨基酸序列的HVR-H3；和第二VH/VL单位，所述第二VH/VL单位包含选自以下的至少一个、至少两个、或所有三个VH HVR序列：(a)含有SEQ ID NO:50的氨基酸序列的HVR-H1；(b)包含SEQ ID NO:51的氨基酸序列的HVR-H2；(c)包含SEQ ID NO:52的氨基酸序列的HVR-H3。

[0200] 一些实施方案中，多特异性抗体包含特异地结合IL-4和IL-13的抗原结合结构域，其中该抗体包含第一VH/VL单位，所述第一VH/VL单位包含选自以下的至少一个、至少两个、或所有三个VL HVR序列：(a)含有SEQ ID NO:15的氨基酸序列的HVR-L1；(b)包含SEQ ID NO:16的氨基酸序列的HVR-L2；和(e)包含SEQ ID NO:17的氨基酸序列的HVR-L3。一些实施方案中，多特异性抗体包含特异地结合IL-4和IL-13的抗原结合结构域，其中该抗体包含第二VH/VL单位，所述第二VH/VL单位包含选自以下的至少一个、至少两个、或所有三个VL HVR序列：(a)含有SEQ ID NO:24的氨基酸序列的HVR-L1；(b)包含SEQ ID NO:25的氨基酸序列的HVR-L2；和(e)包含SEQ ID NO:26的氨基酸序列的HVR-L3。一些实施方案中，多特异性抗体包含特异地结合IL-4和IL-13的抗原结合结构域，其中该抗体包含第二VH/VL单位，所述第二VH/VL单位包含选自以下的至少一个、至少两个、或所有三个VL HVR序列：(a)含有SEQ ID NO:53的氨基酸序列的HVR-L1；(b)包含SEQ ID NO:54的氨基酸序列的HVR-L2；和(e)包含SEQ ID NO:55的氨基酸序列的HVR-L3。

[0201] 一些实施方案中，多特异性抗体包含特异地结合IL-4和IL-13的抗原结合结构域，其中该抗体包含第一VH/VL单位，所述第一VH/VL单位包含选自以下的至少一个、至少两个、或所有三个VL HVR序列：(a)含有SEQ ID NO:15的氨基酸序列的HVR-L1；(b)包含SEQ ID NO:16的氨基酸序列的HVR-L2；和(c)包含SEQ ID NO:17的氨基酸序列的HVR-L3；和第二VH/VL单位，所述第二VH/VL单位包含选自以下的至少一个、至少两个、或所有三个VL HVR序列：(a)含有SEQ ID NO:24的氨基酸序列的HVR-L1；(b)包含SEQ ID NO:25的氨基酸序列的HVR-L2；和(e)包含SEQ ID NO:26的氨基酸序列的HVR-L3。一些实施方案中，多特异性抗体包含特异地结合IL-4和IL-13的抗原结合结构域，其中该抗体包含第一VH/VL单位，所述第一VH/VL单位包含选自以下的至少一个、至少两个、或所有三个VL HVR序列：(a)含有SEQ ID NO:15的氨基酸序列的HVR-L1；(b)包含SEQ ID NO:16的氨基酸序列的HVR-L2；和(c)包含SEQ ID NO:17的氨基酸序列的HVR-L3；和第二VH/VL单位，所述第二VH/VL单位包含选自以下的至少一个、至少两个、或所有三个VL HVR序列：(a)含有SEQ ID NO:53的氨基酸序列的HVR-L1；(b)包含SEQ ID NO:54的氨基酸序列的HVR-L2；和(c)包含SEQ ID NO:55的氨基酸序列的HVR-L3。

[0202] 一些实施方案中，多特异性抗体包含特异地结合IL-4和IL-13的抗原结合结构域，其中该抗体包含第一VH/VL单位，所述第一VH/VL单位包含选自以下的三个VH HVR序列：(a)含有SEQ ID NO:12的氨基酸序列的HVR-H1；(b)包含SEQ ID NO:13或SEQ ID NO:18的氨基酸序列的HVR-H2；(c)包含SEQ ID NO:14的氨基酸序列的HVR-H3；和选自以下的三个VL HVR：(a)包含SEQ ID NO:15的氨基酸序列的HVR-L1；(b)包含SEQ ID NO:16的氨基酸序列的HVR-L2；和(c)包含SEQ ID NO:17的氨基酸序列的HVR-L3。一些实施方案中，多

特异性抗体包含特异地结合 IL-4 和 IL-13 的抗原结合结构域，其中该抗体包含第一 VH/VL 单位，所述第一 VH/VL 单位包含选自以下的三个 VH HVR 序列：(a) 含有 SEQ ID NO:12 的氨基酸序列的 HVR-H1；(b) 包含 SEQ ID NO:13 的氨基酸序列的 HVR-H2；(c) 包含 SEQ ID NO:14 的氨基酸序列的 HVR-H3；和选自以下的三个 VL HVR：(a) 包含 SEQ ID NO:15 的氨基酸序列的 HVR-L1；(b) 包含 SEQ ID NO:16 的氨基酸序列的 HVR-L2；和 (c) 包含 SEQ ID NO:17 的氨基酸序列的 HVR-L3。

[0203] 一些实施方案中，多特异性抗体包含特异地结合 IL-4 和 IL-13 的抗原结合结构域，其中该抗体包含第二 VH/VL 单位，所述第二 VH/VL 单位包含选自以下的三个 VH HVR 序列：(a) 含有 SEQ ID NO:21 或 SEQ ID NO:60 的氨基酸序列的 HVR-H1；(b) 包含 SEQ ID NO:22 的氨基酸序列的 HVR-H2；(c) 包含 SEQ ID NO:23 的氨基酸序列的 HVR-H3；和选自以下的三个 VL HVR：(a) 包含 SEQ ID NO:24 的氨基酸序列的 HVR-L1；(b) 包含 SEQ ID NO:25 的氨基酸序列的 HVR-L2；和 (c) 包含 SEQ ID NO:26 的氨基酸序列的 HVR-L3。

[0204] 一些实施方案中，多特异性抗体包含特异地结合 IL-4 和 IL-13 的抗原结合结构域，其中该抗体包含第二 VH/VL 单位，所述第二 VH/VL 单位包含选自以下的三个 VH HVR 序列：(a) 含有 SEQ ID NO:50 的氨基酸序列的 HVR-H1；(b) 包含 SEQ ID NO:51 的氨基酸序列的 HVR-H2；(c) 包含 SEQ ID NO:52 的氨基酸序列的 HVR-H3；和选自以下的三个 VL HVR：(a) 包含 SEQ ID NO:53 的氨基酸序列的 HVR-L1；(b) 包含 SEQ ID NO:54 的氨基酸序列的 HVR-L2；和 (c) 包含 SEQ ID NO:55 的氨基酸序列的 HVR-L3。

[0205] 一些实施方案中，多特异性抗体包含特异地结合 IL-4 和 IL-13 的抗原结合结构域，其中该抗体包含第一 VH/VL 单位，所述第一 VH/VL 单位包含选自以下的三个 VH HVR 序列：(a) 含有 SEQ ID NO:12 的氨基酸序列的 HVR-H1；(b) 包含 SEQ ID NO:13 或 SEQ ID NO:18 的氨基酸序列的 HVR-H2；(c) 包含 SEQ ID NO:14 的氨基酸序列的 HVR-H3；和选自以下的三个 VL HVR：(a) 包含 SEQ ID NO:15 的氨基酸序列的 HVR-L1；(b) 包含 SEQ ID NO:16 的氨基酸序列的 HVR-L2；和 (c) 包含 SEQ ID NO:17 的氨基酸序列的 HVR-L3；和第二 VH/VL 单位，所述第二 VH/VL 单位包含选自以下的三个 VH HVR 序列：(a) 含有 SEQ ID NO:21 或 SEQ ID NO:60 的氨基酸序列的 HVR-H1；(b) 包含 SEQ ID NO:22 的氨基酸序列的 HVR-H2；(c) 包含 SEQ ID NO:23 的氨基酸序列的 HVR-H3；和选自以下的三个 VL HVR：(a) 包含 SEQ ID NO:24 的氨基酸序列的 HVR-L1；(b) 包含 SEQ ID NO:25 的氨基酸序列的 HVR-L2；和 (c) 包含 SEQ ID NO:26 的氨基酸序列的 HVR-L3。

[0206] 一些实施方案中，多特异性抗体包含特异地结合 IL-4 和 IL-13 的抗原结合结构域，其中该抗体包含第一 VH/VL 单位，所述第一 VH/VL 单位包含选自以下的三个 VH HVR 序列：(a) 含有 SEQ ID NO:12 的氨基酸序列的 HVR-H1；(b) 包含 SEQ ID NO:13 或 SEQ ID NO:18 的氨基酸序列的 HVR-H2；(c) 包含 SEQ ID NO:14 的氨基酸序列的 HVR-H3；和选自以下的三个 VL HVR：(a) 包含 SEQ ID NO:15 的氨基酸序列的 HVR-L1；(b) 包含 SEQ ID NO:16 的氨基酸序列的 HVR-L2；和 (c) 包含 SEQ ID NO:17 的氨基酸序列的 HVR-L3；和第二 VH/VL 单位，所述第二 VH/VL 单位包含选自以下的三个 VH HVR 序列：(a) 含有 SEQ ID NO:50 的氨基酸序列的 HVR-H1；(b) 包含 SEQ ID NO:51 的氨基酸序列的 HVR-H2；(c) 包含 SEQ ID NO:52 的氨基酸序列的 HVR-H3；和选自以下的三个 VL HVR：(a) 包含 SEQ ID NO:53 的氨基酸序列的 HVR-L1；(b) 包含 SEQ ID NO:54 的氨基酸序列的 HVR-L2；和 (c) 包含 SEQ ID NO:55 的

氨基酸序列的 HVR-L3。

[0207] 一些实施方案中，多特异性抗体包含特异地结合 IL-4 和 IL-13 的抗原结合结构域，其中该抗体包含第一 VH/VL 单位，所述第一 VH/VL 单位包含选自以下的三个 VH HVR 序列：(a) 含有 SEQ ID NO:12 的氨基酸序列的 HVR-H1；(b) 包含 SEQ ID NO:13 的氨基酸序列的 HVR-H2；(c) 包含 SEQ ID NO:14 的氨基酸序列的 HVR-H3；和选自以下的三个 VL HVR：(a) 包含 SEQ ID NO:15 的氨基酸序列的 HVR-L1；(b) 包含 SEQ ID NO:16 的氨基酸序列的 HVR-L2；和 (c) 包含 SEQ ID NO:17 的氨基酸序列的 HVR-L3；和第二 VH/VL 单位，所述第二 VH/VL 单位包含选自以下的三个 VH HVR 序列：(a) 含有 SEQ ID NO:21 或 SEQ ID NO:60 的氨基酸序列的 HVR-H1；(b) 包含 SEQ ID NO:22 的氨基酸序列的 HVR-H2；(c) 包含 SEQ ID NO:23 的氨基酸序列的 HVR-H3；和选自以下的三个 VL HVR：(a) 包含 SEQ ID NO:24 的氨基酸序列的 HVR-L1；(b) 包含 SEQ ID NO:25 的氨基酸序列的 HVR-L2；和 (c) 包含 SEQ ID NO:26 的氨基酸序列的 HVR-L3。

[0208] 一些实施方案中，多特异性抗体包含特异地结合 IL-4 和 IL-13 的抗原结合结构域，其中该抗体包含第一 VH/VL 单位，所述第一 VH/VL 单位包含选自以下的三个 VH HVR 序列：(a) 含有 SEQ ID NO:12 的氨基酸序列的 HVR-H1；(b) 包含 SEQ ID NO:13 的氨基酸序列的 HVR-H2；(c) 包含 SEQ ID NO:14 的氨基酸序列的 HVR-H3；和选自以下的三个 VL HVR：(a) 包含 SEQ ID NO:15 的氨基酸序列的 HVR-L1；(b) 包含 SEQ ID NO:16 的氨基酸序列的 HVR-L2；和 (c) 包含 SEQ ID NO:17 的氨基酸序列的 HVR-L3；和第二 VH/VL 单位，所述第二 VH/VL 单位包含选自以下的三个 VH HVR 序列：(a) 含有 SEQ ID NO:50 的氨基酸序列的 HVR-H1；(b) 包含 SEQ ID NO:51 的氨基酸序列的 HVR-H2；(c) 包含 SEQ ID NO:52 的氨基酸序列的 HVR-H3；和选自以下的三个 VL HVR：(a) 包含 SEQ ID NO:53 的氨基酸序列的 HVR-L1；(b) 包含 SEQ ID NO:54 的氨基酸序列的 HVR-L2；和 (c) 包含 SEQ ID NO:55 的氨基酸序列的 HVR-L3。

[0209] 在多个实施方案中，多特异性抗体包含第一 hemimer 和第二 hemimer，所述第一 hemimer 包含结合 IL-4 的第一 VH/VL 单位，其中第一 hemimer 在重链恒定区中包含杵突变，所述第二 hemimer 包含结合 IL-13 的第二 VH/VL 单位，其中第二 hemimer 在重链恒定区中包含臼突变。在多个实施方案中，多特异性抗体包含第一 hemimer 和第二 hemimer，所述第一 hemimer 包含结合 IL-4 的第一 VH/VL 单位，其中第一 hemimer 在重链恒定区中包含臼突变，所述第二 hemimer 包含结合 IL-13 的第二 VH/VL 单位，其中第二 hemimer 在重链恒定区中包含杵突变。在一些实施方案中，包含臼突变的重链恒定区具有 SEQ ID NO:35(IgG1) 或 SEQ ID NO:37(IgG4) 中所示的序列。在一些实施方案中，包含杵突变的重链恒定区具有 SEQ ID NO:34(IgG1) 或 SEQ ID NO:36(IgG4) 中所示的序列。一些实施方案中，多特异性抗体包含第一 hemimer 和第二 hemimer，其中第一 hemimer 包含具有 SEQ ID NO:38 的序列的第一重链和具有 SEQ ID NO:39 的序列的第一轻链，第二 hemimer 包含具有 SEQ ID NO:40 或 58 的序列的第二重链和具有 SEQ ID NO:41 或 59 的序列的第二轻链。一些实施方案中，多特异性抗体包含第一 hemimer 和第二 hemimer，其中第一 hemimer 包含具有 SEQ ID NO:38 的序列的第一重链和具有 SEQ ID NO:39 的序列的第一轻链，第二 hemimer 包含具有 SEQ ID NO:40 的序列的第二重链和具有 SEQ ID NO:41 的序列的第二轻链。

[0210] 在一些实施方案中，根据任何上述实施方案的抗 -IL-4/IL-13 多特异性抗体可以是单克隆抗体，包括嵌合、人源化或人抗体。在一些实施方案中，抗 IL-4/IL-13 多特异性抗

体是抗体片段，例如 Fv、Fab、Fab'、scFv、双抗体或 F(ab')2 片段。在一些实施方案中，抗体是全长抗体，例如完整 IgG1 或 IgG4 抗体或如本文定义的其他抗体类或同种型。

[0211] 在一些实施方案中，根据任何上述实施方案的抗 -IL-4/IL-13 多特异性抗体可以并入单独或组合的如下文章节 1-7 中所述的任何特征。

[0212] 1. 抗体亲和力

[0213] 在一些实施方案中，本文提供的抗体对抗原具有≤ 1μM、≤ 100nM、≤ 10nM、≤ 1nM、≤ 0.1nM、≤ 0.01nM 或≤ 0.001nM（例如 10^{-8}M 或更少，例如 10^{-8}M 至 10^{-13}M，例如 10^{-9}M 至 10^{-13}M）的解离常数 (Kd)。

[0214] 在一些实施方案中，Kd 通过放射性标记抗原结合测定法 (RIA) 进行测量。在一些实施方案中，RIA 用目的抗体的 Fab 形式及其抗原实施。例如，Fabs 对于抗原的溶液结合亲和力通过下述进行测量：在滴定系列的未标记抗原的存在下用最低浓度的 (^{125}I) 标记的抗原平衡 Fab，随后用抗 Fab 抗体包被的板捕获结合的抗原（参见例如 Chen 等人，J. Mol. Biol. 293:865-881(1999)）。为了建立用于该测定法的条件，**MICROTITER®** 多孔板 (Thermo Scientific) 用在 50mM 碳酸钠 (pH 9.6) 中的 5μg/ml 捕获抗 Fab 抗体 (Cappel Labs) 包被过夜，并且随后用在 PBS 中的 2% (w/v) 牛血清白蛋白在室温（约 23℃）封闭二到五小时。在非吸附性板 (Nunc#269620) 中，将 100pM 或 26pM[^{125}I]- 抗原与系列稀释的目的 Fab 混合（例如，与在 Presta 等人，Cancer Res. 57:4593-4599(1997) 中抗 VEGF 抗体 Fab-12 的评价一致）。目的 Fab 随后温育过夜；然而，温育可以继续更长时间（例如约 65 小时），以确保达到平衡。其后，将混合物转移至捕获板用于在室温温育（例如一小时）。随后去除溶液并且将板用在 PBS 中的 0.1% 聚山梨醇酯 20（**TWEEN-20®**）洗涤八次。当板干燥后，加入 150μl/ 孔的闪烁剂 (MICROSCINT-20™;Packard)，并且将板在 TOPCOUNT™γ 计数器 (Packard) 上计数十分钟。选择给出小于或等于 20% 最大结合的每种 Fab 的浓度，用于在竞争结合测定法中使用。

[0215] 根据一些实施方案，Kd 使用**BIACORE®**表面等离子体共振测定法测量。例如，在 25℃使用**BIACORE®-2000**或**BIACORE ®-3000** (BIAcore, Inc., Piscataway, NJ) 实施的测定法，其中以～ 10 反应单位 (RU) 使用固定化抗原 CM5 芯片。在一些实施方案中，根据供应商的说明书，用 N- 乙基 -N'-(3- 二甲基氨基丙基)- 碳化二亚胺盐酸盐 (EDC) 和 N- 羟基琥珀酰亚胺 (NHS) 活化羧甲基化葡聚糖生物传感器芯片 (CM5, BIACORE, Inc.)。将抗原用 10mM 乙酸钠，pH 4.8 稀释至 5μg/ml（～ 0.2μM），之后以 5μl/ 分钟的流速注射，以实现约 10 反应单位 (RU) 的偶联蛋白质。在抗原注射后，注射 1M 乙醇胺以封闭未反应的基团。对于动力学测量，在 25℃以约 25μl/ 分钟的流速注射在具有 0.05% 聚山梨醇酯 20(TWEEN-20™) 表面活性剂的 PBS(PBST) 中两倍系列稀释的 Fab(0.78nM-500nM)。通过同时拟合结合和解离传感图，使用简单的一对一兰米尔结合模型（**BIACORE ®** Evaluation Software 版本 3.2），计算结合速率 (kon) 和解离速率 (koff)。平衡解离常数 (Kd) 计算为 koff/kon 比值。参见，例如，Chen 等，J. Mol. Biol. 293:865-881(1999)。如果通过上文表面等离子体共振测定法结合速率超过 10^6M^{-1}s^{-1}，则结合速率可以通过使用荧光猝灭技术进行测定，所述荧光猝灭技术测量在递增浓度的抗原的存在下在 25℃在 PBS，pH 7.2 中 20nM 抗 - 抗原抗体 (Fab 形式）的荧光发射强度（激发＝ 295nm；发射＝

340nm，16nm 带通）的增加或降低，其中测量可以使用例如分光光度计，例如配备停流的分光光度计 (Aviv Instruments) 或具有搅拌比色杯的 8000 系列 SLM-AMINCO™分光光度计 (ThermoSpectronic)。

[0216] 2. 抗体片段

[0217] 在特定实施方案中，本文提供的抗体是抗体片段。抗体片段包括但不限于 Fab、Fab'、Fab'-SH、F(ab')2、Fv 和 scFv 片段及下文描述的其他片段。关于一些抗体片段的综述，参见 HUDSON 等 NAT. MED. 9:129-134(2003)。关于 scFv 片段的综述，参见例如，Pluckthün, in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds., (Springer-Verlag, New York), pp. 269-315(1994)；也参见 WO 93/16185；和 U. S. Patent Nos. 5,571,894 和 5,587,458。关于包含补救受体结合表位残基且具有增加的体内半衰期的 Fab 和 F(ab')2 片段的讨论，参见美国专利号 5,869,046。

[0218] 双抗体 (diabody) 是具有两个抗原结合位点的抗体片段，其可以是二价或双特异性的。参见例如，EP 404,097；WO 1993/01161；HUDSON 等，NAT. MED. 9:129-134(2003)；和 HOLLINGER 等，PROC. NATL. ACAD. SCI. USA 90:6444-6448(1993)。三抗体 (triabody) 和四抗体 (tetrabody) 也在 Hudson 等人 NAT. MED. 9:129-134(2003) 中描述。

[0219] 单结构域抗体是包含抗体的重链可变结构域的全部或部分或者轻链可变结构域的全部或部分的抗体片段。在特定实施方案中，单结构域抗体是人单结构域抗体 (Domantis, Inc., Waltham, MA；参见例如美国专利号 6,248,516B1)。

[0220] 抗体片段可以通过多种技术进行制备，包括但不限于，完整抗体的蛋白水解消化以及通过重组宿主细胞（例如大肠杆菌或噬菌体）产生，如本文描述的。

[0221] 3. 嵌合和人源化抗体

[0222] 在特定实施方案中，本文提供的抗体是嵌合抗体。特定嵌合抗体例如在 U. S. PATENT NO. 4,816,567；和 MORRISON 等，PROC. NATL. ACAD. SCI. USA, 81:6851-6855(1984)) 中描述。在一个例子中，嵌合抗体包含非人可变区（例如衍生自小鼠、大鼠、仓鼠、兔或非人灵长类动物例如猴的可变区）和人恒定区。在进一步例子中，嵌合抗体是"类别转换的"抗体，其中类或亚类已相对于亲本抗体发生改变。嵌合抗体包括其抗原结合片段。

[0223] 在特定实施方案中，嵌合抗体是人源化抗体。一般地，将非人抗体人源化，以减少对人的免疫原性，同时保留亲本非人抗体的特异性和亲和力。一般地，人源化抗体包含一个或多个可变结构域，其中 HVRs 例如 CDRs（或其部分）衍生自非人抗体，并且 FRs（或其部分）衍生自人抗体序列。人源化抗体任选地还可以包含人恒定区的至少部分。在一些实施方案中，在人源化抗体中的一些 FR 残基被置换为来自非人抗体（例如 HVR 残基所源自的抗体）的相应残基，例如以恢复或改善抗体特异性或亲和力。

[0224] 人源化抗体和制备其的方法例如在 ALMAGRO AND FRANSSON, FRONT. BIOSCI. 13:1619-1633(2008) 中综述，并且例如在下述文献中进一步描述：RIECHMANN 等，NATURE 332:323-329(1988)；QUEEN 等，PROC. NAT'L ACAD. SCI. USA 86:10029-10033(1989)；美国专利号 5,821,337, 7,527,791, 6,982,321, 和 7,087,409；KASHMIRI 等，METHODS36:25-34(2005)（描述特异性决定区 (SDR) 嫁接）；PADLAN, MOL. IMMUNOL. 28:489-498(1991)（描述"表面重建"(resurfacing)）；DALL'ACQUA 等，METHODS

36:43-60(2005)(描述“FR 改组”);和 OSBOURN 等,METHODS 36:61-68(2005) 和 KLIMKA 等,BR. J. CANCER,83:252-260(2000)(描述“引导选择”方法到 FR 改组)。

[0225] 可以用于人源化的人构架区包括但不限于:使用“最佳配合”法选择的构架区(参见,例如,SIMS 等 J. IMMUNOL. 151:2296(1993));衍生自特定亚组的轻或重链可变区的人抗体共有序列的构架区(参见,例如,CARTER 等 PROC. NATL. ACAD. SCI. USA,89:4285(1992);和 PRESTA 等 J. IMMUNOL.,151:2623(1993));人成熟(体细胞成熟的)构架区或人种系构架区(参见,例如,ALMAGRO 和 FRANSSON,FRONT. BIOSCI. 13:1619-1633(2008));和自筛选 FR 文库得到的构架区(参见,例如,BACA 等,J. BIOL. CHEM. 272:10678-10684(1997) 和 ROSOK 等,J. BIOL. CHEM. 271:22611-22618(1996))。

[0226] 4. 人抗体

[0227] 在特定实施方案中,本文提供的抗体是人抗体。人抗体可以使用本领域已知的多种技术产生。人抗体一般描述于 VAN DIJK 和 VAN DE WINKEL,CURR. OPIN. PHARMACOL. 5:368-74(2001) 和 LONBERG,CURR. OPIN. IMMUNOL. 20:450-459(2008)。

[0228] 人抗体可以通过给转基因动物施用免疫原进行制备,所述转基因动物已经修饰为响应抗原攻击而产生完整人抗体或具有人可变区的完整抗体。此类动物一般含有全部或部分的人免疫球蛋白基因座,其替换内源免疫球蛋白基因座、或在染色体外存在或随机整合到动物的染色体内。在此类转基因小鼠中,内源免疫球蛋白基因座一般已灭活。关于用于从转基因动物获得人抗体的方法的综述,参见 LONBERG,NAT. BIOTECH. 23:1117-1125(2005)。还参见例如描述 XENOMOUSE™技术的美国专利号 6,075,181 和 6,150,584;描述 **HUMAB®** 技术的美国专利号 5,770,429;描述 K-M **MOUSE®** 技术的美国专利号 7,041,870,和描述 **VELOCIMOUSE®** 技术的美国专利申请公开号 US 2007/0061900)。来自此类动物生成的完整抗体的人可变区可以进一步修饰,例如与不同人恒定区组合。

[0229] 人抗体还可以通过基于杂交瘤的方法进行制备。用于产生人单克隆抗体的人骨髓瘤和小鼠-人杂交骨髓瘤细胞系已得到描述。(参见,例如,Kozbor J. Immunol.,133:3001(1984);Brodeur 等,Monoclonal Antibody Production Techniques and Applications,pp. 51-63(Marcel Dekker,Inc.,New York,1987);和 Boerner 等,J. Immunol.,147:86(1991).)经由人 B 细胞杂交瘤技术生成的人抗体也在 Li 等人,Proc. Natl. Acad. Sci. USA,103:3557-3562(2006) 中描述。其它方法包括例如描述于美国专利号 7,189,826(描述了从杂交瘤细胞系中产生单克隆人 IgM 抗体)和 Ni,Xiandai Mianyixue,26(4):265-268(2006)(描述了人-人杂交瘤)中的那些方法。人杂交瘤技术(三源杂交瘤技术(trioma technology))还描述于 VOLLMERS 和 BRANDLEIN,HISTOLOGY and HISTOPATHOLOGY,20(3):927-937(2005) 和 VOLLMERS 和 BRANDLEIN,METHODS and FINDINGS IN EXPERIMENTAL and CLINICAL PHARMACOLOGY,27(3):185-91(2005)。

[0230] 人抗体还可以通过分离从人衍生的噬菌体展示文库选择的 Fv 克隆可变结构域序列而生成。此类可变结构域序列随后可以与所需人恒定结构域组合。用于从抗体文库中选择人抗体的技术在下文描述。

[0231] 5. 文库衍生的抗体

[0232] 本文所述抗体可以通过在组合文库中筛选具有一种或多种期望活性的抗体进行

分离。例如，本领域已知多种方法可以用于生成噬菌体展示文库且在此类文库中筛选具有所需结合特征的抗体。此类方法综述在例如 HOOGENBOOM 等，METHODS IN MOLECULAR BIOLOGY 178:1-37(O'BRIEN 等，ED.，HUMAN PRESS, TOTOWA, NJ, 2001) 中，并进一步描述在例如 THE MCCAFFERTY 等，NATURE 348:552-554；CLACKSON 等，NATURE 352:624-628(1991)；MARKS 等，J. MOL. BIOL. 222:581-597(1992)；MARKS 和 BRADBURY, METHODS IN MOLECULAR BIOLOGY248:161-175(LO, ED.，HUMAN PRESS, TOTOWA, NJ, 2003)；SIDHU 等，J. MOL. BIOL. 338(2):299-310(2004)；LEE 等，J. MOL. BIOL. 340(5):1073-1093(2004)；FELLOUSE, PROC. NATL. ACAD. SCI. USA 101(34):12467-12472(2004)；和 LEE 等，J. IMMUNOL. METHODS 284(1-2):119-132(2004) 中。

[0233] 在特定噬菌体展示方法中，VH 和 VL 基因库通过聚合酶链反应 (PCR) 分开克隆，且随机重组在噬菌体文库中，随后可以如 Winter 等人，Ann. Rev. Immunol.，12:433-455(1994) 中所述在所述噬菌体文库中筛选抗原结合性噬菌体。噬菌体一般以单链 Fv(scFv) 片段的形式或以 Fab 片段的形式展示抗体片段。来自经免疫的来源的文库提供了针对免疫原的高亲和力抗体，无需构建杂交瘤。可替代地，可以克隆（例如从人中）幼稚库（**naïve** repertoire)，不经任何免疫接种，以提供针对广泛的非自身以及自身抗原的单个抗体来源，如通过 Griffiths 等，EMBO J, 12:725-734(1993) 描述的。最后，幼稚文库还可以通过下述方式合成地制备：从干细胞中克隆未重排的 V 基因区段，且使用含有随机序列的 PCR 引物以编码高可变 CDR3 区，在体外实现重排，如通过 Hoogenboom 和 Winter, J. Mol. Biol.，227:381-388(1992) 描述的。描述人抗体噬菌体文库的专利出版物包括，例如：美国专利号 5,750,373, 和美国专利公布号 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, 和 2009/0002360。

[0234] 从人抗体文库中分离的抗体或抗体片段在本文中被视为人抗体或人抗体片段。

[0235] 6. 多特异性抗体

[0236] 在特定实施方案中，本文提供的抗体是多特异性抗体，例如双特异性抗体。多特异性抗体是具有针对至少两个不同位点的结合特异性的单克隆抗体。在特定实施方案中，结合特异性之一针对 IL-4，并且另一个针对任何其他抗原。在特定实施方案中，结合特异性之一针对 IL-4，并且另一个针对 IL-13。在特定实施方案中，双特异性抗体可以与 IL-4 的两个不同表位结合。双特异性抗体还可以用于将细胞毒素剂定位至细胞。双特异性抗体可以制备为全长抗体或抗体片段。

[0237] 用于制备多特异性抗体的技术包括但不限于，具有不同特异性的两个免疫球蛋白重链 - 轻链对的重组共表达（参见 Milstein 和 Cuello, Nature 305:537(1983))，WO 93/08829, 和 Traunecker 等，EMBO J. 10:3655(1991))、和“杵进臼 (knob-in-hole)”工程（参见，例如，美国专利号 5,731,168；美国申请公布号 2011/0287009)。多特异性抗体还可以通过下述进行制备：改造静电转向效应 (electrostatic steering effects) 用于制备抗体 Fc- 异二聚体分子 (WO 2009/089004A1)；交联两种或更多种抗体或片段（参见，例如，美国专利号 4,676,980, 和 BRENNAN 等，SCIENCE, 229:81(1985))；使用亮氨酸拉链以产生双特异性抗体（参见，例如，KOSTELNY 等，J. IMMUNOL.，148(5):1547-1553(1992))；在单个 VH/VL 单位中在 CL 结构域和 VH 结构域之间使用弗林 (furin) 可切割拴链（参见，例如，国际专利申请号 PCT/US2012/059810)；使用“双抗体”技术用于制备双特异性抗体片

段（参见，例如，HOLLINGER 等，PROC. NATL. ACAD. SCI. USA, 90:6444-6448(1993)）；使用单链 Fv(sFv) 二聚体（参见，例如 GRUBER 等，J. IMMUNOL., 152:5368(1994)）；和制备三特异性抗体，如例如 TUTT 等 J. IMMUNOL. 147:60(1991) 中所述的。

[0238] 具有三个或更多个功能性抗原结合位点的改造抗体，包括章鱼抗体（“Octopus antibodies”），也包括在本文中（参见，例如 US 2006/0025576A1）。

[0239] 本文的抗体或片段还包括包含抗原结合位点的“双重作用 FAb”或“DAF”，其中所述抗原结合位点与 IL-4 以及另一不同抗原例如 IL-13 结合（参见例如 US 2008/0069820）。

[0240] 杵进臼

[0241] 在例如美国专利号 5,731,168, WO2009/089004, US2009/0182127, US2011/0287009, MARVIN 和 ZHU, ACTA PHARMACOL. SIN. (2005) 26(6):649-658, 和 KONTERMANN(2005) ACTA PHARMACOL. SIN., 26:1-9 中，描述了将杵进臼用作产生多特异性抗体的方法。以下提供简要的非限制性讨论。

[0242] “突起”指从第一多肽表面突出的至少一个氨基酸侧链，由此该氨基酸侧链可以置入相邻界面（即，第二多肽的界面）的互补腔中，例如以稳定该异二聚体，并由此相比同二聚体形成更利于异二聚体形成。突起可以存在于原始界面中，或可以通过合成引入（例如通过改变编码界面的核酸）。在一些实施方案中，编码第一多肽的界面的核酸被改变以编码突起。为此，将编码第一多肽界面中至少一个“原始”氨基酸残基的核酸置换为编码至少一个“输入”氨基酸残基的核酸，其中所述输入氨基酸残基比原始氨基酸残基具有更大的侧链体积。可以明了，可以存在一个以上原始和相应的输入残基。各种氨基酸残基的侧链体积显示在例如 US2011/0287009 的表 1 中。

[0243] 一些实施方案中，用于形成突起的输入残基是选自精氨酸 (R)，苯丙氨酸 (F)，酪氨酸 (Y) 和色氨酸 (W) 的天然氨基酸残基。一些实施方案中，输入残基是色氨酸或酪氨酸。一些实施方案中，用于形成突起的原始残基具有小侧链体积，例如丙氨酸、天冬酰胺、天冬氨酸、甘氨酸、丝氨酸、苏氨酸或缬氨酸。

[0244] “腔”指自第二多肽界面凹入并由此可以容纳相邻第一多肽界面上的相应突起的至少一个氨基酸侧链。腔可以存在于原始界面中，或可以通过合成引入（例如通过改变编码界面的核酸）。在一些实施方案中，编码第二多肽的界面的核酸被改变以编码腔。为此，将编码第二多肽界面中至少一个“原始”氨基酸残基的核酸置换为编码至少一个“输入”氨基酸残基的 DNA，其中所述输入氨基酸残基比原始氨基酸残基具有较小的侧链体积。可以明了，可以存在一个以上原始和相应的输入残基。一些实施方案中，用于形成腔的输入残基是选自丙氨酸 (A)，丝氨酸 (S)，苏氨酸 (T) 和缬氨酸 (V) 的天然氨基酸残基。一些实施方案中，输入残基是丝氨酸、丙氨酸或苏氨酸。一些实施方案中，用于形成腔的原始残基具有大侧链体积，例如酪氨酸、精氨酸、苯丙氨酸或色氨酸。

[0245] 突起“可以置入”腔中，意指突起和腔分别位于第一多肽和第二多肽界面上的空间位置以及突起和腔的大小使得该突起可以位于腔中而不显著地干扰第一多肽和第二多肽在该界面的正常相关性 (association)。由于突起例如 Tyr, Phe 和 Trp 典型地不从界面轴垂直地伸出和具有优选的构象，因此突起与相应腔的对齐可以，在一些情况下，依赖于基于三维结构（例如通过 X 射线晶体学或核磁共振 (NMR) 获得的三维结构）模建该突起 / 腔对。这可以使用本领域中广泛接受的技术实现。

[0246] 一些实施方案中，杵突变在 IgG1 恒定区中是 T366W。一些实施方案中，臼突变在 IgG1 恒定区中包含选自 T366S, L368A 和 Y407V 的一个或多个突变。一些实施方案中，臼突变在 IgG1 恒定区中包含 T366S, L368A 和 Y407V。SEQ ID NO:34 显示具有杵突变的示例性 IgG1 恒定区，SEQ ID NO:35 显示具有臼突变的示例性 IgG1 恒定区。

[0247] 一些实施方案中，杵突变在 IgG4 恒定区中是 T366W。一些实施方案中，臼突变在 IgG4 恒定区中包含选自 T366S, L368A 和 Y407V 的一个或多个突变。一些实施方案中，臼突变在 IgG4 恒定区中包含 T366S, L368A 和 Y407V。SEQ ID NO:36 显示具有杵突变的示例性 IgG4 恒定区，SEQ ID NO:37 显示具有臼突变的示例性 IgG4 恒定区。

[0248] 7. 抗体变体

[0249] 在特定实施方案中，考虑本文提供的抗体的氨基酸序列变体。例如，可能期望改善抗体的结合亲和力和 / 或其他生物性质。抗体的氨基酸序列变体可以通过将合适修饰引入编码抗体的核苷酸序列内、或通过肽合成，进行制备。此类修饰包括例如在抗体的氨基酸序列内的残基的缺失、和 / 或插入和 / 或置换。可以制备缺失、插入和置换的任何组合，以达到最终构建体，条件是最终构建体具有所需特征，例如抗原结合。

[0250] 置换、插入和缺失变体

[0251] 在特定实施方案中，提供了具有一个或多个氨基酸置换的抗体变体。用于置换诱变的目的位点包括 HVRs 和 FRs。保守置换显示于表 1 中标题“保守置换”下。更实质性的变化在表 1 中标题“示例性置换”下提供，并且在下文参考氨基酸侧链种类进一步描述。可以将氨基酸置换引入目的抗体内且就所需活性筛选产物，例如保留 / 改善的抗原结合、减少的免疫原性或改善的 ADCC 或 CDC。

[0252] 表 1

[0253]

原始残基	示例替代	保守替代
Ala(A)	Val ;Leu ;Ile	Val
Arg(R)	Lys ;Gln ;Asn	Lys
Asn(N)	Gln ;His ;Asp, Lys ;Arg	Gln
Asp(D)	Glu ;Asn	Glu
Cys(C)	Ser ;Ala	Ser
Gln(Q)	Asn ;Glu	Asn
Glu(E)	Asp ;Gln	Asp
Gly(G)	Ala	Ala
His(H)	Asn ;Gln ;Lys ;Arg	Arg

Ile(I)	Leu ;Val ;Met ;Ala ;Phe ;正亮氨酸	Leu
Leu(L)	正亮氨酸 ;Ile ;Val ;Met ;Ala ;Phe	Ile
Lys(K)	Arg ;Gln ;Asn	Arg
Met(M)	Leu ;Phe ;Ile	Leu
Phe(F)	Trp ;Leu ;Val ;Ile ;Ala ;Tyr	Tyr
Pro(P)	Ala	Ala
Ser(S)	Thr	Thr
Thr(T)	Val ;Ser	Ser
Trp(W)	Tyr ;Phe	Tyr
Tyr(Y)	Trp ;Phe ;Thr ;Ser	Phe
Val(V)	Ile ;Leu ;Met ;Phe ;Ala ;正亮氨酸	Leu

[0254] 氨基酸可以根据共同侧链性质进行分组：

[0255] (1) 疏水性：正亮氨酸、Met、Ala、Val、Leu、Ile；

[0256] (2) 中性亲水性 :Cys、Ser、Thr、Asn、Gln；

[0257] (3) 酸性 :Asp、Glu；

[0258] (4) 碱性 :His、Lys、Arg；

[0259] (5) 影响链方向的残基 :Gly、Pro；

[0260] (6) 芳香族 :Trp、Tyr、Phe。

[0261] 非保守置换造成这些类型之一的成员交换为另一类型的成员。

[0262] 一类置换变体涉及置换亲本抗体（例如人源化或人抗体）的一个或多个高变区残基。一般地，选择用于进一步研究的所得变体将相对于亲本抗体在特定生物性质（例如增加的亲和力、减少的免疫原性）上具有修饰（例如改善）、和/或基本上了保留亲本抗体的特定生物性质。示例性置换变体是亲和力成熟抗体，其可以例如使用基于噬菌体展示的亲和力成熟技术，例如本文描述的，方便地生成。简言之，使一个或多个 HVR 残基突变，并且将变体抗体展示在噬菌体上且筛选特定生物活性（例如结合亲和力）。

[0263] 可以在 HVRs 中作出改变（例如置换），例如以改善抗体亲和力。此类改变可以在 HVR “热点”（即，由在体细胞成熟过程中高频率经历突变的密码子编码的残基）（参见例如 Chowdhury, Methods Mol. Biol. 207:179-196(2008)）、和/或 SDRs(a-CDRs) 中作出，测试所得到的变体 VH 或 VL 的结合亲和力。通过构建二级文库且从中再选择而进行的亲和力成熟，已例如描述在 HOOGENBOOM 等, METHODS IN MOLECULAR BIOLOGY 178:1-37(O' BRIEN 等, ED., HUMAN PRESS, TOTOWA, NJ, (2001).）中。在亲和力成熟的一些实施方案中，可以通过多种方法（例如易错 PCR、链改组、或寡核苷酸定向诱变）中的任何，将多样性引入选择用

于成熟的可变基因内。随后制备二级文库。随后筛选文库以鉴定具有所需亲和力的任何抗体变体。引入多样性的另一种方法涉及HVR指导的方法，其中几个HVR残基（例如一次4-6个残基）被随机化。参与抗原结合的HVR残基可以例如使用丙氨酸扫描诱变或建模，特别鉴定。特别常靶向CDR-H3和CDR-L3。

[0264] 在特定实施方案中，置换、插入或缺失可以在一个或多个HVRs内发生，只要此类改变基本上不减少抗体结合抗原的能力即可。例如，可以在HVRs中进行基本上不减少结合亲和力的保守改变（例如如本文提供的保守置换）。此类改变可以在HVR“热点”或SDRs外。在上文提供的变体VH和VL序列的特定实施方案中，每个HVR或者是未改变的、或者含有不超过一个、两个或三个氨基酸置换。

[0265] 用于鉴定可以作为靶标进行诱变的抗体残基或区域的一个有用方法称为“丙氨酸扫描诱变”，如通过Cunningham和Wells(1989)Science，244:1081-1085描述的。在此方法中，鉴定残基或靶标残基组（例如荷电残基例如arg、asp、his、lys和glu），并且替换为中性或带负电荷的氨基酸（例如丙氨酸或聚丙氨酸），以确定抗体与抗原的相互作用是否受影响。可以在对该最初置换显示出功能性敏感的氨基酸位置上引入进一步的置换。可替代地或另外地，可以利用抗原-抗体复合物的晶体结构，鉴定在抗体和抗原之间的接触点。此类接触残基和邻近残基可以作为用于置换的候选物被靶向或消除。可以筛选变体，以确定它们是否含有所需性质。

[0266] 氨基酸序列插入包括氨基和/或羧基末端融合（长度范围从一个残基到含有一百个或更多个残基的多肽）、以及单个或多个氨基酸残基的序列内插入。末端插入的例子包括具有N末端甲硫氨酰残基的抗体。抗体分子的其他插入变体包括抗体的N或C末端与酶（例如对于ADEPT）或增加抗体血清半衰期的多肽的融合。

[0267] 糖基化变体

[0268] 在特定实施方案中，改变本文提供的抗体，以增加或减少抗体糖基化的程度。糖基化位点向抗体的添加或缺失可以通过改变氨基酸序列从而产生或去除一个或多个糖基化位点，而方便地完成。

[0269] 当抗体包含Fc区时，可以改变与之附着的碳水化合物。通过哺乳动物细胞产生的天然抗体一般包含分支的、二天线型的寡糖，其一般通过N连接附着于Fc区的CH2结构域的Asn297。参见，例如，Wright等TIBTECH 15:26-32(1997)。寡糖可以包括各种碳水化合物，例如甘露糖、N-乙酰葡糖胺(GlcNAc)、半乳糖和唾液酸，以及附着在二天线型寡糖结构的“茎”中的GlcNAc上的岩藻糖。在一些实施方案中，可以在本文提供的抗体中进行寡糖的修饰，以便产生具有特定改善性质的抗体变体。

[0270] 在一些实施方案中，提供了具有缺乏（直接或间接）附着在Fc区上的岩藻糖的碳水化合物结构的抗体变体。例如，此类抗体中的岩藻糖量可以是1%-80%、1%-65%、5%-65%或20%-40%。如例如WO 2008/077546中所述的，如通过MALDI-TOF质谱法测量的，相对于与Asn297附着的所有糖结构（例如复杂的、杂合的和高甘露糖的结构）的总和，通过计算在Asn297的糖链内岩藻糖的平均量，确定岩藻糖的量。Asn297指位于Fc区的大约位置297上的天冬酰胺残基(Fc区残基的Eu编号）；然而，由于抗体中的微小序列变异，Asn297也可以位于位置297的上游或下游的约±3个氨基酸处，即位置294和300之间。此类岩藻糖基化变体可以具有改善的ADCC功能。参见，例如，美国专利公布号US

2003/0157108(Presta,L.);US 2004/0093621(Kyowa Hakko Kogyo Co.,Ltd)。与“去岩藻糖化的”或“岩藻糖缺陷的”抗体变体有关的出版物的例子包括:US 2003/0157108;WO 2000/61739;WO 2001/29246;US 2003/0115614;US 2002/0164328;US 2004/0093621;US 2004/0132140;US 2004/0110704;US 2004/0110282;US 2004/0109865;WO 2003/085119;WO 2003/084570;WO 2005/035586;WO 2005/035778;WO2005/053742;WO2002/031140;Okazaki 等 J.Mol.Biol.336:1239-1249(2004);Yamane-Ohnuki 等 Biotech.Bioeng.87:614(2004)。能够产生去岩藻糖基化的抗体的细胞系的例子包括具有蛋白质岩藻糖基化缺陷的Lec13CHO细胞(RIPKA等ARCH.BIOCHEM.BIOPHYS.249:533-545(1986);US PAT APPL NO US 2003/0157108 A1,PRESTA,L;和WO 2004/056312 A1,ADAMS等,尤其是实施例11),和敲除细胞系,例如α-1,6-岩藻糖基转移酶基因,FUT8,敲除CHO细胞(参见,例如,YAMANE-OHNUKI等BIOTECH.BIOENG.87:614(2004);KANDA,Y.等,BIOTECHNOL.BIOENG.,94(4):680-688(2006);和WO2003/085107)。

[0271] 进一步提供了具有平分型寡糖(bisected oligosaccharide)的抗体变体,例如其中与抗体的Fc区附着的二天线寡糖通过GlcNAc平分。此类抗体变体可以具有减少的岩藻糖化和/或改善的ADCC功能。此类抗体变体的例子例如在WO 2003/011878(Jean-Mairet等);美国专利号6,602,684(Umana等);和US 2005/0123546(Umana等)中描述。还提供了在与Fc区附着的寡糖中具有至少一个半乳糖残基的抗体变体。此类抗体变体可以具有改善的CDC功能。此类抗体变体例如在WO 1997/30087(Patel等);WO 1998/58964(Raju,S.);和WO 1999/22764(Raju,S.)中描述。

[0272] Fc区变体

[0273] 在特定实施方案中,一个或多个氨基酸修饰可以引入本文提供的抗体的Fc区内,从而生成Fc区变体。Fc区变体可以包括包含在一个或多个氨基酸位置上的氨基酸修饰(例如置换)的人Fc区序列(例如人IgG1、IgG2、IgG3或IgG4Fc区)。

[0274] 在一些实施方案中,抗体恒定区,例如重链恒定区,包含杵突变和/或臼突变以利于多特异性抗体形成。非限制性示例性杵突变和臼突变、和杵进臼技术一般地描述在例如,美国专利号5,731,168,WO2009/089004,US2009/0182127,US2011/0287009,Marvin和Zhu,Acta Pharmacol.Sin.(2005)26(6):649-658,和Kontermann(2005)Acta Pharmacol.Sin.,26:1-9.中。杵突变和臼突变的一些非限制性实例将本文中讨论。

[0275] 在特定实施方案中,本发明提供具有一些但并非所有效应子功能的抗体变体,这使得其成为用于如下应用的期望候选物,在所述应用中抗体在体内的半衰期是重要的,而一些效应子功能(例如补体和ADCC)是不需要的或有害的。可以进行体外和/或体内细胞毒性测定,以证实CDC和/或ADCC活性的减少/耗竭。例如,可以进行Fc受体(FcR)结合测定试验,以确保抗体缺乏FcγR结合(从而可能缺乏ADCC活性),但保留FcRn结合能力。用于介导ADCC的主要细胞即NK细胞仅表达FcγRIII,而单核细胞表达FcγRI、FcγRII和FcγRIII。在造血细胞上的FcR表达概括在Ravetch和Kinet,Annu.Rev.Immunol.9:457-492(1991)的第464页表3中。评估目的分子的ADCC活性的体外测定试验的非限制性例子在美国专利号5,500,362中描述(参见,例如Hellstrom,I.等Proc.Nat'l Acad.Sci.USA 83:7059-7063(1986))和Hellstrom,I等,Proc.Nat'l Acad.Sci.USA 82:1499-1502(1985);5,821,337(参见Bruggemann,M.等,J.Exp.

Med. 166:1351-1361(1987))。可替代地,可以采用非放射性测定试验方法(参见例如,用于流式细胞术的ACTI™非放射性细胞毒性测定试验(CellTechnology, Inc. Mountain View, CA);和CytoTox 96®非放射性细胞毒性测定试验(Promega, Madison, WI)。可以用于此类测定试验的效应细胞包括外周血单个核细胞(PBMC)和自然杀伤(NK)细胞。可替代地或另外地,目的分子的ADCC活性可以在体内评估,例如在动物模型中,例如公开于Clynes等人Proc. Nat'l Acad. Sci. USA 95:652-656(1998)中的。还可以执行C1q结合测定试验,以证实抗体不能结合C1q且因此缺乏CDC活性。参见,例如,WO 2006/029879和WO 2005/100402中C1q和C3c结合ELISA。为了评估补体激活,可以执行CDC测定试验(参见,例如,Gazzano-Santoro等,J. Immunol. Methods 202:163(1996);Cragg, M. S. 等,Blood 101:1045-1052(2003);和Cragg, M. S. 和M. J. Glennie, Blood103:2738-2743(2004))。FcRn结合和体内清除率/半衰期确定还可以使用本领域已知的方法进行(参见,例如,Petkova, S. B. 等,Int'l. Immunol. 18(12):1759-1769(2006))..

[0276] 具有减少的效应子功能的抗体包括具有Fc区残基238、265、269、270、297、327和329中的一个或多个的置换的那些(美国专利号6,737,056)。此类Fc突变体包括在氨基酸位置265、269、270、297和327的两个或更多个上具有置换的Fc突变体,包括具有残基265和297至丙氨酸的置换的所谓"DANA"Fc突变体(美国专利号7,332,581)。

[0277] 描述了具有改善或减少的FcRs结合的特定抗体变体。(参见,例如,美国专利号6,737,056;WO 2004/056312,和Shields等,J. Biol. Chem. 9(2):6591-6604(2001).)

[0278] 在特定实施方案中,抗体变体包含具有改善ADCC的一个或多个氨基酸置换的Fc区,例如在Fc区的位置298、333和/或334上的置换(残基的EU编号)。

[0279] 在一些实施方案中,在Fc区中作出改变,以导致改变的(例如改善或减少的)C1q结合和/或补体依赖性细胞毒性(CDC),例如美国专利号6,194,551,WO 99/51642,和Idusogie等J. Immunol. 164:4178-4184(2000)中所述的。

[0280] 在US2005/0014934A1(Hinton等)中描述了具有增加的半衰期和改善的新生儿Fc受体(FcRn)结合的抗体,所述新生儿Fc受体负责母源IgGs至胎儿的转移(Guyer等,J. Immunol. 117:587(1976)和Kim等,J. Immunol. 24:249(1994))。这些抗体包含其中具有一个或多个置换的Fc区,所述一个或多个置换改善Fc区与FcRn的结合。此类Fc变体包括在如下Fc区残基的一个或多个上具有置换的那些:238、256、265、272、286、303、305、307、311、312、317、340、356、360、362、376、378、380、382、413、424或434,例如Fc区残基434的置换(美国专利号7,371,826)。.

[0281] 关于Fc区变体的其他例子,还参见Duncan&Winter, Nature322:738-40(1988);美国专利号5,648,260;美国专利号5,624,821;和WO 94/29351。

[0282] 一些实施方案中,抗体恒定区包含本文讨论的一个以上突变(例如,杵和/或臼突变和/或增加稳定性的突变和/或减少ADCC的突变,等等)。

[0283] 半胱氨酸工程化抗体变体

[0284] 在特定实施方案中,可能期望制备半胱氨酸工程化抗体,例如"thioMAbs",其中抗体的一个或多个残基由半胱氨酸残基置换。在特定实施方案中,被置换的残基位于抗体的可接近位点上。通过用半胱氨酸置换这些残基,从而将反应性巯基基团置于抗体的可接近位点上,这些巯基基团可以用于使抗体缀合至其他部分例如药物部分或接头-药物部分,

以产生免疫缀合物，如本文进一步描述的。在特定实施方案中，下述残基中的任何一个或多个可以由半胱氨酸残基置换：轻链的V205(Kabat编号)；重链的A118(EU编号)；和重链Fc区的S400(EU编号)。半胱氨酸工程化抗体可以如例如美国专利号7,521,541中所述生成。

[0285] 抗体衍生物

[0286] 在特定实施方案中，本文提供的抗体可以进一步被修饰，以含有本领域已知的且可容易获得的另外非蛋白质性部分。适于衍生化抗体的部分包括但不限于水溶性聚合物。水溶性聚合物的非限制性例子包括但不限于聚乙二醇(PEG)、乙二醇/丙二醇的共聚物、羧甲基纤维素、葡聚糖、聚乙烯醇、聚乙烯吡咯烷酮、聚-1,3-二氧戊环、聚-1,3,6-三噁烷、乙烯/马来酸酐共聚物、聚氨基酸(同聚物或随机共聚物)、和葡聚糖或聚(n-乙烯吡咯烷酮)聚乙二醇、propropylene glycol同聚物、环氧丙烷/环氧乙烷共聚物、聚氧乙基化多元醇(例如甘油)、聚乙烯醇及其混合物。聚乙二醇丙醛，由于其在水中的稳定性，可以在制造中具有优点。聚合物可以具有任何分子量，并且可以是分支或未分支的。附着至抗体的聚合物数目可以改变，并且如果附着超过一个聚合物，那么它们可以是相同或不同分子。一般而言，用于衍生化的聚合物的数目和/或类型可以基于如下考虑进行确定，所述考虑包括但不限于待改善的抗体的特定性质或功能，抗体衍生物是否用于在限定条件下的治疗中，等。

[0287] 在一些实施方案中，提供了抗体和可以通过暴露于辐射线而被选择性加热的非蛋白质性部分的缀合物。在一些实施方案中，非蛋白质性部分是碳纳米管(KAM等，PROC. NATL. ACAD. SCI. USA 102:11600-11605(2005))。辐射线可以具有任何波长，并且包括但不限于，这样的波长，所述波长不伤害普通细胞、但使非蛋白质性部分加热至可杀死抗体-非蛋白质性部分附近的细胞的温度。

[0288] 重组方法和组合物

[0289] 抗体可以使用重组方法和组合物进行生产，例如如美国专利号4,816,567中所述的。在一些实施方案中，提供了编码本文描述的抗IL-4抗体的分离核酸。在一些实施方案中，提供了编码本文描述的抗IL-13抗体的分离核酸。在一些实施方案中，提供了编码本文描述的抗IL-4/IL-13双特异性抗体的分离核酸。此核酸可以编码包含抗体的VL的氨基酸序列和/或包含抗体的VH的氨基酸序列(例如抗体的轻和/或重链)。在一些实施方案中，提供了包含此核酸的一个或多个载体(例如表达载体)。在一些实施方案中，提供了包含此核酸的宿主细胞。在一个此实施方案中，宿主细胞包含(例如已转化了)：(1)包含编码含有抗体VL的氨基酸序列和含有抗体VH的氨基酸序列的核酸的载体，或(2)包含编码含有抗体VL的氨基酸序列的核酸的第一载体、和包含编码含有抗体VH的氨基酸序列的核酸的第二载体。

[0290] 在一些实施方案中，宿主细胞是真核的，例如中国仓鼠卵巢(CHO)细胞或淋巴样细胞(例如Y0、NS0、Sp20细胞)。在一些实施方案中，提供了制备抗体的方法，其中所述方法包括在适于抗体表达的条件下培养如上文提供的包含编码抗体的核酸的宿主细胞，且任选从宿主细胞(或宿主细胞培养基)中回收抗体。

[0291] 在一些实施方案中，提供了制备多特异性抗体的方法，其中所述方法包括在适于抗体表达的条件下培养包含编码多特异性抗体的核酸的宿主细胞，且任选从宿主细胞(或宿主细胞培养基)中回收多特异性抗体。在一些实施方案中，提供制备多特异性抗体的方

法，其中所述方法包括在适于第一 VH/VL 单位表达的条件下培养包含编码多特异性抗体的第一 VH/VL 单位（包括恒定区（如果有的话），有时称作“hemimer”或“半抗体”）的核酸的第一宿主细胞，且任选从宿主细胞（或宿主细胞培养基）中回收第一 VH/VL 单位；和在适于第二 VH/VL 单位表达的条件下培养包含编码多特异性抗体的第二 VH/VL 单位（包括恒定区（如果有的话））的核酸的第二宿主细胞，且任选从宿主细胞（或宿主细胞培养基）中回收第二 VH/VL 单位。在一些实施方案中，该方法还包括从分离的第一 VH/VL 单位和分离的第二 VH/VL 单位组装多特异性抗体。该组装可以包括，在一些实施方案中，氧化还原步骤以在两个 VH/VL 单位（或 hemimers）之间形成分子内二硫键。生产多特异性抗体的非限制性示例方法描述在例如 US 2011/0287009, US 2007/0196363, US2007/0178552, 美国专利号 5,731,168, WO 96/027011, WO 98/050431, 和 ZHU 等，1997, PROTEIN SCIENCE6:781-788 中。非限制性示例方法也描述在以下实施例中。

[0292] 对于抗 IL-4 抗体或抗 -IL-4/IL-13 双特异性抗体的重组生产，分离编码抗体，例如如上所述的抗体，的核酸，并且插入一个或多个载体内用于进一步克隆和 / 或在宿主细胞中表达。此核酸可以使用常规程序（例如通过使用能够与编码抗体重和轻链的基因特异性结合的寡核苷酸探针）容易地分离且测序。

[0293] 用于抗体编码载体的克隆或表达的合适宿主细胞包括本文描述的原核或真核细胞。例如，特别是当不需要糖基化和 Fc 效应子功能时，抗体可以在细菌中生产。对于抗体片段和多肽在细菌中的表达，参见例如美国专利号 5,648,237, 5,789,199, 和 5,840,523。（还参见 CHARLTON, METHODS IN MOLECULAR BIOLOGY, VOL. 248 (B. K. C. LO, ED., HUMANA PRESS, TOTOWA, NJ, 2003), PP. 245-254, 描述抗体片段在大肠杆菌中的表达）。在表达后，抗体可以在可溶级分中从细菌细胞糊分离且可以进一步纯化。

[0294] 除了原核生物外，真核微生物例如丝状真菌或酵母是用于抗体编码载体的合适克隆或表达宿主，包括其糖基化途径已被人源化”的真菌和酵母菌株，这导致具有部分或全人糖基化模式的抗体生产。参见 Gerngross, Nat. Biotech. 22:1409-1414 (2004), 和 Li 等，Nat. Biotech. 24:210-215 (2006).

[0295] 用于糖基化抗体表达的合适宿主细胞也可以源自多细胞生物（无脊椎动物和脊椎动物）。无脊椎动物细胞的例子包括植物和昆虫细胞。已鉴定了众多杆状病毒株，其可以与昆虫细胞结合使用，特别用于草地贪夜蛾 (Spodoptera frugiperda) 细胞的转染。

[0296] 植物细胞培养物也可以用作宿主。参见，例如，美国专利号 5,959,177；6,040,498；6,420,548；7,125,978；和 6,417,429（描述用于在转基因植物中生产抗体的 PLANTIBODIES™ 技术）。

[0297] 脊椎动物细胞也可以用作宿主。例如，悬浮生长适应化的哺乳动物细胞系可以是有用的。有用的哺乳动物宿主细胞系的其他例子是 SV40 转化的猴肾 CV1 系 (COS-7)；人胚肾系（如例如 Graham 等人，J. Gen Virol. 36:59 (1977) 中所述的 293 或 293 细胞）；幼仓鼠肾细胞 (BHK)；小鼠塞尔托利细胞（如例如 Mather, Biol. Reprod. 23:243-251 (1980) 中所述的 TM4 细胞）；猴肾细胞 (CV1)；非洲绿猴肾细胞 (VERO-76)；人宫颈癌细胞 (HELA)；犬肾细胞 (MDCK；Buffalo 大鼠肝细胞 (BRL 3A)；人肺细胞 (W138)；人肝细胞 (Hep G2)；小鼠乳房肿瘤 (MMT 060562)；如例如 Mather 等人，Annals N. Y. Acad. Sci. 383:44-68 (1982) 中所述的 TRI 细胞；MRC 5 细胞；和 FS4 细胞。其他有用的哺乳动物宿主细胞系包括中国仓鼠卵

巢(CHO)细胞,包括DHFR-CHO细胞(Urlaub等,Proc. Natl. Acad. Sci. USA 77:4216(1980));和骨髓瘤细胞系例如YO、NSO和Sp2/0。对于适合于抗体生产的一些哺乳动物宿主细胞系的综述,参见例如,Yazaki和Wu,Methods in Molecular Biology,Vol. 248(B. K. C. Lo,ed.,Humana Press,Totowa,NJ),pp. 255-268(2003)。

[0298] 示例性测定法

[0299] 结合测定法及其他测定法

[0300] 在一些实施方案中,可以就其抗原结合活性,例如通过已知方法例如ELISA、蛋白质印迹等,测试本文提供的抗体。

[0301] 在一些实施方案中,竞争测定法可以用于鉴定与本文所述IL-4抗体竞争结合IL-4的抗体。在一些实施方案中,竞争测定法可以用于鉴定与本文所述IL-4/IL-13双特异性抗体竞争结合IL-4和/或IL-13的抗体。一些实施方案中,此类竞争抗体与结合IL-4的抗体结合相同的表位(例如线性或构象表位),其中所述结合IL-4的抗体包含含有SEQ ID NO:9的VH氨基酸序列和含有SEQ ID NO:10的VL氨基酸序列。一些实施方案中,此类竞争抗体与结合IL-13的抗体结合相同的表位(例如线性或构象表位),其中所述结合IL-13的抗体包含含有SEQ ID NO:19的VH氨基酸序列和含有SEQ ID NO:20的VL氨基酸序列。一些实施方案中,此类竞争抗体与结合IL-13的抗体结合相同的表位(例如线性或构象表位),其中所述结合IL-13的抗体包含含有SEQ ID NO:49的VH氨基酸序列和含有SEQ ID NO:48的VL氨基酸序列。用于抗体所结合的表位的作图的详细示例性方法在MORRIS(1996)"EPITOPE MAPPING PROTOCOLS,"METHODS IN MOLECULAR BIOLOGY VOL. 66(HUMANA PRESS,TOTOWA,NJ)中提供。

[0302] 在示例性竞争测定法中,在溶液中温育固定化的IL-4,所述溶液包含与IL-4结合的第一标记的抗体(例如,包含含有SEQ ID NO:9的VH氨基酸序列和含有SEQ ID NO:10的VL氨基酸序列的抗体)、和待测试其与第一抗体竞争结合IL-4的能力的第二未标记的抗体。第二抗体可以存在于杂交瘤上清液中。作为对照,在溶液中温育固定化的IL-4,所述溶液包含第一标记的抗体但不包含第二未标记的抗体。在允许第一抗体与IL-4结合的条件下温育后,去除过量的未结合的抗体,测量与固定化的IL-4结合的标记量。如果与固定的IL-4结合的标记量在测试样品中相对于在对照样品中有实质性的减少,那么这指示第二抗体与第一抗体竞争结合IL-4。参见Harlow和Lane(1988)Antibodies:A Laboratory Manual ch. 14(Cold Spring Harbor Laboratory,Cold Spring Harbor,NY).

[0303] 在再一示例性竞争测定法中,在溶液中温育固定化的IL-13,所述溶液包含与IL-13结合的第一标记的抗体(例如,包含含有SEQ ID NO:19的VH氨基酸序列和含有SEQ ID NO:20的VL氨基酸序列的抗体,或包含含有SEQ ID NO:49的VH氨基酸序列和含有SEQ ID NO:48的VL氨基酸序列的抗体)、和待测试其与第一抗体竞争结合IL-13的能力的第二未标记的抗体。第二抗体可以存在于杂交瘤上清液中。作为对照,在溶液中温育固定化的IL-13,所述溶液包含第一标记的抗体但不包含第二未标记的抗体。在允许第一抗体与IL-13结合的条件下温育后,去除过量的未结合的抗体,测量与固定化的IL-13结合的标记量。如果与固定的IL-13结合的标记量在测试样品中相对于在对照样品中有实质性的减少,那么这指示第二抗体与第一抗体竞争结合IL-13。

[0304] 活性测定法

[0305] 在一些实施方案中，提供了用于鉴定具有生物活性的抗 IL-4 抗体和抗 -IL-4/IL-13 双特异性抗体的测定法。生物活性可以包括，例如，抑制 IL-4 结合 IL-4 受体、抑制 IL-4- 诱导的 STAT6 磷酸化、抑制 IL-4 诱导的细胞增殖、抑制 IL-4- 诱导的 B 细胞向 IgE 的类型转换、在哮喘中的活性、和在 IPF 中的活性。在一些实施方案中，生物活性包括，例如，抑制 IL-13 结合 IL-13 受体（例如，包含 IL-4Rα 和 IL-13Rα1 的异二聚体受体）、抑制 IL-13- 诱导的 STAT6 磷酸化、抑制 IL-13 诱导的细胞增殖、抑制 IL-13- 诱导的 B 细胞向 IgE 的类型转换、抑制 IL-13- 诱导的粘液产生、在哮喘中的活性、和在 IPF 中的活性。还提供了在体内和 / 或在体外具有此类生物活性的抗体。用于检测这些生物活性的非限制性示例测定法在本文描述和 / 或是本领域已知的。

[0306] 免疫缀合物

[0307] 在一些实施方案中，提供了包含与一或多个细胞毒性剂缀合的抗 IL-4 抗体或抗 -IL-4/IL-13 双特异性抗体的免疫缀合物。非限制性示例细胞毒性剂包括化学治疗剂或药物、生长抑制剂、毒素（例如蛋白质毒素，细菌、真菌、植物或动物来源的酶促活性毒素，或其片段）、和放射性同位素。

[0308] 在一些实施方案中，免疫缀合物是抗体 - 药物缀合物 (ADC)，其中抗体缀合至一种或多种药物，包括但不限于类美登素 (maytansinoid)（参见，例如，美国专利号 5,208,020,5,416,064 和欧洲专利 EP 0 425 235 B1）；澳瑞他汀 (auristatin) 例如单甲基澳瑞他汀药物部分 DE 和 DF(MMAE 和 MMAF)（参见，例如，美国专利号 5,635,483 和 5,780,588, 和 7,498,298）；多拉司他汀 (dolastatin)；加利车霉素 (calicheamicin) 或其衍生物（参见，例如，美国专利号 5,712,374,5,714,586,5,739,116,5,767,285,5,770,701,5,770,710,5,773,001, 和 5,877,296；HINMAN 等，CANCER RES. 53:3336-3342(1993)；和 LODE 等，CANCER RES. 58:2925-2928(1998)）；蒽环类例如道诺霉素 (daunomycin) 或多柔比星（参见，例如，KRATZ 等，CURRENT MED. CHEM. 13:477-523(2006)；JEFFREY 等，BIOORGANIC&MED. CHEM. LETTERS 16:358-362(2006)；TORGOV 等，BIOCONJ. CHEM. 16:717-721(2005)；NAGY 等，PROC. NATL. ACAD. SCI. USA 97:829-834(2000)；DUBOWCHIK 等，BIOORG. &MED. CHEM. LETTERS12:1529-1532(2002)；KING 等，J. MED. CHEM. 45:4336-4343(2002)；和美国专利号 6,630,579)；氨甲蝶呤；长春地辛 (vindesine)；紫杉烷 (taxane) 例如多西他赛 (docetaxel)、紫杉醇 (paclitaxel)、larotaxel、tesetaxel 和 ortataxel；单端孢霉烯；和 CC1065。

[0309] 在一些实施方案中，免疫缀合物包含缀合至酶促活性毒素或其片段的如本文描述的抗体，所述酶促活性毒素或其片段包括但不限于白喉毒素 A 链、白喉毒素的非结合活性片段、外毒素 A 链（来自铜绿假单胞菌 (Pseudomonas aeruginosa)）、蓖麻毒蛋白 A 链、相思豆毒蛋白 A 链、蒴莲根毒蛋白 A 链、α- 帚曲霉素、油桐 (Aleurites fordii) 蛋白质、香石竹毒蛋白、美洲商陆 (Phytolaca americana) 蛋白质 (PAPI、PAPII 和 PAP-S)、苦瓜 (momordica charantia) 抑制剂、麻疯树毒蛋白、巴豆毒蛋白、肥皂草 (sapaonaria officinalis) 抑制剂、多花白树毒蛋白、mitogellin、局限曲菌素、酚霉素、依诺霉素和单端孢菌毒素类。

[0310] 在一些实施方案中，免疫缀合物包含缀合至放射性原子的如本文描述的抗体，以形成放射性缀合物。多种放射性同位素可用于放射性缀合物的产生。例子包括 At^{211}、I^{131}、I^{125}、Y^{90}、Re^{186}、Re^{188}、Sm^{153}、Bi^{212}、P^{32}、Pb^{212} 和 Lu 的放射性同位素。当放射性缀合物用于检测

时，它可以包含用于闪烁研究的放射性原子，例如 tc99m 或 I123，或用于核磁共振（NMR）成像（也称为磁共振成像，MRI）的自旋标记，例如碘 -123、碘 -131、铟 -111、氟 -19、碳 -13、氮 -15、氧 -17、钆、锰或铁。

[0311] 抗体和细胞毒素剂的缀合物可以使用多种双官能蛋白质偶联剂进行制备，例如 3-(2- 吡啶二巯基）丙酸 N- 琥珀酰亚胺酯（SPDP）、4-(N- 马来酰亚胺基甲基）环己烷 -1- 羧酸琥珀酰亚胺酯（SMCC）、亚氨基硫烷（IT）、亚氨酸酯的双官能衍生物（例如二甲基己二酸酯 HCl）、活性酯（例如辛二酸二琥珀酰亚胺酯）、醛（例如戊二醛）、双 - 叠氮基化合物（例如双（对叠氮基苯甲酰基）己二胺）、双 - 重氮鎓衍生物（例如双（对重氮鎓苯甲酰基）- 乙二胺）、二异氰酸酯（例如 2,6- 二异氰酸甲苯酯）、和双 - 活性氟化合物（例如 1,5- 二氟 -2,4- 二硝基苯）。例如，蓖麻毒蛋白免疫毒素可以如 VITETTA 等，SCIENCE 238:1098(1987) 中所述进行制备。碳 -14 标记的 1- 异硫氰基苯甲基 -3- 甲基二亚乙基三胺五乙酸（MX-DTPA）是用于放射性核素与抗体缀合的示例性螯合剂。参见，例如，WO94/11026. 接头可以是促进细胞毒药物在细胞中释放的“可断裂接头”。例如，可以使用对酸敏感的接头、对肽酶敏感的接头、光敏接头、二甲基接头或含二硫键的接头（CHARI 等，CANCER RES. 52:127-131(1992)；美国专利号 5,208,020）。

[0312] 免疫缀合物或 ADCs 在本文中明确考虑，但不限于，用交联接头试剂制备的此类缀合物，包括但不限于 BMPS、EMCS、GMBS、HBVS、LC-SMCC、MBS、MPBH、SBAP、SIA、SIAB、SMCC、SMPB、SMPH、磺基 -EMCS、磺基 -GMBS、磺基 -KMUS、磺基 -MBS、磺基 -SIAB、磺基 -SMCC 和磺基 -SMPB 和 SVSB（琥珀酰亚胺基 -(4- 乙烯基砜）苯甲酸酯），其是商购可得的（例如来自 Pierce Biotechnology, Inc., Rockford, IL., U.S.A）。

[0313] 用于诊断和检测的方法和组合物

[0314] 在特定实施方案中，本文提供的任何抗 IL-4 抗体可以用于检测生物样品中 IL-4 的存在。在特定实施方案中，本文提供的任何抗 IL-4/IL-13 双特异性抗体可以用于检测生物样品中 IL-4 和 / 或 IL-13 的存在。如本文使用的术语“检测”涵盖定量或定性检测。在特定实施方案中，生物样品包括细胞或组织，例如血清、血浆、鼻拭子、支气管肺泡灌洗液、和痰。

[0315] 在一些实施方案中，提供了抗 IL-4 抗体用于在诊断或检测方法中使用。在进一步方面，提供了检测生物样品中 IL-4 的存在的方法。在特定实施方案中，该方法包括在允许抗 IL-4 抗体与 IL-4 结合的条件下，使生物样品与如本文描述的抗 IL-4 抗体接触，并且检测是否在抗 IL-4 抗体和 IL-4 之间形成复合物。此方法可以是体外或体内方法。在一些实施方案中，抗 IL-4 抗体用于选择如下疗法的合格受试者，所述疗法使用抗 IL-4 抗体或抗 -IL-4/IL-13 双特异性抗体或任何其它 TH2 途径抑制剂来进行，例如，当 IL-4 是用于患者选择的生物标记时。

[0316] 在一些实施方案中，提供了抗 IL-4/IL-13 双特异性抗体用于在诊断或检测方法中使用。在进一步方面，提供了检测生物样品中 IL-4 和 / 或 IL-13 的存在的方法。在一些实施方案中，该方法包括在允许抗 IL-4/IL-13 双特异性抗体与 IL-4 和 / 或 IL-13 结合的条件下，使生物样品与如本文描述的抗 IL-4/IL-13 双特异性抗体接触，并且检测是否在抗 IL-4/IL-13 双特异性抗体和 IL-4 和 / 或 IL-13 之间形成复合物。此方法可以是体外或体

内方法。在一些实施方案中，抗 IL-4/IL-13 双特异性抗体用于选择如下疗法的合格受试者，所述疗法使用抗 -IL-4/IL-13 双特异性抗体或任何其它 TH2 途径抑制剂来进行，例如，当 IL-4 和 / 或 IL-13 是用于患者选择的生物标记时。

[0317] 可以使用抗 IL-4 抗体或抗 IL-4/IL-13 双特异性抗体诊断的示例性病症在本文中提供。

[0318] 在特定实施方案中，提供了标记的抗 IL-4 抗体。在特定实施方案中，提供了标记的抗 IL-4/IL-13 双特异性抗体。标记包括但不限于，可以直接检测的标记或部分（例如荧光、生色、电子致密、化学发光和放射性标记），以及可以间接（例如通过酶促反应或分子相互作用）检测的部分，例如酶或配体。示例性标记包括但不限于，放射性同位素 ^{32}P、^{14}C、^{125}I、^{3}H 和 ^{131}I，荧光团例如稀土螯合物或荧光素及其衍生物，罗丹明及其衍生物，丹磺酰，伞形酮，萤光素酶例如萤火虫萤光素酶和细菌萤光素酶（美国专利号 4,737,456），萤光素，2,3- 二氢酞嗪二酮，辣根过氧化物酶 (HRP)，碱性磷酸酶，β - 半乳糖苷酶，葡糖淀粉酶，溶菌酶，糖氧化酶例如葡萄糖氧化酶、半乳糖氧化酶和 6- 磷酸葡萄糖脱氢酶，杂环氧化酶例如尿酸酶和黄嘌呤氧化酶，与采用过氧化氢以氧化染料前体的酶例如 HRP、乳过氧化物酶或微过氧化物酶偶联，生物素 / 抗生物素蛋白，自旋标记，噬菌体标记，稳定自由基等。

[0319] 药物制剂

[0320] 可以制备如本文描述的抗 IL-4 抗体和 / 或抗 IL-4/IL-13 双特异性抗体的药物制剂，例如通过使具有所需纯度的抗体与一种或多种任选的药学可接受的载体 (REMINGTON'S PHARMACEUTICAL SCIENCES 16TH EDITION, OSOL, A. ED. (1980)) 混合，以冻干制剂或水溶液形式制备。药学可接受的载体在采用的剂量和浓度下对接受者一般是无毒的，并且包括但不限于：缓冲剂例如磷酸盐、柠檬酸盐和其他有机酸；抗氧化剂包括抗坏血酸和甲硫氨酸；防腐剂（例如十八基二甲基苄基氯化铵；氯己双铵；苯扎氯铵；苄索氯铵；苯酚、丁醇或苯甲醇；对羟基苯甲酸烷基酯例如对羟基苯甲酸甲或丙酯；儿茶酚；间苯二酚；环己醇；3- 戊醇；和间甲酚）；低分子量（小于约 10 个残基）多肽；蛋白质例如血清白蛋白、明胶或免疫球蛋白；亲水聚合物例如聚乙烯吡咯烷酮；氨基酸例如甘氨酸、谷氨酰胺、天冬酰胺、组氨酸、精氨酸或赖氨酸；单糖、二糖及其他碳水化合物，包括葡萄糖、甘露糖或糊精；螯合剂例如 EDTA；糖例如蔗糖、甘露醇、海藻糖或山梨糖醇；成盐抗衡离子例如钠；金属络合物（例如 Zn- 蛋白质络合物）；和 / 或非离子型表面活性剂例如聚乙二醇 (PEG)。示例性药学可接受的载体在本文中进一步包括间质 (insterstitial) 药物分散剂例如可溶性中性 - 活性透明质酸酶糖蛋白 (sHASEGP)，例如人可溶性 PH-20 透明质酸酶糖蛋白，例如 rHuPH20（**HYLENEX®**，Baxter International, Inc.）。在美国专利出版号 2005/0260186 和 2006/0104968 中描述了某些示例性 sHASEGPs 和使用方法，包括 rHuPH20。在一些实施方案中，将 sHASEGP 与一种或多种另外的葡糖胺聚糖酶例如软骨素酶组合。

[0321] 示例性冻干抗体制剂在美国专利号 6,267,958 中描述。水性抗体制剂包括美国专利号 6,171,586 和 WO2006/044908 中所述的那些，后面的制剂包括组氨酸 - 乙酸盐缓冲液。

[0322] 本文制剂还可以针对所治疗的特定适应症按照需要含有一种以上的活性成分，优选具有互补活性且不会不利地彼此影响的那些。例如，可能期望进一步与抗 IL-4 抗体和 / 或抗 IL-4/IL-13 双特异性抗体一起提供控制剂和 / 或 TH2 途径抑制剂。此类活性成分适于以对于预期目的有效的量组合存在。

[0323] 活性成分可以截留在例如通过凝聚技术(coacervation)或通过界面聚合制备的微囊中(例如分别地羟甲基纤维素或明胶-微囊和聚-(甲基丙烯酸甲酯)微囊)、在胶体药物递送系统(例如脂质体、白蛋白微球体、微乳液、纳米颗粒和纳米囊)中、或在粗乳液中。此类技术公开于REMINGTON'S PHARMACEUTICAL SCIENCES 16TH EDITION,OSOL,A. ED.(1980)中。

[0324] 可以制备持续释放的制剂。持续释放的制剂的合适例子包括含有抗体的固体疏水聚合物的半透性基质,所述基质可以为成形物品例如薄膜或微胶囊的形式。

[0325] 待用于体内施用的制剂一般是无菌的。无菌性可以通过例如经由无菌过滤膜过滤而容易地达到。

[0326] 治疗方法和组合物

[0327] 本文提供的任何抗IL-4抗体均可以用于本文描述的治疗方法中。本文提供的任何抗IL-4/IL-13双特异性抗体均可以用于本文描述的治疗方法中。

[0328] 在一些实施方案中,提供了抗IL-4抗体和/或抗IL-4/IL-13双特异性抗体用作药物。在一些实施方案中,提供了抗IL-4抗体和/或抗IL-4/IL-13双特异性抗体用于治疗哮喘、IPF、呼吸病症、嗜酸性粒细胞病症、IL-13介导的病症、或IL-4介导的病症。在一些实施方案中,提供了抗IL-4抗体和/或抗IL-4/IL-13双特异性抗体用于治疗方法中。在一些实施方案中,提供了抗IL-4抗体或抗IL-4/IL-13双特异性抗体用于治疗患有如下病症的个体的方法中:哮喘、呼吸病症、嗜酸性粒细胞病症、IL-13介导的病症、或IL-4介导的病症,所述方法包括向个体施用有效量的抗IL-4抗体或抗IL-4/IL-13双特异性抗体。在一些实施方案中,所述方法还包括向个体施用有效量的至少一种其它的治疗剂,例如如下述的其它治疗剂。

[0329] 根据上述任何实施方案的"个体"优选是人。

[0330] 在一些实施方案中,提供了抗IL-4抗体和/或抗IL-4/IL-13双特异性抗体用于制备或生产药物的用途。一些实施方案中,所述药物用于治疗哮喘、呼吸病症、嗜酸性粒细胞病症、IL-13介导的病症、或IL-4介导的病症。在再一实施方案中,药物用于治疗哮喘、IPF、呼吸病症、嗜酸性粒细胞病症、IL-13介导的病症、或IL-4介导的病症的方法中,所述方法包括向患有哮喘、呼吸病症、嗜酸性粒细胞病症、IL-13介导的病症、或IL-4介导的病症的个体施用有效量的药物。在一个该实施方案中,所述方法还包括向个体施用有效量的至少一种其它的治疗剂,例如如下述的其它治疗剂。

[0331] 在一些实施方案中,提供包含本文所述任何抗IL-4抗体和/或抗IL-4/IL-13双特异性抗体的药物制剂,例如用于任何上述治疗方法中。在一些实施方案中,提供包含本文所述任何抗IL-4抗体和/或抗IL-4/IL-13双特异性抗体和可药用载体的药物制剂。在一些实施方案中,提供包含本文所述任何抗IL-4抗体和/或抗IL-4/IL-13双特异性抗体和至少一种其它治疗剂(例如下文描述的其它治疗剂)的药物制剂。

[0332] 本文提供的抗体可以单独或与其它活性剂组合地在治疗中使用。例如,本文提供的抗体可以与至少一种其它治疗剂共施用。在特定实施方案中,其它治疗剂是TH2抑制剂。在一些实施方案中,其它治疗剂是哮喘炎症的控制剂,例如皮质类固醇、白三烯受体拮抗剂、LABAs、皮质类固醇/LABA联合组合物、茶碱(theophylline)、色甘酸钠(cromolyn sodium)、萘多罗米钠(nedocromil sodium)、奥马珠单抗(omalizumab)、LAMAs、MABA(例如

双功能毒蕈碱拮抗剂-β2激动剂)、5-脂加氧酶活化蛋白质(FLAP)抑制剂、或酶PDE-4抑制剂。

[0333] 上述组合治疗涵盖联合施用(其中两个或更多个治疗剂包括在相同或分开的制剂中),和分开施用,在此情况下,抗IL-4抗体和/或抗IL-4/IL-13双特异性抗体的施用可以在其它治疗剂(一种或多种)施用之前、同时和/或之后进行。在一些实施方案中,抗IL-4抗体和/或抗IL-4/IL-13双特异性抗体的施用和其它治疗剂的施用可以发生在彼此的大约一个月内、或大约一、两或三周内、或大约一、二、三、四、五、或六天内。

[0334] 在一些实施方案中,抗IL-4抗体和/或抗IL-4/IL-13双特异性抗体用于治疗癌症,例如成胶质细胞瘤或非霍奇金淋巴瘤。在一些实施方案中,本文提供的抗体可以与放射治疗联合使用。

[0335] 抗IL-4抗体和/或抗IL-4/IL-13双特异性抗体(和任何其它治疗剂)可以通过任何合适方法进行施用,包括肠胃外、肺内和鼻内、和如果需要局部治疗的话,损伤内施用。肠胃外输注包括肌内、静脉内、动脉内、腹膜内或皮下施用。给药可以通过任何合适途径,例如通过注射,例如静脉内或皮下注射(部分取决于施用是短暂的还是长期的)。本文考虑各种给药方案,包括但不限于,单次或经过多个时间点的多次施用、快速浓注施用(bolus administration)和脉冲输注。

[0336] 抗IL-4抗体和/或抗IL-4/IL-13双特异性抗体将以符合良好医疗实践的方式配制,给药和施用。在此考虑的因素包括待治疗的特定病症、待治疗的特定哺乳动物、个体患者的临床状况、病症原因、活性剂递送部位、施用方法、施用时间安排和医学从业者已知的其他因素。抗体无需但可以任选地与目前用于预防或治疗所讨论的病症的一种或多种活性剂配制在一起。此类其他活性剂的有效量取决于存在于制剂中的抗体量、病症或治疗的类型、和上文讨论的其他因素。这些活性剂一般可以以与本文描述相同的剂量和施用途径使用,或以本文描述的剂量的约1-99%使用,或以经验/临床上确定为合适的任何剂量和任何途径使用。

[0337] 对于疾病预防或治疗,抗IL-4抗体和/或抗IL-4/IL-13双特异性抗体的合适剂量(当单独或与一种或多种其他的另外治疗剂组合使用时)将取决于待治疗的疾病类型、抗体类型、疾病严重性和病程、抗体是施用用于预防还是治疗目的、先前疗法、患者的临床史和对抗体的应答、和主治医师的判断。本发明抗体适宜一次或经过一系列治疗而施用于患者。本领域技术人员可以基于疾病类型和严重度来确定抗体的合适剂量。抗IL-13抗体剂量的非限制性示例描述在例如PCT公布号WO 2012/083132中。抗体剂量的一般指导可以见于例如BAI等,CLINICAL PHARMACOKINETICS,51:119-135(2012)和DENG等,EXPERT OPIN.DRUG METAB.TOXICOL.8(2):141-160(2012).。抗体疗法的进展可以通过常规技术和测定法监控。

[0338] 应当理解,可以使用免疫缀合物代替或加上抗IL-4抗体或抗IL-4/IL-13双特异性抗体,实施上述任何制剂或治疗方法。

[0339] 制造品

[0340] 在一些实施方案中,提供含有可以用于治疗、预防和/或诊断上述病症的材料的制造品。制造品可以包含容器和在容器上或与容器结合的标签或包装说明书。合适的容器包括例如瓶、小瓶、注射器、IV溶液袋等。容器可以由多种材料例如玻璃或塑料形成。容器

容纳单独或与对于治疗、预防和/或诊断所述状况有效的另外组合物组合的组合物，且可以具有无菌进入口（例如容器可以是具有可被皮下注射针穿透的塞子的静脉溶液袋或小瓶）。组合物中至少一种活性剂是抗-IL-4抗体和/或抗-IL-4/IL-13双特异性抗体。标签或包装说明书指示组合物用于治疗所选择的状况。此外，制造品可以包含(a)含有组合物的第一容器，其中所述组合物包含抗-IL-4抗体和/或抗-IL-4/IL-13双特异性抗体；和(b)含有组合物的第二容器，其中所述组合物包含其它的细胞毒性剂或另外的治疗剂。在一些实施方案中，制造品可以进一步包含指示组合物可以用于治疗特定状况的包装说明书。可替代地或另外地，制造品可以进一步包括包含药学可接受的缓冲液的第二（或第三）容器，所述药学可接受的缓冲液例如抑菌注射用水(BWFI)、磷酸盐缓冲盐水、林格氏溶液和葡萄糖溶液。它可以进一步包括从商业和用户观点来看希望的其他材料，包括其他缓冲液、稀释剂、滤器、针头和注射器。

[0341] 应当理解，任何上述制成品可以包括替代抗IL-4抗体或抗IL-4/IL-13双特异性抗体的免疫缀合物，或可以除抗IL-4抗体或抗IL-4/IL-13双特异性抗体之外还包括免疫缀合物。

实施例

[0342] 以下是本发明方法和组合物的实例。应当理解，鉴于上文提供的一般描述，可以实施多种其他实施方案。

[0343] 实施例1-一些方法和试剂

[0344] 表面等离子体共振(SPR)BIAcore亲合力测量

[0345] 在Biacore 3000仪器(GE Healthcare)上，使用表面等离子体共振(SPR)，测量了抗IL-4、抗IL-13和抗IL-4/IL-13双特异性抗体的结合动力学。使用厂商提供的方案，通过基于胺的偶联，将抗人Fc(GE Healthcare)固定在CM5传感器芯片上。以1200共振单位(RU)的水平捕获抗体。

[0346] 测量了与浓度0、3.13、6.25、12.50、25.0、和50.0nM的人IL-4、食蟹猴IL-4、人IL-13、人IL-13R130Q(SEQ ID NO:31)、和食蟹猴IL-13的双特异性结合。使用2分钟注射时间和30μl/min流速，在25℃温度，以10mM HEPES,pH 7.4,150mM NaCl和0.005% Tween 20的运行缓冲液，记录了细胞因子结合的传感图。注射后，在运行缓冲液中对细胞因子自抗体的解离进行了1000秒监测。在结合周期之间通过注射60μl的3M氯化镁，再生表面。在扣除仅含有运行缓冲液的空白对照后，使用1:1Langmuir结合模型，利用厂商提供的软件，分析针对细胞因子与抗IL-13/抗IL-4双特异性抗体的结合所观察到的传感图，以计算动力学和结合常数。

[0347] 表面等离子体共振(SPR)BIAcore结合竞争试验

[0348] 在Biacore 3000仪器(GE Healthcare)上，使用表面等离子体共振(SPR)，检测了抗IL-4/IL-13双特异性抗体对人IL-13和人IL-13Rα2结合的抑制作用。使用厂商提供的方案，通过基于胺的偶联，将抗人IL-13固定在CM5传感器芯片上。在流动池4(FC4)上以985共振单位(RU)的水平固定了IL-13，并随后使用1M乙醇胺-HCl封闭未反应的位点。FC3用作测量的参照池，其通过活化和随后以乙醇胺封闭而预备。使用2分钟注射时间和30μl/min流速，在25℃温度，以10mM HEPES,pH 7.4,150mM NaCl和0.005% Tween 20的

运行缓冲液，记录了 IL-13Rα2（根据本领域标准方法制备和纯化的、带组氨酸标签的重组人 IL-13Rα2）结合的传感图。为了确定 IL-13Rα2 与 IL-13 结合的结合常数，针对浓度从 12.5 至 200nM 变化（2 倍稀释）的一系列 IL-13Rα2 溶液，记录了传感图。注射后，在运行缓冲液中对受体自细胞因子的解离进行了 600 秒监测。在结合周期之间通过注射 60μl 的 10mM 甘氨酸 -HCl pH 1.7，再生表面。

[0349] 为了评价在抗 IL-4/IL-13 双特异性抗体存在下 IL-13Rα2 与 IL-13 的结合，加入一个额外步骤——注射 60μl 250nM 抗 IL-4/IL-13 双特异性抗体，以评价在竞争性抗体存在下受体与细胞因子的结合。在扣除仅含有运行缓冲液的空白对照后，使用 1:1Langmuir 结合模型，利用厂商提供的软件，分析在竞争性抗体不存在和存在的情况下针对受体与细胞因子的结合所观察到的传感图，以计算动力学和结合常数。

[0350] ELISA 结合竞争试验

[0351] 为了确定抗体是否抑制 IL-4 与 IL-4 受体（IL-4R）结合，使用了 ELISA 测定法。在 96 孔板中，将 150μg/mL(1000nM) 抗体溶液三倍系列稀释在测试缓冲液（磷酸盐缓冲盐水 [PBS]，pH 7.5，含有 0.05% Tween 20 和 0.5% 牛血清白蛋白 [BSA]）中，以提供 0.0009，0.003，0.008，0.02，0.07，0.21，0.62，1.9，5.6，16.7，50.0，和 150μg/mL（分别为，0.0056，0.017，0.05，0.15，0.46，1.37，4.12，12.3，37，111，333，和 1000nM）。每个稀释物的体积均为 35μL。向每个孔中加入 35μL 11.6ng/mL(780pM) 生物素化 IL4 溶液。室温孵育混合物 40 分钟。孵育后，将孔中的内容物转移到用 50μL 2.0μg/mL 可溶性 IL-4R 蛋白（R&D Systems，Cat.No.230-4R/CF）在 PBS 中的溶液包被过夜并用含有 1% BSA 的 PBS 封闭的 96 孔 Nunc Maxisorp 板（Roskilde，Denmark）。40 分钟孵育后，在洗涤缓冲液（含有 0.05% Tween20 的 1X PBS）中洗涤板 5 次。然后每孔接受 50μL 链霉亲和素辣根过氧化物酶溶液（Caltag Laboratories，Invitrogen；Carlsbad，CA），孵育 40 分钟。用洗涤缓冲液洗涤 5 次后，向每孔加入 50μL 四甲基联苯胺（TMB）底物（KPL；Gaithersburg，MD）。几分钟后，加入 50μL 1N HCl 溶液以终止该反应。使用 Spectra Max 340 读板器（Molecular Devices；Sunnyvale，CA）在 450nM 读板。对于每个样品，相对于浓度，绘制 450nM 读取的光密度（OD）图。在 Kaleidagraph（Synergy Software；Reading，PA）中绘制曲线并使用 4 参数拟合进行拟合或点到点绘图。

[0352] 为了确定是否抗体抑制 IL-13 与 IL-13Rα1 受体的结合，除了使用生物素化 IL-13R130Q(SEQ ID NO:31) 替代生物素化的 IL-4、和使用可溶性 IL-13Rα1-Fc 蛋白（R&D Systems，Cat.No.146-IR-100）替代可溶性 IL-4R-Fc，基本上如上述进行 ELISA 试验。

[0353] 为了确定是否抗体抑制 IL-13 与 IL-13Rα2 受体的结合，除了使用生物素化 IL-13 替代生物素化的 IL-4、和使用可溶性 IL-13Rα2-Fc 蛋白（R&D Systems，Cat.No.614-IR-100）替代可溶性 IL-4R-Fc，基本上如上述进行 ELISA 试验。

[0354] 质粒构建和抗体表达

[0355] 如前述（SIMMONS 等，2002，J IMMUNOL METHODS 263，133 - 147），将抗体克隆到表达载体中。对于重链和轻链两者，在编码成熟抗体的序列之前为具有翻译起始强度 1 的 STII 信号序列。为了表达蛋白，30℃在 LB(100μg/ml 羧苄青霉素）中生长在适宜的 W3110 衍生物（Reilly 和 Yansura，2010，Antibody Engineering（Berlin，Heidelberg：Springer Berlin Heidelberg））中的过夜培养物，1:100 稀释到 CRAP 培养基（100μg/ml 羧苄青

霉素）中并30℃生长24小时。对于更大的制备，在10L发酵罐中，例如如前述（SIMMONS等，2002，J IMMUNOL METHODS 263，133－147），生长培养物。

[0356] 对于非还原条件下SDS-PAGE分析，收获200μl过夜培养物并重悬在100μl NR-裂解缓冲液（88μl PopCulture Reagent(Novagen)，10μl 100mM碘乙酰胺，2μl lysonase试剂（EMD Biosciences））中。在室温温育10分钟后，9300rcf离心样品2'，将50μl上清液转移到新鲜管中，与相同体积的2x SDS样品缓冲液（Invitrogen）混合。在将10μl样品上样到NuPAGE4-12％Bis-Tris/MES凝胶（Invitrogen）上前，95℃加热样品5'，16000rcf离心1'。通过iBlot(Invitrogen)将凝胶转移到硝酸纤维素膜上，用IRDye800CW缀合的抗人IgG F(c)抗体（Rockland）进行免疫印迹，用LiCOR Odyssey成像仪成像。

[0357] 对于完全还原的细胞样品，将细胞沉淀重悬在R-裂解缓冲液（10μl1M DTT，88μl PopCulture Reagent(Novagen)，2μl lysonase）中，室温温育10分钟，之后样品与2x SDS样品缓冲液混合。除了使用IRDye800CW缀合的抗人抗体（Rockland）用于免疫检测之外，如前所述对Western印迹进行成像。

[0358] 双特异性抗体的纯化和组装

[0359] 使用GEA的Niro-Soavi匀浆器（Bedford，NH，U.S.A），匀浆大肠杆菌全细胞培养液。所得的匀浆物然后通过加入聚乙烯亚胺絮凝剂至终浓度0.4％而进行提取，用纯化的水稀释并室温混合16小时。离心澄清提取物，使用冷却至15℃的0.2μm无菌过滤器过滤后，上样至预平衡的（25mM Tris，25mM NaCl 5mM EDTA pH 7.1）蛋白A柱上。用平衡缓冲液和0.4M磷酸钾pH 7.0洗涤柱子，并最后用100mM乙酸pH 2.9洗脱。然后在组装反应中合并蛋白A池。

[0360] 分开的半抗体蛋白A池分别用0.2M精氨酸调理，用1.5M Tris碱将pH调整至pH 8.0，合并，以200x摩尔过量于双特异性抗体的量加入L-还原型谷胱甘肽（GSH），20℃温育48小时。温育后，通过阴离子交换层析步骤和阳离子交换层析步骤，纯化组装的双特异性抗体。浓缩阳离子交换洗脱物，将缓冲液更换为最终制剂缓冲液。

[0361] 通过完整和还原的质谱分析对抗体进行分析性表征

[0362] 使用偶联nano-Chip-LC系统的Agilent 6210ESI-TOF质谱仪，通过LC/MS分析，获得双特异性抗体的还原的和完整的质量。经和不经之前TCEP还原的双特异性样品，以每次注射大约5ng抗体的量，通过RP HPLC脱盐用于直接线上MS分析。针对还原和非还原样品所得的质谱显示出了多重荷电蛋白离子的分布，使用MassHunter工作站软件／定量分析B.03.01(AGILENT TECHNOLOGIES INC.2009)将该质谱解卷积至零电荷态。

[0363] 分析性大小排阻层析

[0364] 使用TosoHaas TSK G3000SW_{XL}柱（7.8x 300mm），分离大小变体，用由0.2M磷酸钾和0.25M氯化钾（pH 6.2）组成的移动相进行等度洗脱。所述分离在室温以0.5mL/min流速进行。在280nm监控柱流出液。使用Dionex Corporation的Chromeleon软件v6.80SR11，获得高分子量物（HMWS）（主峰）和低分子量物（LMWS）的峰面积的相对百分数。

[0365] 毛细管电泳－十二烷基硫酸钠分析（CE-SDS）

[0366] 首先用柠檬酸－磷酸缓冲液pH 6.6稀释双特异性样品，用SDS和N-乙基马来酰亚胺70℃处理3分钟。冷却后，将样品在50℃用3-(2-呋喃甲酰基）喹啉-2-甲醛）(FQ)在过量氰化钾存在下标记10分钟。通过缓冲液更换淬灭标记反应，然后用1％SDS处理。

非还原样品在70℃加热5分钟。还原的样品用50mM二硫苏糖醇(DTT)在70℃处理10分钟。

[0367] 使用Beckman PA 800CE系统(具有50μm直径、未包被的熔融石英毛细管(fused silica capillary)),通过CE SDS分析了非还原的和还原的样品。电动态地(electrokinetically)(以5kV,40秒)注射样品,并在15kV恒压以反向极性进行35分钟分离。毛细管温度维持在40℃。通过LIF检测,监测标记的成分的迁移;在488nm激发,在600nm监测发射。

[0368] 细胞培养(TF-1细胞)

[0369] 在增湿温育箱中以37℃和5%CO2将人TF-1(红白血病细胞,R&D Systems,Minneapolis,MN)培养在包含RPMI 1640(GENENTECH MEDIA PREPARATION FACILITY,SOUTH SAN FRANCISCO,CA)的生长培养基中,其中所述生长培养基含有10%热灭活胎牛血清(FBS)(目录号SH30071.03,HYCLONE LABORATORIES,INC.,LOGAN,UT);以及1X青霉素:链霉素:谷氨酰胺(目录号10378-016,GIBCO INVITROGEN CORP.,CARLSBAD,CA)和2ng/mL rhGM-CSF(目录号215-GM,R&D SYSTEMS,MINNEAPOLIS,MN)。测试培养基是不含2ng/mL rhGM-CSF的生长培养基。将细胞因子以如下终浓度加入所述的测试培养基中:0.2ng/ml人IL-4(目录号204-IL,R&D SYSTEMS,MINNEAPOLIS,MN),10ng/ml人IL-13(GENENTECH,SO.SAN FRANCISCO,CA),10ng/ml人IL-13R130Q(GENENTECH,SO.SAN FRANCISCO,CA),2ng/ml食蟹猴IL-4(GENENTECH,SO.SAN FRANCISCO,CA),和20ng/ml食蟹猴IL-13(GENENTECH,SO.SAN FRANCISCO,CA)。

[0370] 实施例2-产生结合IL-4的抗体

[0371] 使用商业可得人IL-4(R&D SYSTEMS,MINNEAPOLIS,MN),产生了选择性结合人白介素-4(IL-4)的一组抗体。用0.5μg IL-4(重悬在磷酸盐缓冲盐水(PBS)中的单磷酰脂质A和海藻糖二棒分枝菌酸(dicorynomycolate)(MPL™+TDM)基佐剂(CORIXA,HAMILTON,MT)中,总共25μl),以3至4天间隔,注射5只BALB/c小鼠的每个后脚垫。在7次加强后取血清样品,通过酶联免疫吸附测定法(ELISA)确定滴度,以鉴定具有IL-4阳性免疫反应的小鼠。通过脚垫(0.5μg在25μl中/脚垫),腹膜腔(2μg在100μl中),和静脉内(1μg在50μl中)途径,使用在PBS中的佐剂,再加强免疫小鼠两次。最后一次加强后三天,处死表现出ELISA阳性血清滴度的动物,并且使用电融合(Cyto Pulse Sciences,Inc.,Glen Burnie,MD),将脾细胞的单细胞悬浮液与小鼠骨髓瘤细胞系P3X63Ag.U.1(美国典型培养物保藏中心,Manassas,VA)融合。使用次黄嘌呤-氨甲喋呤-胸苷(HAT)选择,在来自**ClonaCell®**杂交瘤选择试剂盒(StemCell Technologies,Inc.,Vancouver,BC,Canada)的培养基D中,从未融合的脾、腘淋巴结或骨髓瘤细胞中,选择出融合的杂交瘤细胞。将杂交瘤细胞培养在**ClonaCell®**杂交瘤选择试剂盒的培养基E中,细胞培养物上清液用于进一步表征和筛选。为了筛选产生的1921个杂交瘤细胞系,如前面所述(BAKER,K.N.,等,TRENDS BIOTECHNOL.20,149-156(2002)),实施了酶联免疫吸附测定法(ELISA)。

[0372] 我们鉴定了克隆19C11,其以≤10pM的亲合力结合人IL-4(通过表面等离子体共振(SPR)分析确定)。为了确定19C11是否阻断人IL-4与IL-4Rα结合,将生物素化的IL-4(0.17nM)与50μl的来自克隆19C11的系列稀释IgG上清液(1000,200,40,8,1.6,和0.32nM,终浓度)或对照抗体预先混合。在30分钟室温孵育后,将混合物转移到含有固

定化的可溶性人 IL-4Rα (R&D Systems,Minneapolis,MN) 的 Nunc Maxisorp 板中。为了固定化,4℃在磷酸盐缓冲盐水 (PBS) 中用 2μg/ml IL-4Rα 包被板子过夜,以固定可溶性人 IL-4Rα。用 200μL 稀释在 PBS 中的 0.5%牛血清白蛋白 (SIGMA,ST.LOUIS,MO) 溶液封闭板子,之后加入抗体 /IL-4。加入抗体 /IL-4 混合物后,室温温育板子 60 分钟。温育后,用含有 0.05%聚山梨醇酯 20(Sigma) 的 PBS 洗涤板子 3 次。将辣根过氧化物酶缀合的链霉亲和素 (Jackson ImmunoResearch,West Grove,PA) 以 1:5000 稀释在测试缓冲液中,向每孔中加入 100μL。30 分钟室温温育后,如上所述洗涤板子。加入 100μL TMB 底物,温育板子 5 至 15 分钟。通过加入 1N 磷酸终止反应。使用 Spectra Max 340 读板器 (Molecular Devices ;Sunnyvale,CA) 在 OD450 读 ELISA 板。使用 Kaleidagraph 绘图软件 (Synergy Software,Reading,PA) 绘制曲线。

[0373] 为了确定 19C11 是否封闭 IL-4 诱导的 TF-1 细胞增殖,将系列稀释的纯化 19C11 或无关对照抗体与 IL-4 及 TF-1 细胞一起孵育。48 小时孵育后,每个样品接受 ^{3}H- 胸苷,并在 4 小时孵育后确定 ^{3}H- 胸苷的掺入。

[0374] 19C11 阻断了生物素化 IL-4 与 IL-4Rα 的结合 (图 1A),提示 IL-4 上的表位与参与结合 IL-4Rα 的区域重叠。19C11 也抑制了 IL-4- 诱导的 TF-1 细胞增殖 (图 1B)。对于阻断 IL-4 诱导的 TF-1 细胞增殖,IC50 经确定为 0.014μg/ml,而 IC90 经确定为 0.07μg/ml(数据未显示)。随后通过将高变区嫁接到具有选择点突变的人 Vκ-1/VHIII 受体构架中,人源化 19C11。在人源化过程中保留了 19C11 的结合亲合力、表位、和细胞活性 (数据未显示)。

[0375] 实施例 3-19C11 的人源化

[0376] 将来自 mu19C11 的高变区 (HVRs) 嫁接入人 VL kappa I(huKI),VL kappa III(huKIII),VH 亚组 I(huVH1) 和 VH 亚组 III(huVHIII) 共有受体构架,以产生 CDR 嫁接物 (19C11-κ1 嫁接物,19C11-κ3 嫁接物,19C11-VH1 嫁接物,19C11-VH3 嫁接物)(见图 10 至 13)。在 VL 结构域中,嫁接了如下区域 :位置 24-34(HVRL1,SEQ ID NO:15),50-56(HVRL2,SEQ ID NO:16) 和 89-97(HVRL3,SEQ ID NO:17)。在 VH 结构域中,嫁接了位置 26-35b(HVRH1,SEQ ID NO:12),49-65(HVRH2,SEQ ID NO:13) 和 95-102(HVRH3,SEQ ID NO:14)。

[0377] 使用针对各高变区的分开寡核苷酸,通过 Kunkel 诱变,作为 IgG 表达构建体,产生了这些 19C11- 嫁接物。通过 DNA 测序,鉴定了正确克隆。为了潜在地加强 19C11 嫁接物的亲合力和功能,在 VH 结构域嫁接物中恢复了一些鼠 vernier 构架位置 (见图 12 和 13)。特别地,19C11-VH1 嫁接物的位置 67,69 和 71 和 19C11-VH3 嫁接物的位置 69,71 和 78 被多样化,以产生 19C11-VH1.L,19C11-VH1.FFL,19C11-VH3.LA, 和 19C11-VH3.FLA。此外,将突变 D62S 和 F63V 引入 19C11-VH3.LA 的 CDR-H2,产生 19C11-VH3.LA.SV(见图 13)。

[0378] 为了筛选目的,最初在 6 孔板中在 293 细胞中产生 IgG 变体。使用 FuGene 系统将编码 VL 和 VH 的载体 (各 2μg) 转移到 293 细胞中。将 6μl FuGene 与不含 PBS 的 100μl DMEM 培养基混合,室温温育 5 分钟。向此混合物中加入每条链 (2μg),室温孵育 20 分钟,然而转移到 6 孔板用于在 37℃和 5% CO2 中转染过夜。下一日,移出含有转染混合物的培养基,替换为 2ml 细胞培养基,例如含有 FBS 的 DMEM。再孵育细胞 5 天,之后以 1000rpm 5 分钟收获培养基,使用 0.22μm 低蛋白结合滤器无菌过滤。样品储存在 4℃。

[0379] 使用 BIAcore™-A100,通过表面等离子体共振测定亲合力。抗人 Fcγ 抗体 (大约～

7000RU) 在 10mM 乙酸钠 pH 4.8 中固定在 CM5 芯片上。293 细胞中表达的人源化 19C11IgG 变体通过抗人 Fcγ 抗体捕获。然后以 30μL/min 流速注射重组 IL-4。每次注射后，使用 3M MgCL2，再生芯片。通过从人源化 19C11 变体 IgG 流动池中扣除对照流动池，校正结合反应。使用同步拟合 kon 和 koff 的 1:1Languir 模型，进行动力学分析。

[0380] 制备了十二种不同的人源化 19C11 变体，每个人源化轻链 (19C11-κ1 嫁接物，19C11-κ3 嫁接物) 分别与每个人源化重链 (19C11-VH1 嫁接物，19C11-VH1.L, 19C11-VH1.FFL, 19C11-VH3 嫁接物，19C11-VH3.LA, 和 19C11-VH3.FLA) 组合。通过 SPR 测试了十二种人源化 19C11 变体的 IL-4 亲合力，以及嵌合 19C11 (其中鼠可变区与人 IgG 恒定区组合) 的 IL-4 亲合力 (图 14)。大多数变体保持了小于 10pM 的 IL-4 亲和力，除了 19C11-VH1 嫁接物 / κ1 嫁接物，19C11-VH3 嫁接物 / κ1 嫁接物，19C11-VH3.FLA/ κ1 嫁接物，和 19C11-VH3 嫁接物 / κ3 嫁接物。19C11-VH1.FFL/ κ3 嫁接物和 19C11-VH3.FLA/ κ3 嫁接物具有 11pM 的 IL-4 亲合力。

[0381] 19C11-VH3.LA.SV/ κ1 嫁接物被选择用于进一步研究。SEQ ID NOS:9 和 10 中分别显示了人源化抗体 19C11-VH3.LA.SV/ κ1 嫁接物 (在以下实施例中称作抗 -IL-4) 的重链和轻链可变区序列。SEQ ID NOS:12 至 14 中显示了抗体 19C11-VH3.LA.SV/ κ1 嫁接物的重链高变区 (HVRs)，SEQ ID NOS:15 至 17 中显示轻链 HVRs。

[0382] 实施例 4- 产生 IL-4/IL-13IgG1 双特异性抗体

[0383] 我们以前已经建立了在大肠杆菌中产生具有两种不同轻链的人 IgG1 双特异性抗体的技术 (YU 等，2011, SCI TRANSL MED 3, 84RA44)。该方法利用杵进臼技术 (RIDGWAY 等，1996, PROTEIN ENG. 9, 617 - 621 ; ATWELL 等，1997, J MOL BIOL 270, 26 - 35) 促进免疫球蛋白重链的异二聚化。为了可以使用两种不同轻链而无轻链错配，我们在分开的大肠杆菌细胞中分别以 hemimer 的形式培养了各臂。我们应用该方法，通过将抗 -IL-4 和抗 -IL-13 亲本抗体亚克隆到载体中，允许抗 IL-4 臂表达为人 IgG1 臼和抗 -IL-13 臂表达为人 IgG1 杵，产生抗 -IL-4/IL-13 双特异性抗体。SEQ ID NO:34 中显示 IgG1 杵恒定区的序列，SEQ ID NO:35 中显示 IgG1 臼恒定区的序列。

[0384] 对于双特异性抗体的抗 IL-13Fab，我们以之前已经产生并表征过的 lebrikizumab 为基础。参见，例如，PCT 公布号 WO 2005/062967A2。以低于 10pM 检测限的 Biacore- 源 Kd，Lebrikizumab 结合可溶性人 IL-13。lebrikizumab 与 IL-13 的结合不抑制该细胞因子与 IL-13Rα1 的结合，但确实阻断能够进行信号传导的异源二聚体 IL-4Rα / IL-13Rα1 复合物的随后形成 (ULTSCH, M. 等，2013, J. MOL. BIOL., DX. DOI. ORG/10. 1016/J. JMB. 2013. 01. 024 ; CORREN 等，2011, N. ENGL. J. MED. 365, 1088 - 1098)。

[0385] 为了抗体表达，使用大肠杆菌菌株 64B4。30℃在 LB(100μg/ml 羧苄青霉素) 中生长过夜培养物，1:100 稀释到 5ml CRAP 培养基 (100μg/ml 羧苄青霉素)(SIMMONS 等，2002, J. IMMUNOL. METHODS, 263:133-147) 中并 30℃培养 24 小时。表达后，可溶性级分进行 SDS-PAGE，之后抗 Fc 免疫染色以分析半抗体物的形成。杵和臼突变两者均导致显著的半抗体物。为了将规模放大到 10L 发酵罐，将开始的起子培养物 (500ml) 培养到静止相，用于接种 10L 发酵罐 (SIMMONS 等，2002, J. IMMUNOL. METHODS, 263:133-147)。

[0386] 抗 IL-13IgG1 杵 hemimer 在大肠杆菌中的最初表达比预计的低。以前已经证实，Fab 序列的随机诱变和 / 或置换疏水性表面残基可以导致改善的 Fab 稳定性和折叠

(FORSBERG 等，1997, J. BIOL. CHEM.，272:12430-12436；DEMAREST 等，2006, PROTEIN ENG. DES. SEL.，19:325-336；KUGLER 等，2009, PROTEIN ENG. DES. SEL.，22:135-147)。

[0387] 在大肠杆菌细胞中表达变体，通过非还原 SDS-PAGE 和随后的抗 Fc 免疫印迹分析了非还原的全细胞提取物。使用Odyssey® (LiCOR Biosciences) 定量 hemimer 带，并相对于 lebrikizumab 信号进行了标化。

[0388] 发现重链和轻链中的几个变化可以改善 hemimer 产量和 / 或折叠。选择了其中一个改变，即，轻链中的 M4L。此外，在重链中引入 Q1E 改变。在单个 hemimer 中组合这两个变化，发现得到的 hemimer 具有优于野生型 hemimer 的改善产量和折叠。lebrikizumab Q1E 重链可变区的序列示于 SEQ ID NO:19 中，lebrikizumab M4L 轻链可变区的序列示于 SEQ ID NO:20 中。使用这些变体区域构建抗 IL-4/IL-13IgG1 双特异性抗体。

[0389] 使用先前描述的方法，例如，在美国专利公布号 2011/0287009 和国际专利申请号 PCT/US2012/059810 中描述的方法，通过氧化还原化学，从分离的半抗体组装了完整的双特异性抗体。

[0390] 实施例 5- 产生 IL-4/IL-13IgG4 双特异性抗体

[0391] 在建立了人 IgG1 同种型抗 IL-4/IL-13 双特异性抗体的产生后，我们将该双特异性平台变为人 IgG4 同种型。我们希望产生人 IgG4 抗体形式的抗 -IL-4/IL-13 双特异性抗体以匹配 lebrikizumab 的同种型，该抗 IL-13 抗体 lebrikizumab 已经在中度至重度的不受控哮喘治疗中显示出临床益处 (Corren 等 .，2011, N. Engl. J. Med. 365, 1088 - 1098)。

[0392] 相对于 IgG1，IgG4 的重 - 轻链间二硫键由不连续的二硫键形成。该不连续二硫键连接模式对于大肠杆菌蛋白是不常见的 (BERKMEN, 2005, J. BIOL. CHEM. 280, 11387-11394). 。此外，S228 残基可以使 IgG4 的铰链区失稳定，且 IgG4 的 CH3 二聚体界面含有失稳定性 R409 残基 (Dall'Acqua et al., 1998, Biochemistry 37, 9266-9273)(EU 编号惯例）我们设计了几种构建体以解剖 IgG4Fc 区序列、链间二硫键模式、和 CH3R409 对半抗体在大肠杆菌中的功能性表达和随后的双特异性分子组装的影响。在每种情况下，我们均在铰链区中引入了稳定性 S228P 突变以减少组装后 Fab 臂交换 (STUBENRAUCH 等，2010, DRUG METAB. DISPOS. 38, 84-91)。我们首先将具有相应杵 / 臼突变（杵 :T366W；臼 :T366S, L368A, Y407V) 的 IgG4Fc 区嫁接到 IgG1Fab 上，以评价 IgG4Fc 区对半抗体功能性表达的影响。对于两种抗体，抗 -IL-4 和抗体 -IL-13，这均产生了与 IgG1 同种型相似量的二硫键连接的物质（图 2C 和 2D)，说明 Fc 区中同种型的差异不影响半抗体在大肠杆菌中的功能性表达。接着我们将重链的整个恒定区转变为 IgG4 亚类。尽管这导致了功能性表达的半抗体的减少，但证实了，大肠杆菌原则上能够在抗体恒定区中从非连续半胱氨酸形成分子内二硫键。

[0393] 由于位置 409 可能对于 CH3 稳定性是重要的 (Dall'Acqua et al., 1998, Biochemistry 37, 9266 - 9273)，并且由于在该阶段 R409 对于下游组装过程的影响不清楚，所以我们也设计了具有 R409K 突变的构建体，以重新产生 IgG1 同种型中的 CH3 界面。对于两种抗体，这部分地挽救了 IgG4 同种型功能性表达的轻微下降（图 2C 和 2D)。

[0394] 实施例 6- 组装和纯化 IL-4/IL-13 双特异性抗体

[0395] 为了比较不同双特异性抗体构建体的组装，我们生长了以 IgG1, IgG4 和 IgG4R409K 形式表达半抗体的培养物。通过蛋白 A 层析纯化半抗体后，混合 hemimer 对，通

过异二聚体化的杵/臼对的氧化还原化学步骤形成了完整双特异性抗体。通过阴离子和阳离子交换层析步骤，移出过量的半抗体。最终层析步骤后，将物质以45g/l配制在0.2M精氨酸琥珀酸盐pH5.5，0.02%-聚山梨醇酯20中。为了证实组装的抗体从半抗体物转移为稳定的完整抗体，我们通过大小排阻层析进行了抗体表征。所有三个构建体均以相应于完整150kDa抗体的保留时间洗脱（图3A）。此外，没有检测到显著量的聚集物（对于IgG1/IgG4/IgG4R409K，0.6/0.4/0.4%）并仅检测到痕量的低分子量物（对于IgG1/IgG4/IgG4R409K，0.2/0/4.4%），说明两种同种型均能够用于组装具有低聚集倾向的抗体。

[0396] 双特异性组装过程中一个步骤是形成铰链二硫键。由于大小排阻层析不能解析链间二硫键的氧化态，我们对抗体进行了毛细管电泳-十二烷基硫酸钠分析（CE-SDS），发现所有三种形式均以相似效率形成了铰链-二硫键。对于IgG1，IgG4和IgG4R409K，分别观察到89.3%，91.4%和86.7%的物质为完全氧化的构象（图3B）。接着我们还原了样品，重新通过CE-SDS进行分析，以确定轻链和重链的相应比率（图3C）。所有三种形式均具有相似的和预期的轻链（对于IgG1/IgG4/IgG4R409K，31.3/31.4/30.9%）和重链（对于IgG1/IgG4/IgG4R409K，65.8/64.9/65.4%）分布，进一步证实天然抗体构象的存在。

[0397] 为了确保在组装过程中产生异二聚物，我们通过质谱分析了最终的双特异性分子。完整的和还原的质量总结在表2、图4和表3中。对于所有三种双特异性抗体，实验质量与理论质量紧密匹配，未能检测到相应于同二聚物的任何质量。反相HPLC测定法进一步证实，这些抗体是双特异性的，没有同二聚体抗体的迹象（数据未显示）。

[0398] 表2非还原抗IL-4/IL-13双特异性抗体的质谱分析

[0399]

	理论质量 (Da)	实验质量 (Da)
抗-IL-4/IL-13 IgG1 双特异性	145298.4	145304.5
抗-IL-4 IgG1 同二聚体	144798.6	n.o.
抗-IL-13 IgG1 同二聚体	145798.3	n.o.
抗-IL-4/IL-13 IgG4 双特异性	144923.7	144929.6
抗-IL-4 IgG4 同二聚体	144423.9	n.o.
抗-IL-13 IgG4 同二聚体	145423.5	n.o.
抗-IL-4/IL-13 $IgG4_{R409K}$ 双特异性	144867.7	144874.0
抗-IL-4 $IgG4_{R409K}$ 同二聚体	144367.8	n.o.
抗-IL-13 $IgG4_{R409K}$ 同二聚体	145367.5	n.o.

[0400] n.o. 未观察到

[0401] 表3：还原的抗IL-4/IL-13双特异性抗体的质谱分析

[0402]

	理论质量 (Da)	实验质量 (Da)
抗-IL-4 LC IgG1	23522	23521
抗-IL-4 HC IgG1	48893	48893
抗-IL-13 LC IgG1	23815	23815
抗-IL-13 HC IgG1	49100	49099
抗-IL-4 LC IgG4	23522	23523
抗-IL-4 HC IgG4	48706	48708
抗-IL-13 LC IgG4	23815	23816
抗-IL-13 HC IgG4	48913	48914
抗-IL-4 LC $IgG4_{R409K}$	23522	23523
抗-IL-4 HC $IgG4_{R409K}$	48678	48679
抗-IL-13 LC $IgG4_{R409K}$	23815	23816
抗-IL-13 HC $IgG4_{R409K}$	48885	48886

[0403] LC 轻链，HC 重链

[0404] 由于未能在 R409 和 R409K IgG4 双特异性杵进臼抗体组装中检测到任何显著差异，故所有其它研究均使用野生型 (R409)IgG4 双特异性抗体形式。

[0405] 实施例 7-IL-4/IL-13IgG1 双特异性抗体的生化表征

[0406] 接着，我们表征了 IgG1 和 IgG4 双特异性抗体以评价是否它们对 IL-4 和 IL-13 的结合亲合力以及它们阻断 IL-4 和 IL-13 结合其受体的能力是相当的。IgG1 和 IgG4 双特异性抗体对 IL-4 和 IL-13 的亲合力如实施例 1 所述通过 Biacore 测量，发现它们是相当的 (表 4) 并与亲本抗体是相似的，说明结合配体的能力不受双特异性形式或同种型的影响。

[0407] 抗 IL-4/IL-13 双特异性抗体以高亲合力结合人 IL-13、人 IL-13R130Q(SEQ ID NO:31)，和食蟹猴 IL-13。针对这些细胞因子分别计算了 0.056，0.142，和 0.048(nM) 的解离常数。表 4 提供了动力学常数。其它 SPR 实验显示，抗 IL-4/IL-13 双特异性抗体以高亲合力结合人 IL-4 和食蟹猴 IL-4。针对这些细胞因子分别计算了 0.046 和 0.076nM 的解离常数。表 4 提供了动力学常数。

[0408] 表 4 抗 IL-4/IL-13 双特异性抗体的结合动力学

[0409]

同种型	配体	$K_{on}/10^5 (M^{-1}s^{-1})$	$k_{off}/10^{-4} (s^{-1})$	K_d (nM)
IgG1	人 IL-4	134.4±49.8	0.848±0.05	0.068±0.020
IgG4	人 IL-4	287.00±4.58	1.327±0.058	0.046±0.001
IgG1	人 IL-13	71.4±4.0	0.170±0.119	0.023±0.015
IgG4	人 IL-13	53.73±2.1	0.301±0.109	0.056±0.020
IgG4	人 IL-13 R130Q	1.84±0.13	0.262±0.036	0.142±0.013
IgG4	cyno IL-4	201.67±39.15	1.507±0.153	0.076±0.013
IgG4	cyno IL-13	60.80±4.94	0.283±0.202	0.048±0.036

[0410] 为了确保双特异性分子能阻断细胞因子与其受体的结合，使用了基本上如实施

例 1 所述的 ELISA 结合竞争测定法。抗 -IL-4/IL-13 双特异性抗体抑制了生物素化的人 IL-4(5.8ng/mL) 与人 IL 4R 的直接结合 (见图 15)。以 0.035 至 25 μg/mL(0.23 至 167nM) 的双特异性抗体,观察到生物素化 IL-4 结合 IL4R 的减少。

[0411] 相反地,抗 -IL-4/IL-13 双特异性抗体不抑制生物素化的人 IL-13(0.625 μg/mL) 与人 IL-13Rα1 的直接结合 (见图 16)。以测试的浓度加入双特异性抗体,没有观察到生物素化的人 IL-13 与 IL-13Rα1 结合的减少。

[0412] 抗 -IL-4/IL-13 双特异性抗体不实质性地抑制生物素化的人 IL-13(0.056 μg/mL) 与人 IL-13Rα2 的直接结合 (见图 17)。观察到生物素化的人 IL-13 与 IL-13Rα2 结合的部分减少。

[0413] 如实施例 1 所述,使用 SPR 观察 IL-13Rα2 与 IL-13 的结合。将一系列浓度的 IL-13Rα2 注射到固定化的 IL-13 上,收集传感图。基于这些传感图,观察到 0.365nM 的结合常数 (Kd)(kon = $24.27x10^4 \pm 0.49Ms^{-1}$, koff = $0.891x10^{-4} \pm 0.026s^{-1}$)。之前已经在分开的 SPR 实验中证实了抗 IL-4/IL-13 双特异性抗体以高亲合力 (Kd = 56pM) 结合人 IL-13(见表 4)。为了检查对 IL-13Rα2 结合 IL-13 的抑制作用,将 250nM 抗 -IL-4/IL-13 双特异性抗体注射到固定化的 IL-13 上,之后注射 IL-13Rα2。双特异性抗体的结合没有阻止 IL-13Rα2 与固定化的 IL-13 结合 (见图 18)。IL-13Rα2 与双特异性抗体 :IL-13 复合物结合的 Kd 是 1.09nM(kon = $10.06x10^4 \pm 0.56Ms^{-1}$, koff = $1.10x10^{-4} \pm 0.12s^{-1}$)。固定化 IL-13 用双特异性抗体预饱和仅仅适度地破坏了 IL-13Rα2 与 IL-13 的结合,说明双特异性抗体对 IL13 与 IL-13Rα2 结合的抑制作用不显著。

[0414] 因此,类似于亲本抗 -IL-4 和抗 IL-13 抗体,双特异性抗体完全地抑制了 IL-4 与 IL-4Rα 的结合,并且不实质性地抑制 IL-13 与 IL-13Rα1 或 IL-13Rα2 的结合。这些发现说明,IL-13 和 IL-4 臂的结合表位和单价亲合力均在双特异性抗体中得到保留。

[0415] 实施例 8- 在细胞测定法中中和 IL-4 和 IL-13 活性。

[0416] 在体外细胞测定法中评价了抗 -IL-4/IL-13IgG1 和抗 IL-4/IL-13IgG4 双特异性抗体两者的活性,在所述测定法中人 IL-4 和 IL-13 诱导 TF-1 细胞增殖。如下所述评价了每种双特异性抗体阻断人 IL-4 和人 IL-13 单独和组合地诱导 TF-1 细胞增殖的能力。

[0417] 在 96 孔组织培养板 (目录号 353072,FALCON BD,FRANKLIN LAKES,NJ) 中,在 50μl 含有细胞因子的测定培养基中将抗体 3.3 倍系列稀释。37℃温育板子 30 分钟。在测定培养基中洗涤 TF-1 细胞两次,并以 2.5x 10^5细胞 /ml 的终体积重悬。向每孔加入 50μl 细胞,得到总体积 100μl。板子在增湿保温箱中 37℃和 5% CO2 中孵育 4 天,之后每孔加入 1μCi ^3H 胸苷。再孵育 4 小时后,使用液体闪烁计数器,通过细胞相关的 ^3H 胸苷掺入,测量了增殖。从一式两份样品获得的结果以平均值表达。使用 KaleidaGraph(SYNERGY SOFTWARE,READING,PA) 制图。

[0418] 抗 -IL-4/IL-13IgG1 和抗 -IL-4/IL-13IgG4 双特异性抗体两者均以剂量依赖性方式抑制了人 IL-4 和 IL-13 诱导的 TF-1 细胞增殖,两种不同双特异性抗体在体外中和 IC50 方面没有显著差异 (图 5 和表 5)。

[0419] 表 5 :抗 IL-4/IL-13 双特异性抗体的 TF-1 增殖抑制测定试验的 IC50

[0420]

	IC_{50} (μg/ml)		
	IL-4	IL-13	IL-4 + IL-13
IgG1 双特异性	0.06	0.03	0.07
IgG4 双特异性	0.05	0.03	0.05

[0421] 进行了相似分析，以确定针对食蟹猴 IL-4 和 IL-13 诱导的 TF-1 细胞增殖，抗 -IL-4/IL-13IgG1 和抗 -IL-4/IL-13IgG4 双特异性抗体是否以剂量依赖性方式发挥抑制作用（图 6）。

[0422] 实施例 9- 在食蟹猴中的药代动力学研究

[0423] 我们评价了在向食蟹猴单次静脉内 (IV) 或皮下 (SC) 施用后 IgG4 和 IgG1 抗 -IL-4/IL-13 双特异性抗体的体内药代动力学。在食蟹猴中的该药代动力学 (PK) 研究获得实验动物保护和使用协会 (IACUC) 批准。在 Charles River 实验室 (CRL) 临床前服务站 (Reno, NV)，进行了抗 -IL-4/IL-13IgG4 的 PK 研究。来自 CRL 库存的总共 15 只雌性食蟹猴 (2.2 - 2.6kg) 随机分成 5 组 (n = 3/ 组)。组 1 的动物接受对照运载体 (vehicle) 的静脉内 (IV) 和皮下 (SC) 给药。组 2, 3, 和 4 的动物分别接受 10, 30, 和 100mg/kg 抗 -IL-4/IL-13IgG4 的单剂 IV 快速浓注 (bolus) 给药。组 5 的动物接受 10mg/kg 抗 -IL-4/IL-13IgG4 的 SC 给药。

[0424] 在 Shin Nippon 生物化学实验室 (SNBL)USA(Everett, WA)，进行了抗 -IL-4/IL-13IgG1 的 PK 研究。来自 SNBL 库存的总共 12 只雌性食蟹猴 (2.4 - 3.1kg) 随机分成 4 组 (n = 3/ 组)。组 1 的动物接受对照运载体 (vehicle) 的静脉内 (IV) 给药。组 2, 3, 和 4 的动物分别接受 10, 30, 和 60mg/kg 抗 -IL-4/IL-13IgG1 的单剂 IV 快速浓注给药。

[0425] 对于两个研究，在不同时间点收集血清样品直到给药后 4-5 周，通过定量限 0.078 μg/mL 的 ELISA 评价了抗 IL-4/IL-13IgG4 或抗 -IL-4/IL-13IgG1 的浓度，并通过桥式 ELISA 评价了抗治疗性抗体 (ATA)。对于 PK 数据收集，将研究第 1 日转化为 PK 第 0 日，以指示剂量施用开始。在该实际 (in life) 给药日后的所有时间点均计算为研究日减 1。使用 2 室分析，采用 **WinNonlin®** 的 5.2.1 版本 (Pharsight ;Mountain View, CA)，分析了每个动物的血清浓度数据。

[0426] 抗 IL-4/IL-13IgG4 和抗 IL-4/IL-13IgG1 双特异性抗体的血清浓度 - 时间曲线显示了双相处置 (biphasic disposition) 和跨测试剂量范围的线性药代动力学（图 7A 和 7B)。对于两种抗体，中央室的最初体积与血清体积相似，说明限制的分布。如针对人 IgG4 和 IgG1 抗体在食蟹猴中预期的一样，两种抗体均具有相对慢的清除 (CL) 和长终末半衰期 (terminal half-life)（平均 CL = 5.79 至 6.70mL/ 天 /kg 对于抗 -IL-4/IL-13IgG4, 3.59 至 4.09mL/ 天 /kg 对于抗 -IL-4/IL-13IgG1)。基于针对 10mg/kg 剂量组计算的曲线下面积 (AUC)，抗 IL-4/IL-13IgG4 抗体的 SC 生物利用率是 95.1%。在 50% 的抗 -IL-4/IL-13IgG4 给药的动物中检测到存在抗治疗性抗体 (ATA)，包括在 100mg/kg IV 剂量组中的所有 3 只动物，ATA 的存在看起来与第 14 天后抗 -IL-4/IL-13IgG4 的清除增加相关。在抗 IL-4/IL-13IgG1 处理的动物中检测到低 ATA 发生率，其看起来不影响 PK。总之，抗 -IL-4/IL-13IgG4 和抗 -IL-4/IL-13IgG1 双特异性抗体的药代动力学是相似的，并与其它人源化 IgG1 和 IgG4 单克隆抗体在食蟹猴中的药代动力学是相当的。

[0427] 实施例 10- 在食蟹猴哮喘模型中的肺分配

[0428] 我们在食蟹猴哮喘模型中评价了 IgG4 相对于 IgG1 抗 -IL-4/IL-13 双特异性抗体在肺分配上的潜在差异。在该哮喘模型中，对 Ascaris suum(A. suum) 天然敏化的食蟹猴进行 A. suum 提取物的气雾剂攻击，以引发可以模拟暴露于变应原的哮喘患者的变应性炎症反应。

[0429] 在食蟹猴中的该肺分配研究获得 IACUC 批准。在 CRL 临床前服务站 (Reno, NV)，进行了比较抗 -IL-4/IL-13IgG4 和抗 -IL-4/IL-13IgG1 的该研究。研究由两个不同部分组成。第一部分，来自 CRL 库存的食蟹猴 (3-10kg) 接受基线 Ascaris suum(A suum) 气雾剂攻击以确定 A suum 攻击在每只动物中引发合适气道反应的适宜性。在整个攻击期间监测动物的窘迫迹象，在该部分中不给予抗体。4 周后，开始第二部分，总共 7 只雄性食蟹猴随机分为 2 组 (在 IgG4 组 n = 3 ;在 IgG1 组 n = 4)。然后，这些食蟹猴通过 IV 快速浓注给药在第 1 研究日和第 8 研究日接受 10mg/kg 抗 -IL-4/IL-13IgG4 或抗 -IL-4/IL-13IgG1。随后，在第 9 研究日，通过气雾剂吸入 A suum，攻击动物。在不同时间点直到给药后 23 天，收集支气管肺泡灌洗液 (BAL) 和血清样品，通过定量限 0.078 μg/mL 的 ELISA 分析抗 -IL-4/IL-13IgG4 或抗 -IL-4/IL-13IgG1 浓度。对于数据计算，将研究第 1 日转化为 PK 第 0 日，以指示剂量施用开始。在该实际 (in life) 给药日后的所有时间点均计算为研究日减 1。在 BAL 和血清中测量尿素和白蛋白以分别估计上皮衬液 (ELF) 浓度以及就炎症诱导的血管渗漏进行校正。也通过 ELISA 在血清中测量了 Ascaris 特异性 IgE。如 RENNARD 等，1986, J. APPL. PHYSIOL., 60(2) :532-538 所述，使用 BAL 和血清尿素浓度数据估计了稀释因子。

[0430] 在第 1 和 8 研究日进行 10mg/kg IV 施用和第 9 研究日用 A. suum 提取物进行肺攻击后，我们比较了抗 IL-4/IL-13IgG4 和抗 -IL-4/3IgG1 抗体的血清浓度和上皮衬液 (ELF) 浓度。如例如 RENNARD 等，1986, J. APPL. PHYSIOL., 60(2) :532-538. 所述，针对 BAL 液体收集程序固有的稀释，校正 BAL 液 IgG 浓度数据，从而获得 ELF 中 IgG 浓度值。抗 -IL-4/IL-13IgG4 和抗 -IL-4/IL-13IgG1 双特异性抗体的血清与肺分配在整个研究长度上是相当的 (图 8)。在变应原攻击前，两种抗体的 ELF 浓度均大约为 IgG 血清浓度的 1% -4%，说明仅小部分系统抗体达到 ELF。第 9 研究日 A. suum 的吸入攻击看起来导致两种抗体的增加的肺分配。然而，当将 IgG 浓度相对于 ELF 中的白蛋白浓度标化并将这些值与血清 IgG 浓度比较时，这些数据表明，在呼吸攻击后增加的 ELF IgG 浓度是由攻击诱导的非特异性大分子血管渗漏导致的。

[0431] 实施例 11- 抗 -IL-4, 抗 -IL-13, 和抗 -IL-4/IL-13 抗体在小鼠变应性气道炎症和哮喘模型中的功效

[0432] 在本研究中使用了 8 只 BALB/c 小鼠 (Charles River Laboratories)。在第 0 日，所有小鼠用 100 μl 无菌 PBS 中在 2mg 明矾中的 50 μg 三硝基苯基 - 卵白蛋白 (trinitrophenyl-ovalbumin, TNP-OVA) 腹膜内 (IP) 免疫。免疫后第 35 天开始，所有小鼠每日用 PBS 中 1% TNP-OVA 通过雾化器进行 30 分钟的气雾剂攻击，连续 7 日。如图 9A 所示，第 37 天开始，每日用单克隆抗体 (mAbs) 处理小鼠，其中在每次气雾剂攻击 (7 天) 前 4 小时 IP 施用所述抗体。

[0433] 第 42 天，麻醉下对所有小鼠进行眶后取血以最终获得 200 μl 血清 (以测量研究期间达到的 TNP-OVA- 特异性 IgE, IgG1, 和抗体血清浓度)。在异氟烷麻醉下进行小鼠眶内采血以获得血清样品用于 TNP-OVA 特异性免疫球蛋白和血清 TARC(胸腺活化调节趋化因

子，thymus and activation regulated chemokine）的 ELISA 测量。收集支气管肺泡灌洗液样品用于差异计数。用冷 PBS 灌洗肺，之后通过 FACS 分析。将肺切成片，然后通过金属捣碎机捣碎，以获得单细胞悬液，然后通过 0.7μm 尼龙滤器过滤。肺样品重悬在 5ml 中。将固定体积的细胞悬液加入固定浓度的 FITC 标记的荧光珠中，在流式细胞仪上分析，每样品收集 5000 个珠事件以获得细胞计数。对于肺的定量和表型分析，用抗表面白细胞标记物的荧光染料标记的 mAb（CD44-FTC，CD4-APC，CCR3-Pe 和 CD4-APC，或 CD11c-FITC，CD11b-PE 和 Gr-1-APC；BD Biosciences，San Jose，CA），染色每样品的 3 百万个肺细胞。在 BD FACSCalibur（BD，San Jose，CA）上运行样品，并在 Flowjo 软件（Ashland，OR）上分析。

[0434] 图 9B-9E 中显示实验结果。抗 -IL-4/IL-13 双特异性抗体的施用与抗 IL-4 抗体相比以更大程度抑制了肺嗜酸性粒细胞（$p = 0.0381$），并且看起来比抗 IL-13 抗体以更大程度抑制肺嗜酸性粒细胞，尽管该差异没有达到统计学显著性（$p = 0.1803$）（图 9B）。相似地，抗 -IL-4/IL-13 双特异性抗体的施用与抗 IL-4 抗体（$p = 0.0031$）或抗 IL-13 抗体（$p = 0.0135$）相比以更大程度抑制了支气管肺泡灌洗液中的嗜酸性粒细胞（图 9C）。与对照处理相比，施用抗 IL-4 抗体或抗 IL-4/IL-13 双特异性抗体看起来减少 TNP-OVA- 特异性 IgE，尽管该结果没有达到统计学显著性（图 9D）。最后，抗 -IL-4/IL-13 双特异性抗体的施用与抗 IL-4 抗体或抗 IL-13 抗体（分别为 $p<0.0001$ 和 $p = 0.0323$）相比以更大程度抑制了血清 TARC 水平（图 9E）。

[0435] 讨论

[0436] 在此，我们应用了以前开发的杵进臼双特异性抗体平台产生抗细胞因子 IL-4 和 IL-13 的人 IgG1 和人 IgG4 双特异性抗体。鉴于 IL-4 和 IL-13 的重叠的和独特的生物学、以及抗 IL-13 抗体在中度至重度哮喘治疗中的活性，靶向 IL-4 和 IL-13 两者的双特异性抗体可能是优于抗 IL-13 的用于哮喘治疗的改良疗法。实施例 11 中给出的数据支持了此假设。我们的抗 -IL-4/IL-13 双特异性抗体是抗 IL-13 抗体 lebrikizumab 的延伸，lebrikizumab 在中度至重度不受控哮喘中在 II 期研究中显示了临床功效。由于 lebrikizumab 是人 IgG4 抗体，我们使用了杵进臼双特异性抗体平台和人 IgG4 以使我们的抗 IL-4/IL-13 双特异性抗体的同种型与 lebrikizumab 的匹配。

[0437] 人 IgG1 和 IgG4 同种型的一个关键区别是 CH3 二聚体界面，其影响二聚体稳定性。差异由位置 409 驱动。我们的结果证实，杵进臼突变与 IgG4CH3 结构域中的 Arg409，在半抗体表达和双特异抗体组装方面，均是相容的。我们未检测到两种不同同种型在组装效率或在最终抗体物质的质量上的任何显著差异。

[0438] 尽管在哺乳动物细胞中成熟建立了各种同种型的人抗体的表达，但是一直较少尝试在大肠杆菌中表达不同人抗体同种型，因此，全长或半抗体人 IgG4 同种型在大肠杆菌中的表达并没有充分的文献报道。在此，我们针对这些抗 IL-4/IL-13 双特异性抗体证实，人 IgG4hemimer 可以成功地在大肠杆菌细胞中大量地表达，并和人 IgG1 双特异性抗体一样容易地组装为双特异性抗体。

[0439] 杵进臼技术的标志之一是，在最终双特异性分子中单价亲本抗体的生物物理性质的保持。IgG1 和 IgG4 双特异性抗体均保留了亲本 Fab 的靶表位和结合性质，包括对 IL-4 或 IL-13 靶细胞因子的高亲合力，从而在体外细胞测定试验中导致高效力。

[0440] 食蟹猴中药代动力学研究证实 IgG1 和 IgG4 双特异性抗体两者的缓慢清除和相似

的终末半衰期。此外，IgG1 和 IgG4 双特异性抗体两者以如下水平相当地从血清向肺分配，所述水平使得可以完全中和肺中的致病性 IL-4 和 IL-13，这对于哮喘治疗是重要的。尽管在食蟹猴中与 IgG1 双特异性抗体相比 IgG4 双特异性抗体表现出具有更高 ATA 率，但是考虑到我们研究中使用的动物数量小以及缺乏人源化抗体在食蟹猴中相对于在人中免疫原性的明确关系，有关我们的抗 IL-4/IL-13IgG4 和 IgG1 双特异性抗体在人中的相对免疫原性，不能作出任何结论。然而，应当注意，除了抗体 Fab 的 CDR 区外，我们的双特异性抗体由全人 IgG1 和 IgG4 序列组成，其应当在人中表现出极小的免疫原性。因此，我们产生的双特异性抗体是临床开发哮喘和 IPF 及其它呼吸病症治疗的良好候选者。而且，基于本文提供的体内数据，自然可以达到治疗人病症，例如哮喘、IPF 和其它呼吸病症的方法。

[0441] 不同人同种型的抗体可以因在结合血清补体蛋白和免疫效应细胞上的 Fcγ 受体方面的差异而具有非常不同的体外和体内性质 (Nirula, A. et al., 2011, Curr Opin Rheumatol 23, 119 - 124)。特别地，人 IgG1 同种型抗体可以有效地激活补体系统和与 Fcγ 受体结合以触发抗体依赖性细胞毒性 (ADCC)，而人 IgG4 同种型抗体不激活补体系统并具有减少的 ADCC。重要的是，抗体效应子功能中的这些性质要求抗体糖基化（在哺乳动物细胞中在表达过程中产生）。在细菌细胞例如大肠杆菌中产生的抗体无论何种同种型均由于缺乏抗体糖基化而缺乏抗体效应子功能 (Jung, S. T. et al., 2011, Curr. Opin. Biotechnol. 22, 858 - 867 ; Simmons, L. C., et al., 2002, J Immunol Methods 263, 133 - 147)。尽管在本研究中产生的双特异性抗体在大肠杆菌中产生并因此缺乏糖基化和 Fc 效应子功能，但是本文所述双特异性抗体也可以在哺乳动物细胞中产生。该方法可以将用于这些抗体的杵进臼双特异性抗体平台有效地延伸至包括完全糖基化的双特异性抗 IL-4/IL-13 人 IgG1 和 IgG4 抗体同种型，并且这又可以提供广泛范围的具有不同效应子功能的治疗性双特异性抗体。

[0442] 尽管为了用于理解清楚的目的本发明已通过举例说明和实施例一定详细地进行了描述，但说明书和实施例不应解释为限制本发明的范围。本文引用的所有专利和科学文献的公开内容明确地整体引入作为参考。

[0443] 序列表

[0444]

SEQ ID NO:	描述	序列
1	mu19C11 VH	QIQLVQSGPE LKKPGETVKI SCKASGYTFT DYSMHWMKQA PGKGLKWMVW INTETGEPTY ADDFKGRFAF SLETSANTAY LKINNLKNED TATYFCARGG IFYGMDYWGQ GTSVTVSS
2	mu19C11 VL	SIVMTQTPKF LLISAGDRVT ITCKASQSVI NDAAWYQQKP GQSPRLLIYY TSHRYTGVPD RFTGSGYGTD FTFTISTVQA EDLAVYFCQQ DYTSPWTFGG GTKLEIKR
3	hu19C11 VH1 嫁接物	QVQLVQSGAE VKKPGASVKV SCKASGYTFT DYSMHWVRQA PGQGLEWMVW INTETGEPTY ADDFKGRVTI TRDTSTSTAY LELSSLRSED TAVYYCARGG IFYGMDYWGQ GTLVTVSS
4	hu19C11 VH1.L 嫁接物	QVQLVQSGAE VKKPGASVKV SCKASGYTFT DYSMHWVRQA PGQGLEWMVW INTETGEPTY ADDFKGRVTI TLDTSTSTAY LELSSLRSED TAVYYCARGG IFYGMDYWGQ GTLVTVSS
5	hu19C11 VH1.FFL 嫁接物	QVQLVQSGAE VKKPGASVKV SCKASGYTFT DYSMHWVRQA PGQGLEWMVW INTETGEPTY ADDFKGRPTF TLDTSTSTAY LELSSLRSED TAVYYCARGG IFYGMDYWGQ GTLVTVSS
6	hu19C11 VH3 嫁接物	EVQLVESGGG LVQPGGSLRL SCAASGYTFT DYSMHWVRQA PGKGLEWVVW INTETGEPTY ADDFKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG IFYGMDYWGQ GTLVTVSS
7	hu19C11 VH3.FLA 嫁接物	EVQLVESGGG LVQPGGSLRL SCAASGYTFT DYSMHWVRQA PGKGLEWVVW INTETGEPTY ADDFKGRFTF SLDNSKNTAY LQMNSLRAED TAVYYCARGG IFYGMDYWGQ GTLVTVSS
8	hu19C11 VH3.LA 嫁接物	EVQLVESGGG LVQPGGSLRL SCAASGYTFT DYSMHWVRQA PGKGLEWVVW INTETGEPTY ADDFKGRFTI SLDNSKNTAY LQMNSLRAED TAVYYCARGG IFYGMDYWGQ GTLVTVSS
9	hu19C11 VH3.LA.SV 嫁接物	EVQLVESGGG LVQPGGSLRL SCAASGYTFT DYSMHWVRQA PGKGLEWVVW INTETGEPTY ADSVKGRFTI SLDNSKNTAY LQMNSLRAED TAVYYCARGG IFYGMDYWGQ GTLVTVSS
10	hu19C11 VL κ1 嫁接物	DIQMTQSPSS LSASVGDRVT ITCKASQSVI NDAAWYQQSP GKAPKLLIYY TSHRYTGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ DYTSPWTFGQ GTKVEIKR
11	hu19C11 VL κ3 嫁接物	EIVLTQSPAT LSLSPGERAT LSCKASQSVI NDAAWYQQSP GQAPRLLIYY TSHRYTGIPA RFSGSGSGTD FTLTISSLEP EDFAVYYCQQ DYTSPWTFGQ GTKVEIKR
12	19C11 HVRH1	GYTFTDYSMH
13	19C11 HVRH2.SV	VWINTETGEPTYADSVKG
14	19C11 HVRH3	GGIFYGMDY
15	19C11 HVRL1	KASQSVINDAA
16	19C11 HVRL2	YTSHRYT
17	19C11 HVRL3	QQDYTSPWT
18	19C11 HVRH2	VWINTETGEPTYADDFKG
56	lebrikizumab VH	QVTLRESGPA LVKPTQTLTL TCTVSGFSLS AYSVNWIRQP PGKALEWLAM IWGDGKIVYN SALKSRLTIS KDTSKNQVVL TMTNMDPVDT ATYYCAGDGY YPYAMDNWGQ GSLVTVSS
57	lebrikizumab VL	DIVMTQSPDS LSVSLGERAT INCRASKSVD SYGNSFMHWY QQKPGQPPKL LIYLASNLES GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQQNNEDPR TFGGGTKVEI KR
19	lebrikizumab VH Q1E	EVTLRESGPA LVKPTQTLTL TCTVSGFSLS AYSVNWIRQP PGKALEWLAM IWGDGKIVYN SALKSRLTIS KDTSKNQVVL TMTNMDPVDT ATYYCAGDGY YPYAMDNWGQ GSLVTVSS
20	lebrikizumab VL M4L	DIVLTQSPDS LSVSLGERAT INCRASKSVD SYGNSFMHWY QQKPGQPPKL LIYLASNLES GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQQNNEDPR TFGGGTKVEI KR
21	lebrikizumab HVRH1	GFSLSAYSVNW
60	lebrikizumab HVRH1 (替代)	AYSVN

[0445]

22	lebrikizumab HVRH2	MIWGDGSIVYNSALKS
23	lebrikizumab HVRH3	DGYYPYAMDN
24	lebrikizumab HVRL1	RASKSVDSYGNSFMH
25	lebrikizumab HVRL2	LASNLES
26	lebrikizumab HVRL3	QQNNEDPRT
27	人IL-4 前体 (Swiss-Prot 登录号 P05112.1)	MGLTSQLLPP LFFLLACAGN FVHGHKCDIT LQEIIKTLNS LTEQKTLCTE LTVTDIFAAS KNTTEKETFC RAATVLRQFY SHHEKDTRCL GATAQQFHRH KQLIRFLKRL DRNLWGLAGL NSCPVKEANQ STLENFLERL KTIMREKYSK CSS
28	人IL-4 成熟形式 (无信号序列)	HKCDIT LQEIIKTLNS LTEQKTLCTE LTVTDIFAAS KNTTEKETFC RAATVLRQFY SHHEKDTRCL GATAQQFHRH KQLIRFLKRL DRNLWGLAGL NSCPVKEANQ STLENFLERL KTIMREKYSK CSS
29	人IL-13 前体 (Swiss-Prot 登录号 P35225.2)	MALLLT TVIALTCLGG FASPGPVPPS TALRELIEEL VNITQNQKAP LCNGSMVWSI NLTAGMYCAA LESLINVSGC SAIEKTQRML SGFCPHKVSA GQFSSLHVRD TKIEVAQFVK DLLLHLKKLF REGRFN
30	人IL-13 成熟形式 (无信号序列)	SP GPVPPSTALR ELIEELVNIT QNQKAPLCNG SMVWSINLTA GMYCAALESL INVSGCSAIE KTQRMLSGFC PHKVSAGQFS SLHVRDTKIE VAQFVKDLLL HLKKLFREGR FN
31	人IL-13 R130Q 成熟形式	LTCLGGFASP GPVPPSTALR ELIEELVNIT QNQKAPLCNG SMVWSINLTA GMYCAALESL INVSGCSAIE KTQRMLSGFC PHKVSAGQFS SLHVRDTKIE VAQFVKDLLL HLKKLFREGQ FN
32	食蟹猴IL-13 前体 (GenBank 登录号 ABG75889.1)	MALLLTMVIA LTCLGGFASP SPVPPSTALK ELIEELVNIT QNQKAPLCNG SMVWSINLTA GVYCAALESL INVSGCSAIE KTQRMLNGFC PHKVSAGQFS SLRVRDTKIE VAQFVKDLLV HLKKLFREGQ FN
33	食蟹猴IL-4 前体 (Swiss-Prot 登录号 P79339.2); 成熟形式是 氨基酸 25-153	MGLTSQLLPP LFFLLACAGN FVHGHKCDIT LQEIIFTLNS LTEQKTLCTK LTITDILAAS KNTTEKETFC RAATVLRQFY SHHEKDTRCL GATAQQFHRH KQLIRFLKRL DRNLWGLAGL NSCPVKEANQ STLENFLERL KTIMREKYSK CSS
34	IgG1 T366W 重链恒定区	ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKRVEP KSCDKTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSREE MTKNQVSLWC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV

[0446]

		LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK
35	IgG1 T366S/ L368A/Y407V 重链恒定区	ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP KSCDKTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSREE MTKNQVSLSC AVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLV SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK
36	IgG4 T366W / S228P 重链恒定区	ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTKT YTCNVDHKPS NTKVDKRVES KYGPPCPPCP APEFLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSQED PEVQFNWYVD GVEVHNAKTK PREEQFNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS SIEKTISKAK GQPREPQVYT LPPSQEEMTK NQVSLWCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSRL TVDKSRWQEG NVFSCSVMHE ALHNHYTQKS LSLSLGK
37	IgG4 T366S/ L368A/Y407V/ S228P 重链恒定区	ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTKT YTCNVDHKPS NTKVDKRVES KYGPPCPPCP APEFLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSQED PEVQFNWYVD GVEVHNAKTK PREEQFNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS SIEKTISKAK GQPREPQVYT LPPSQEEMTK NQVSLSCAVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLVSRL TVDKSRWQEG NVFSCSVMHE ALHNRYTQKS LSLSLGK
38	hu19C11 IgG4 T366S/ L368A/Y407V/ S228P 重链	EVQLVESGGG LVQPGGSLRL SCAASGYTFT DYSMHWVPQA PGKGLEWVVN INTETGEPTY ADSVKGRFTI SLDNSKNTAY LQMNSLRAED TAVYYCARGG IFYGMDYWGQ GTLVTVSSAS TKGPSVFPLA PCSRSTSEST AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTKTYT CNVDHKPSNT KVDKRVESKY GPPCPPCPAP EFLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSQEDPE VQFNWYVDGV EVHNAKTKPR EEQFNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKGLPSSI EKTISKAKGQ PREPQVYTLP PSQEEMTKNQ VSLSCAVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLVSRLTV DKSRWQEGNV FSCSVMHEAL HNHYTQKSLS LSLGK
39	hu19C11 轻链	DIQMTQSPSS LSASVGDEVT ITCKASQSVI NDAAWYQQKP GKAPKLLIYY TSHRYTGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ DYTSPWTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
40	lebrikizumab Q1E IgG4 T366W/S228P 重链	EVTLRESGFA LVKPTQTLTL TCTVSGFSLS AYSVNWIRQP PGKALEWLAM IWGDGKIVYN SALKSRLTIS KDTSKNQVVL TMTNMDPVDT ATYYCAGDGY YPYAMDNWGQ GSLVTVSSAS TKGPSVFPLA PCSRSTSEST AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTKTYT CNVDHKPSNT KVDKRVESKY GPPCPPCPAP EFLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSQEDPE VQFNWYVDGV EVHNAKTKPR EEQFNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKGLPSSI EKTISKAKGQ PREPQVYTLP PSQEEMTKNQ VSLWCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSRLTV DKSRWQEGNV FSCSVMHEAL HNHYTQKSLS LSLGK
41	lebrikizumab M4L 轻链	DIVLTQSPDS LSVSLGERAT INCRASKSVD SYGNSFMHWY QQKPGQPPKL LIYLASNLES GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQQNNEDPR TFGGGTKVEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV THQGLSSPVT KSFNRGEC

[0447]

58	lebrikizumab IgG4 T366W/ S228P 重链	QVTLRESGPA LVKPTQTLTL TCTVSGFSLS AYSVNWIRQP PGKALEWLAM IWGDGKIVYN SALKSRLTIS KDTSKNQVVL TMTNMDPVDT ATYYCAGLGY YPYAMDNWGQ GSLVTVSSAS TKGPSVFPLA PCSRSTSEST AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTKTYT CNVDHKPSNT KVDKRVESKY GPPCPPCPAP EFLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSQEDPE VQFNWYVDGV EVHNAKTKPR EEQFNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKGLPSSI EKTISKAKGQ PREPQVYTLP PSQEEMTKNQ VSLWCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSRLTV DKSRWQEGNV FSCSVMHEAL HNHYTQKSLS LSLGK
59	lebrikizumab 轻链	DIVMTQSPDS LSVSLGERAT INCRASKSVD SYGNSFMHWY QQKPGQPPKL LIYLASNLES GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQQNNEDPR TFGGGTKVEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV THQGLSSPVT KSFNRGEC
42	IL-13 表位, SEQ ID NO: 29的氨基酸68至75 (SEQ ID NO: 30的氨基酸50至57)	ESLINVSG
43	IL-13 表位, SEQ ID NO: 29的氨基酸63至74 (SEQ ID NO: 30的氨基酸45至56)	YCAALESLINVS
44	抗-IL-13 mu1IH4 VL	DIVLTQSPAS LAVSLGQPAT ISCRASQSVS TSSYSYMHWY QQTPGQPPKL LIKYASNLES GIPARFSGSG SGTDFTLNIH PVEEEDTATY YCQHSWEIPY TFGGGT
45	抗-IL-13 mu1IH4 VH	QVTLKESGPG ILQPSQTLSL TCSFSGFSLS TSDMGVGWIR QPSGKGLEWL AHIWWDDVKR YNPALKSRLT ISKDTSSSQV FLKIASVDTA DTATYYCARI GTNYGYDGLF DYWGQGTTLT VSS
46	抗-IL-13 hu1IH4v6 轻链	DIVMTQSPDS LAVSLGERAT INCRASQSVS TSSYSYMHWY QQKPGQPPKL LIKYASNLES GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQHSWEIPY TFGQGTKVEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV THQGLSSPVT KSFNRGEC
47	抗-IL-13 hu1IH4v6 重链	EVQLVESGPA LVKPTQTLTL TCTFSGFSLS TSDMGVGWIR QPPGKALEWL AHIWWDDVKR YNPALKSRLT ISKDTSKNQV VLTMTNMDPV DTATYYCARI GTNYGYDALF DYWGQGTLVT VSSASTKGPS VFPLAPSSKS TSGGTAALGC LVKDYFPEPV TVSWNSGALT SGVHTFPAVL QSSGLYSLSS VVTVPSSSLG TQTYICNVNH KPSNTKVDKK VEPKSCDKTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS REEMTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK
48	抗-IL-13 hu1IH4v6 VL	DIVMTQSPDS LAVSLGERAT INCRASQSVS TSSYSYMHWY QQKPGQPPKL LIKYASNLES GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQHSWEIPY TFGQGTKVEI K
49	抗-IL-13 hu1IH4v6 VH	EVQLVESGPA LVKPTQTLTL TCTFSGFSLS TSDMGVGWIR QPPGKALEWL AHIWWDDVKR YNPALKSRLT ISKDTSKNQV VLTMTNMDPV DTATYYCARI GTNYGYDALF DYWGQGTLVT VSS

[0448]

50	hu11H4v6 HVRH1	GFSLSTSDMGVG
51	hu11H4v6 HVRH2	AHIWWDDVERYNPALKS
52	hu11H4v6 HVRH3	ARIGTNYGYDALFDY
53	hu11H4v6 HVRL1	RASQSVSTSSYSYMN
54	hu11H4v6 HVRL2	YASNLES
55	hu11H4v6 HVRL3	QHSWEIPYT

[0001]

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Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
20 25 30
Ser Met His Trp Met Lys Gln Ala Pro Gly Lys Gly Leu Lys Trp Met
35 40 45
Val Trp Ile Asn Thr Glu Thr Gly Glu Pro Thr Tyr Ala Asp Asp Phe
50 55 60
Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Asn Thr Ala Tyr
65 70 75 80
Leu Lys Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala Thr Tyr Phe Cys
85 90 95
Ala Arg Gly Gly Ile Phe Tyr Gly Met Asp Tyr Trp Gly Gln Gly Thr
100 105 110
Ser Val Thr Val Ser Ser
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Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Ser Val Ile Asn Asp
20 25 30
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[0002]

35 40 45
Tyr Tyr Thr Ser His Arg Tyr Thr Gly Val Pro Asp Arg Phe Thr Gly
50 55 60
Ser Gly Tyr Gly Thr Asp Phe Thr Phe Thr Ile Ser Thr Val Gln Ala
65 70 75 80
Glu Asp Leu Ala Val Tyr Phe Cys Gln Gln Asp Tyr Thr Ser Pro Trp
85 90 95
Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
100 105
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1 5 10 15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
20 25 30
Ser Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45
Val Trp Ile Asn Thr Glu Thr Gly Glu Pro Thr Tyr Ala Asp Asp Phe
50 55 60
Lys Gly Arg Val Thr Ile Thr Arg Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80
Leu Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Gly Gly Ile Phe Tyr Gly Met Asp Tyr Trp Gly Gln Gly Thr
100 105 110
Leu Val Thr Val Ser Ser
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1 5 10 15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
20 25 30
Ser Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met

[0003]

35 40 45
Val Trp Ile Asn Thr Glu Thr Gly Glu Pro Thr Tyr Ala Asp Asp Phe
50 55 60
Lys Gly Arg Val Thr Ile Thr Leu Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80
Leu Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Gly Gly Ile Phe Tyr Gly Met Asp Tyr Trp Gly Gln Gly Thr
100 105 110
Leu Val Thr Val Ser Ser
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Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
20 25 30
Ser Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Val Trp Ile Asn Thr Glu Thr Gly Glu Pro Thr Tyr Ala Asp Asp Phe
50 55 60
Lys Gly Arg Phe Thr Phe Thr Leu Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80
Leu Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Gly Gly Ile Phe Tyr Gly Met Asp Tyr Trp Gly Gln Gly Thr
100 105 110
Leu Val Thr Val Ser Ser
115

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1 5 10 15

[0004]

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
20 25 30

Ser Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Val Trp Ile Asn Thr Glu Thr Gly Glu Pro Thr Tyr Ala Asp Asp Phe
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Gly Gly Ile Phe Tyr Gly Met Asp Tyr Trp Gly Gln Gly Thr
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Leu Val Thr Val Ser Ser
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20 25 30
Ser Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Val Trp Ile Asn Thr Glu Thr Gly Glu Pro Thr Tyr Ala Asp Asp Phe
50 55 60
Lys Gly Arg Phe Thr Phe Ser Leu Asp Asn Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Gly Gly Ile Phe Tyr Gly Met Asp Tyr Trp Gly Gln Gly Thr
100 105 110
Leu Val Thr Val Ser Ser
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[0005]

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20 25 30
Ser Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Val Trp Ile Asn Thr Glu Thr Gly Glu Pro Thr Tyr Ala Asp Asp Phe
50 55 60
Lys Gly Arg Phe Thr Ile Ser Leu Asp Asn Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Gly Gly Ile Phe Tyr Gly Met Asp Tyr Trp Gly Gln Gly Thr
100 105 110
Leu Val Thr Val Ser Ser
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Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
20 25 30
Ser Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Val Trp Ile Asn Thr Glu Thr Gly Glu Pro Thr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Leu Asp Asn Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Gly Gly Ile Phe Tyr Gly Met Asp Tyr Trp Gly Gln Gly Thr
100 105 110
Leu Val Thr Val Ser Ser
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[0006]

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Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Ser Val Ile Asn Asp
20 25 30
Ala Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Tyr Thr Ser His Arg Tyr Thr Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Asp Tyr Thr Ser Pro Trp
85 90 95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
100 105
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Glu Arg Ala Thr Leu Ser Cys Lys Ala Ser Gln Ser Val Ile Asn Asp
20 25 30
Ala Ala Trp Tyr Gln Gln Lys Pro Gly Glu Ala Pro Arg Leu Leu Ile
35 40 45
Tyr Tyr Thr Ser His Arg Tyr Thr Gly Ile Pro Ala Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65 70 75 80
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Asp Tyr Thr Ser Pro Trp
85 90 95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
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[0007]

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Gly Tyr Thr Phe Thr Asp Tyr Ser Met His
1 5 10
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1 5 10 15
Lys Gly

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Gly Gly Ile Phe Tyr Gly Met Asp Tyr
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[0008]

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1 5 10 15
Lys Gly

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Thr Leu Thr Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Ala Tyr
20 25 30
Ser Val Asn Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu Trp Leu
35 40 45
Ala Met Ile Trp Gly Asp Gly Lys Ile Val Tyr Asn Ser Ala Leu Lys
50 55 60
Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val Val Leu
65 70 75 80
Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
85 90 95
Gly Asp Gly Tyr Tyr Pro Tyr Ala Met Asp Asn Trp Gly Gln Gly Ser
100 105 110
Leu Val Thr Val Ser Ser
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[0009]

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20 25 30
Gly Asn Ser Phe Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
35 40 45
Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Asp
50 55 60
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
65 70 75 80
Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Asn Asn
85 90 95
Glu Asp Pro Arg Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
100 105 110
<210> 21
<211> 11
<212> PRT
<213> 人工序列
<220>
<223> 人工序列的描述：合成的 肽
<400> 21
Gly Phe Ser Leu Ser Ala Tyr Ser Val Asn Trp
1 5 10
<210> 22
<211> 16
<212> PRT
<213> 人工序列
<220>
<223> 人工序列的描述：合成的 肽

<400> 22
Met Ile Trp Gly Asp Gly Lys Ile Val Tyr Asn Ser Ala Leu Lys Ser
1 5 10 15
<210> 23
<211> 10
<212> PRT
<213> 人工序列
<220>
<223> 人工序列的描述：合成的 肽
<400> 23

[0010]

Asp Gly Tyr Tyr Pro Tyr Ala Met Asp Asn
1 5 10
<210> 24
<211> 15
<212> PRT
<213> 人工序列
<220>
<223> 人工序列的描述：合成的 肽
<400> 24
Arg Ala Ser Lys Ser Val Asp Ser Tyr Gly Asn Ser Phe Met His
1 5 10 15
<210> 25
<211> 7
<212> PRT
<213> 人工序列
<220>
<223> 人工序列的描述：合成的 肽
<400> 25
Leu Ala Ser Asn Leu Glu Ser
1 5
<210> 26
<211> 9
<212> PRT
<213> 人工序列
<220>
<223> 人工序列的描述：合成的 肽
<400> 26
Gln Gln Asn Asn Glu Asp Pro Arg Thr
1 5
<210> 27
<211> 153
<212> PRT
<213> 智人(Homo sapiens)
<400> 27
Met Gly Leu Thr Ser Gln Leu Leu Pro Pro Leu Phe Phe Leu Leu Ala
1 5 10 15
Cys Ala Gly Asn Phe Val His Gly His Lys Cys Asp Ile Thr Leu Gln
20 25 30
Glu Ile Ile Lys Thr Leu Asn Ser Leu Thr Glu Gln Lys Thr Leu Cys
35 40 45
Thr Glu Leu Thr Val Thr Asp Ile Phe Ala Ala Ser Lys Asn Thr Thr
50 55 60
Glu Lys Glu Thr Phe Cys Arg Ala Ala Thr Val Leu Arg Gln Phe Tyr
65 70 75 80

[0011]

Ser His His Glu Lys Asp Thr Arg Cys Leu Gly Ala Thr Ala Gln Gln
85 90 95
Phe His Arg His Lys Gln Leu Ile Arg Phe Leu Lys Arg Leu Asp Arg
100 105 110
Asn Leu Trp Gly Leu Ala Gly Leu Asn Ser Cys Pro Val Lys Glu Ala
115 120 125
Asn Gln Ser Thr Leu Glu Asn Phe Leu Glu Arg Leu Lys Thr Ile Met
130 135 140
Arg Glu Lys Tyr Ser Lys Cys Ser Ser
145 150

<210> 28
<211> 129
<212> PRT
<213> 智人
<400> 28
His Lys Cys Asp Ile Thr Leu Gln Glu Ile Ile Lys Thr Leu Asn Ser
1 5 10 15
Leu Thr Glu Gln Lys Thr Leu Cys Thr Glu Leu Thr Val Thr Asp Ile
20 25 30
Phe Ala Ala Ser Lys Asn Thr Thr Glu Lys Glu Thr Phe Cys Arg Ala
35 40 45
Ala Thr Val Leu Arg Gln Phe Tyr Ser His His Glu Lys Asp Thr Arg
50 55 60
Cys Leu Gly Ala Thr Ala Gln Gln Phe His Arg His Lys Gln Leu Ile
65 70 75 80
Arg Phe Leu Lys Arg Leu Asp Arg Asn Leu Trp Gly Leu Ala Gly Leu
85 90 95
Asn Ser Cys Pro Val Lys Glu Ala Asn Gln Ser Thr Leu Glu Asn Phe
100 105 110
Leu Glu Arg Leu Lys Thr Ile Met Arg Glu Lys Tyr Ser Lys Cys Ser
115 120 125
Ser

<210> 29
<211> 132
<212> PRT
<213> 智人
<400> 29
Met Ala Leu Leu Leu Thr Thr Val Ile Ala Leu Thr Cys Leu Gly Gly
1 5 10 15
Phe Ala Ser Pro Gly Pro Val Pro Pro Ser Thr Ala Leu Arg Glu Leu
20 25 30
Ile Glu Glu Leu Val Asn Ile Thr Gln Asn Gln Lys Ala Pro Leu Cys
35 40 45

[0012]

Asn Gly Ser Met Val Trp Ser Ile Asn Leu Thr Ala Gly Met Tyr Cys
50 55 60
Ala Ala Leu Glu Ser Leu Ile Asn Val Ser Gly Cys Ser Ala Ile Glu
65 70 75 80
Lys Thr Gln Arg Met Leu Ser Gly Phe Cys Pro His Lys Val Ser Ala
85 90 95
Gly Gln Phe Ser Ser Leu His Val Arg Asp Thr Lys Ile Glu Val Ala
100 105 110
Gln Phe Val Lys Asp Leu Leu Leu His Leu Lys Lys Leu Phe Arg Glu
115 120 125
Gly Arg Phe Asn
130

<210> 30
<211> 114
<212> PRT
<213> 智人
<400> 30
Ser Pro Gly Pro Val Pro Pro Ser Thr Ala Leu Arg Glu Leu Ile Glu
1 5 10 15
Glu Leu Val Asn Ile Thr Gln Asn Gln Lys Ala Pro Leu Cys Asn Gly
20 25 30
Ser Met Val Trp Ser Ile Asn Leu Thr Ala Gly Met Tyr Cys Ala Ala
35 40 45
Leu Glu Ser Leu Ile Asn Val Ser Gly Cys Ser Ala Ile Glu Lys Thr
50 55 60
Gln Arg Met Leu Ser Gly Phe Cys Pro His Lys Val Ser Ala Gly Gln
65 70 75 80
Phe Ser Ser Leu His Val Arg Asp Thr Lys Ile Glu Val Ala Gln Phe
85 90 95
Val Lys Asp Leu Leu Leu His Leu Lys Lys Leu Phe Arg Glu Gly Arg
100 105 110
Phe Asn

<210> 31
<211> 122
<212> PRT
<213> 智人
<400> 31
Leu Thr Cys Leu Gly Gly Phe Ala Ser Pro Gly Pro Val Pro Pro Ser
1 5 10 15
Thr Ala Leu Arg Glu Leu Ile Glu Glu Leu Val Asn Ile Thr Gln Asn
20 25 30
Gln Lys Ala Pro Leu Cys Asn Gly Ser Met Val Trp Ser Ile Asn Leu
35 40 45

[0013]

Thr Ala Gly Met Tyr Cys Ala Ala Leu Glu Ser Leu Ile Asn Val Ser
50 55 60
Gly Cys Ser Ala Ile Glu Lys Thr Gln Arg Met Leu Ser Gly Phe Cys
65 70 75 80
Pro His Lys Val Ser Ala Gly Gln Phe Ser Ser Leu His Val Arg Asp
85 90 95
Thr Lys Ile Glu Val Ala Gln Phe Val Lys Asp Leu Leu Leu His Leu
100 105 110
Lys Lys Leu Phe Arg Glu Gly Gln Phe Asn
115 120

<210> 32
<211> 132
<212> PRT
<213> 食蟹猴(Macaca fascicularis)
<400> 32

Met Ala Leu Leu Leu Thr Met Val Ile Ala Leu Thr Cys Leu Gly Gly
1 5 10 15
Phe Ala Ser Pro Ser Pro Val Pro Pro Ser Thr Ala Leu Lys Glu Leu
20 25 30
Ile Glu Glu Leu Val Asn Ile Thr Gln Asn Gln Lys Ala Pro Leu Cys
35 40 45
Asn Gly Ser Met Val Trp Ser Ile Asn Leu Thr Ala Gly Val Tyr Cys
50 55 60
Ala Ala Leu Glu Ser Leu Ile Asn Val Ser Gly Cys Ser Ala Ile Glu
65 70 75 80
Lys Thr Gln Arg Met Leu Asn Gly Phe Cys Pro His Lys Val Ser Ala
85 90 95
Gly Gln Phe Ser Ser Leu Arg Val Arg Asp Thr Lys Ile Glu Val Ala
100 105 110
Gln Phe Val Lys Asp Leu Leu Val His Leu Lys Lys Leu Phe Arg Glu
115 120 125
Gly Gln Phe Asn
130

<210> 33
<211> 153
<212> PRT
<213> 食蟹猴
<400> 33

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

[0014]

Thr Lys Leu Thr Ile Thr Asp Ile Leu Ala Ala Ser Lys Asn Thr Thr
50 55 60
Glu Lys Glu Thr Phe Cys Arg Ala Ala Thr Val Leu Arg Gln Phe Tyr
65 70 75 80
Ser His His Glu Lys Asp Thr Arg Cys Leu Gly Ala Thr Ala Gln Gln
85 90 95
Phe His Arg His Lys Gln Leu Ile Arg Phe Leu Lys Arg Leu Asp Arg
100 105 110
Asn Leu Trp Gly Leu Ala Gly Leu Asn Ser Cys Pro Val Lys Glu Ala
115 120 125
Asn Gln Ser Thr Leu Glu Asn Phe Leu Glu Arg Leu Lys Thr Ile Met
130 135 140
Arg Glu Lys Tyr Ser Lys Cys Ser Ser
145 150

<210> 34
<211> 330
<212> PRT
<213> 人工序列
<220>
<223> 人工序列的描述：合成的 多肽
<400> 34
Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1 5 10 15
Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30
Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45
Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60
Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65 70 75 80
Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95
Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
100 105 110
Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
115 120 125
Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
130 135 140
Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
145 150 155 160
Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
165 170 175
Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu

[0015]

180 185 190
His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
195 200 205
Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
210 215 220
Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
225 230 235 240
Met Thr Lys Asn Gln Val Ser Leu Trp Cys Leu Val Lys Gly Phe Tyr
245 250 255
Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
260 265 270
Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
275 280 285
Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
290 295 300
Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
305 310 315 320
Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
325 330

<210> 35
<211> 330
<212> PRT
<213> 人工序列
<220>
<223> 人工序列的描述: 合成的 多肽
<400> 35
Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1 5 10 15
Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30
Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60
Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65 70 75 80
Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95
Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
100 105 110
Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
115 120 125
Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys

[0016]

130 135 140
Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
145 150 155 160
Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
165 170 175
Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
180 185 190
His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
195 200 205
Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
210 215 220
Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
225 230 235 240
Met Thr Lys Asn Gln Val Ser Leu Ser Cys Ala Val Lys Gly Phe Tyr
245 250 255
Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
260 265 270
Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
275 280 285
Leu Val Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
290 295 300
Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
305 310 315 320
Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
325 330

<210> 36
<211> 327
<212> PRT
<213> 人工序列
<220>
<223> 人工序列的描述：合成的 多肽
<400> 36
Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
1 5 10 15
Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30
Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45
Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60
Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr
65 70 75 80
Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

[0017]

Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro
100 105 110
Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
115 120 125
Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
130 135 140
Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp
145 150 155 160
Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe
165 170 175
Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
180 185 190
Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu
195 200 205
Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
210 215 220
Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys
225 230 235 240
Asn Gln Val Ser Leu Trp Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
245 250 255
Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
260 265 270

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
275 280 285
Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser
290 295 300
Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
305 310 315 320
Leu Ser Leu Ser Leu Gly Lys
325

<210> 37
<211> 327
<212> PRT
<213> 人工序列
<220>
<223> 人工序列的描述：合成的 多肽
<400> 37
Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
1 5 10 15
Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30
Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

[0018]

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60
Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr
65 70 75 80
Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95
Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro
100 105 110
Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
115 120 125
Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
130 135 140
Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp
145 150 155 160
Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe
165 170 175
Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
180 185 190
Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu
195 200 205
Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
210 215 220
Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys
225 230 235 240
Asn Gln Val Ser Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro Ser Asp
245 250 255
Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
260 265 270
Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Val Ser
275 280 285

Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser
290 295 300
Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
305 310 315 320
Leu Ser Leu Ser Leu Gly Lys
325

<210> 38
<211> 445
<212> PRT
<213> 人工序列
<220>
<223> 人工序列的描述：合成的 多肽
<400> 38

[0019]

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
20 25 30
Ser Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Val Trp Ile Asn Thr Glu Thr Gly Glu Pro Thr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Leu Asp Asn Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Gly Gly Ile Phe Tyr Gly Met Asp Tyr Trp Gly Gln Gly Thr
100 105 110
Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
115 120 125
Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly
130 135 140
Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145 150 155 160
Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
165 170 175
Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
180 185 190
Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser
195 200 205
Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys
210 215 220
Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu
225 230 235 240
Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
245 250 255
Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln
260 265 270
Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
275 280 285
Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu
290 295 300

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
305 310 315 320
Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys
325 330 335
Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser

[0020]

340 345 350

Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Ser Cys Ala Val Lys

355 360 365

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln

370 375 380

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly

385 390 395 400

Ser Phe Phe Leu Val Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln

405 410 415

Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn

420 425 430

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys

435 440 445

<210> 39

<211> 214

<212> PRT

<213> 人工序列

<220>

<223> 人工序列的描述：合成的 多肽

<400> 39

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly

1 5 10 15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Ser Val Ile Asn Asp

20 25 30

Ala Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile

35 40 45

Tyr Tyr Thr Ser His Arg Tyr Thr Gly Val Pro Ser Arg Phe Ser Gly

50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro

65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Asp Tyr Thr Ser Pro Trp

85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala

100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly

115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala

130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln

145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser

165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr

[0021]

180 185 190
Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205
Phe Asn Arg Gly Glu Cys
210
<210> 40
<211> 445
<212> PRT
<213> 人工序列
<220>
<223> 人工序列的描述：合成的 多肽
<400> 40
Glu Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1 5 10 15
Thr Leu Thr Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Ala Tyr
20 25 30
Ser Val Asn Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu Trp Leu
35 40 45
Ala Met Ile Trp Gly Asp Gly Lys Ile Val Tyr Asn Ser Ala Leu Lys
50 55 60
Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val Val Leu
65 70 75 80
Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
85 90 95
Gly Asp Gly Tyr Tyr Pro Tyr Ala Met Asp Asn Trp Gly Gln Gly Ser
100 105 110
Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
115 120 125
Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly
130 135 140
Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145 150 155 160
Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
165 170 175
Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
180 185 190
Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser
195 200 205
Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys
210 215 220
Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu
225 230 235 240
Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
245 250 255

[0022]

Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln
260 265 270
Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
275 280 285
Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu
290 295 300
Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
305 310 315 320
Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys
325 330 335
Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
340 345 350
Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Trp Cys Leu Val Lys
355 360 365
Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
370 375 380
Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
385 390 395 400
Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln
405 410 415
Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
420 425 430
His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys
435 440 445

<210> 41
<211> 218
<212> PRT
<213> 人工序列
<220>
<223> 人工序列的描述：合成的 多肽
<400> 41
Asp Ile Val Leu Thr Gln Ser Pro Asp Ser Leu Ser Val Ser Leu Gly
1 5 10 15
Glu Arg Ala Thr Ile Asn Cys Arg Ala Ser Lys Ser Val Asp Ser Tyr
20 25 30
Gly Asn Ser Phe Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
35 40 45
Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Asp
50 55 60
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
65 70 75 80
Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Asn Asn
85 90 95
Glu Asp Pro Arg Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg

[0023]

100 105 110
Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
115 120 125
Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
130 135 140
Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
145 150 155 160
Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
165 170 175
Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
180 185 190
His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
195 200 205
Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 42
<211> 8
<212> PRT
<213> 智人
<400> 42
Glu Ser Leu Ile Asn Val Ser Gly
1 5

<210> 43
<211> 12
<212> PRT
<213> 智人
<400> 43
Tyr Cys Ala Ala Leu Glu Ser Leu Ile Asn Val Ser
1 5 10

<210> 44
<211> 106
<212> PRT
<213> 人工序列
<220>
<223> 人工序列的描述: 合成的 多肽
<400> 44
Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
1 5 10 15
Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Gln Ser Val Ser Thr Ser
20 25 30
Ser Tyr Ser Tyr Met Asn Trp Tyr Gln Gln Thr Pro Gly Gln Pro Pro
35 40 45
Lys Leu Leu Ile Lys Tyr Ala Ser Asn Leu Glu Ser Gly Ile Pro Ala
50 55 60

[0024]

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
65 70 75 80
Pro Val Glu Glu Glu Asp Thr Ala Thr Tyr Tyr Cys Gln His Ser Trp
85 90 95
Glu Ile Pro Tyr Thr Phe Gly Gly Gly Thr
100 105

<210> 45
<211> 123
<212> PRT
<213> 人工序列
<220>
<223> 人工序列的描述：合成的 多肽
<400> 45
Gln Val Thr Leu Lys Glu Ser Gly Pro Gly Ile Leu Gln Pro Ser Gln
1 5 10 15
Thr Leu Ser Leu Thr Cys Ser Phe Ser Gly Phe Ser Leu Ser Thr Ser
20 25 30
Asp Met Gly Val Gly Trp Ile Arg Gln Pro Ser Gly Lys Gly Leu Glu
35 40 45
Trp Leu Ala His Ile Trp Trp Asp Asp Val Lys Arg Tyr Asn Pro Ala
50 55 60
Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Ser Ser Gln Val
65 70 75 80
Phe Leu Lys Ile Ala Ser Val Asp Thr Ala Asp Thr Ala Thr Tyr Tyr
85 90 95
Cys Ala Arg Ile Gly Thr Asn Tyr Gly Tyr Asp Gly Leu Phe Asp Tyr
100 105 110
Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser
115 120

<210> 46
<211> 218
<212> PRT
<213> 人工序列
<220>
<223> 人工序列的描述：合成的 多肽
<400> 46
Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
1 5 10 15
Glu Arg Ala Thr Ile Asn Cys Arg Ala Ser Gln Ser Val Ser Thr Ser
20 25 30
Ser Tyr Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
35 40 45
Lys Leu Leu Ile Lys Tyr Ala Ser Asn Leu Glu Ser Gly Val Pro Asp
50 55 60

[0025]

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
65 70 75 80
Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln His Ser Trp
85 90 95
Glu Ile Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
100 105 110
Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
115 120 125
Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
130 135 140
Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
145 150 155 160
Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
165 170 175
Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
180 185 190
His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
195 200 205
Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 47
<211> 453
<212> PRT
<213> 人工序列
<220>
<223> 人工序列的描述：合成的 多肽
<400> 47

Glu Val Gln Leu Val Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1 5 10 15
Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ser
20 25 30
Asp Met Gly Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
35 40 45
Trp Leu Ala His Ile Trp Trp Asp Asp Val Lys Arg Tyr Asn Pro Ala
50 55 60
Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65 70 75 80
Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
85 90 95
Cys Ala Arg Ile Gly Thr Asn Tyr Gly Tyr Asp Ala Leu Phe Asp Tyr
100 105 110
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
115 120 125
Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly

[0026]

130 135 140
Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
145 150 155 160
Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
165 170 175
Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
180 185 190
Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
195 200 205
Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
210 215 220
Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
225 230 235 240
Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
245 250 255
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
260 265 270
Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
275 280 285
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
290 295 300
Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
305 310 315 320
Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
325 330 335
Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
340 345 350
Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
355 360 365
Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
370 375 380
Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
385 390 395 400
Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
405 410 415
Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
420 425 430
Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
435 440 445
Leu Ser Pro Gly Lys
450
<210> 48
<211> 111
<212> PRT

[0027]

<213> 人工序列
<220>
<223> 人工序列的描述：合成的 多肽
<400> 48

Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
1 5 10 15
Glu Arg Ala Thr Ile Asn Cys Arg Ala Ser Gln Ser Val Ser Thr Ser
20 25 30
Ser Tyr Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
35 40 45
Lys Leu Leu Ile Lys Tyr Ala Ser Asn Leu Glu Ser Gly Val Pro Asp
50 55 60
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
65 70 75 80
Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln His Ser Trp
85 90 95
Glu Ile Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105 110

<210> 49
<211> 123
<212> PRT
<213> 人工序列
<220>
<223> 人工序列的描述：合成的 多肽
<400> 49

Glu Val Gln Leu Val Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1 5 10 15
Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ser
20 25 30
Asp Met Gly Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
35 40 45
Trp Leu Ala His Ile Trp Trp Asp Asp Val Lys Arg Tyr Asn Pro Ala
50 55 60
Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65 70 75 80
Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
85 90 95
Cys Ala Arg Ile Gly Thr Asn Tyr Gly Tyr Asp Ala Leu Phe Asp Tyr
100 105 110
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 50
<211> 12
<212> PRT

[0028]

<213> 人工序列
<220>
<223> 人工序列的描述：合成的 肽
<400> 50
Gly Phe Ser Leu Ser Thr Ser Asp Met Gly Val Gly
1 5 10
<210> 51
<211> 17
<212> PRT
<213> 人工序列
<220>
<223> 人工序列的描述：合成的 肽
<400> 51
Ala His Ile Trp Trp Asp Asp Val Lys Arg Tyr Asn Pro Ala Leu Lys
1 5 10 15
Ser

<210> 52
<211> 15
<212> PRT
<213> 人工序列
<220>
<223> 人工序列的描述：合成的 肽
<400> 52
Ala Arg Ile Gly Thr Asn Tyr Gly Tyr Asp Ala Leu Phe Asp Tyr
1 5 10 15
<210> 53
<211> 16
<212> PRT
<213> 人工序列
<220>
<223> 人工序列的描述：合成的 肽
<400> 53
Arg Ala Ser Gln Ser Val Ser Thr Ser Ser Tyr Ser Tyr Met Asn
1 5 10 15
<210> 54
<211> 7
<212> PRT
<213> 人工序列
<220>
<223> 人工序列的描述：合成的 肽
<400> 54
Tyr Ala Ser Asn Leu Glu Ser
1 5

[0029]

<210> 55
<211> 9
<212> PRT
<213> 人工序列
<220>
<223> 人工序列的描述：合成的 肽
<400> 55
Gln His Ser Trp Glu Ile Pro Tyr Thr
1 5
<210> 56
<211> 118
<212> PRT
<213> 人工序列
<220>
<223> 人工序列的描述：合成的 多肽
<400> 56
Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1 5 10 15
Thr Leu Thr Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Ala Tyr
20 25 30
Ser Val Asn Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu Trp Leu
35 40 45
Ala Met Ile Trp Gly Asp Gly Lys Ile Val Tyr Asn Ser Ala Leu Lys
50 55 60
Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val Val Leu
65 70 75 80
Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
85 90 95
Gly Asp Gly Tyr Tyr Pro Tyr Ala Met Asp Asn Trp Gly Gln Gly Ser
100 105 110
Leu Val Thr Val Ser Ser
115
<210> 57
<211> 112
<212> PRT
<213> 人工序列
<220>
<223> 人工序列的描述：合成的 多肽
<400> 57
Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ser Val Ser Leu Gly
1 5 10 15
Glu Arg Ala Thr Ile Asn Cys Arg Ala Ser Lys Ser Val Asp Ser Tyr
20 25 30
Gly Asn Ser Phe Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro

[0030]

35 40 45
Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Asp
50 55 60
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
65 70 75 80
Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Asn Asn
85 90 95
Glu Asp Pro Arg Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
100 105 110

<210> 58
<211> 445
<212> PRT
<213> 人工序列
<220>
<223> 人工序列的描述：合成的 多肽
<400> 58
Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1 5 10 15
Thr Leu Thr Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Ala Tyr
20 25 30
Ser Val Asn Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu Trp Leu
35 40 45
Ala Met Ile Trp Gly Asp Gly Lys Ile Val Tyr Asn Ser Ala Leu Lys
50 55 60
Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val Val Leu
65 70 75 80
Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
85 90 95
Gly Asp Gly Tyr Tyr Pro Tyr Ala Met Asp Asn Trp Gly Gln Gly Ser
100 105 110
Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
115 120 125
Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly
130 135 140
Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145 150 155 160
Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
165 170 175
Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
180 185 190
Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser
195 200 205
Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys
210 215 220

[0031]

Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu
225 230 235 240
Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
245 250 255
Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln
260 265 270
Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
275 280 285
Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu
290 295 300
Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
305 310 315 320
Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys
325 330 335
Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
340 345 350
Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Trp Cys Leu Val Lys
355 360 365
Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
370 375 380
Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
385 390 395 400
Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln
405 410 415
Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
420 425 430
His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys
435 440 445

<210> 59
<211> 218
<212> PRT
<213> 人工序列
<220>
<223> 人工序列的描述：合成的 多肽
<400> 59
Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ser Val Ser Leu Gly
1 5 10 15
Glu Arg Ala Thr Ile Asn Cys Arg Ala Ser Lys Ser Val Asp Ser Tyr
20 25 30
Gly Asn Ser Phe Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
35 40 45
Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Asp
50 55 60
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser

[0032]

65 70 75 80
Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Asn Asn
85 90 95
Glu Asp Pro Arg Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
100 105 110
Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
115 120 125
Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
130 135 140
Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
145 150 155 160
Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
165 170 175
Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
180 185 190
His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
195 200 205
Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 60
<211> 5
<212> PRT
<213> 人工序列
<220>
<223> 人工序列的描述：合成的 肽
<400> 60
Ala Tyr Ser Val Asn
1 5

<210> 61
<211> 107
<212> PRT
<213> 人工序列
<220>
<223> 人工序列的描述：合成的 多肽
<400> 61
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Tyr
20 25 30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro

[0033]

65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Ser Tyr Pro Thr
85 90 95
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
100 105
<210> 62
<211> 107
<212> PRT
<213> 人工序列
<220>
<223> 人工序列的描述：合成的 多肽
<400> 62
Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
20 25 30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35 40 45
Tyr Gly Ala Ser Thr Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65 70 75 80
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Asn Asn Trp Pro Thr
85 90 95
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
100 105
<210> 63
<211> 109
<212> PRT
<213> 人工序列
<220>
<223> 人工序列的描述：合成的 多肽
<400> 63
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30
Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45
Gly Trp Ile Asn Pro Gly Asn Gly Asn Thr Asn Tyr Ala Gln Lys Phe
50 55 60
Gln Gly Arg Val Thr Ile Thr Arg Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys

[0034]

85 90 95
Ala Arg Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
100 105
<210> 64
<211> 110
<212> PRT
<213> 人工序列
<220>
<223> 人工序列的描述：合成的 多肽
<400> 64
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ser Val Ile Ser Ser Lys Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Gly Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
100 105 110

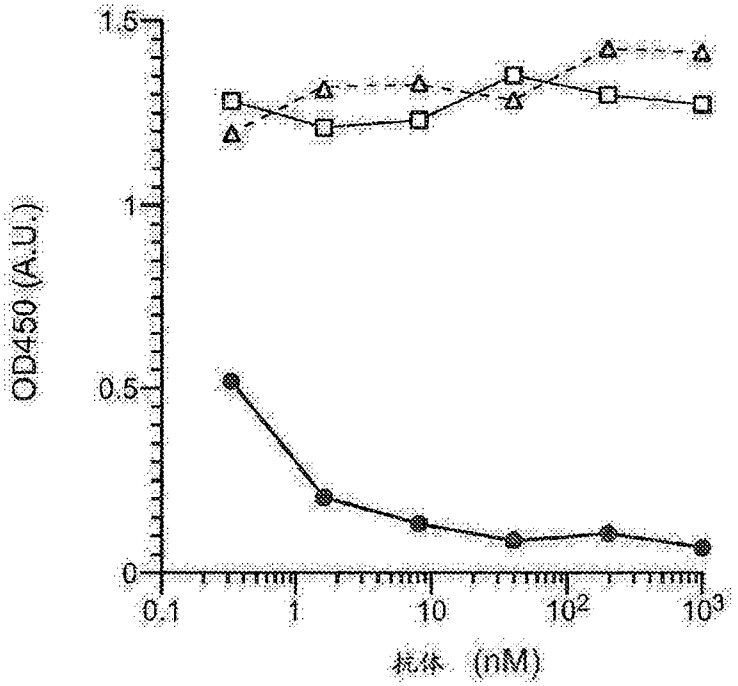
1.5
1
0.5
0
OD450 (A.U.)
0.1
1
10
10^2
10^3
抗体 (nM)

图 1A

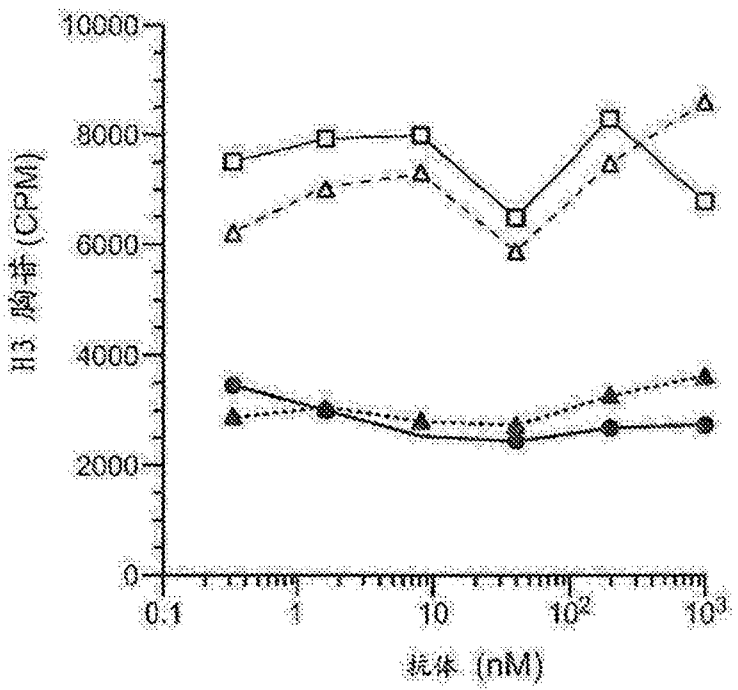
10000
8000
6000
4000
2000
0
H3 胸苷 (CPM)
0.1
1
10
10^2
10^3
抗体 (nM)

图 1B

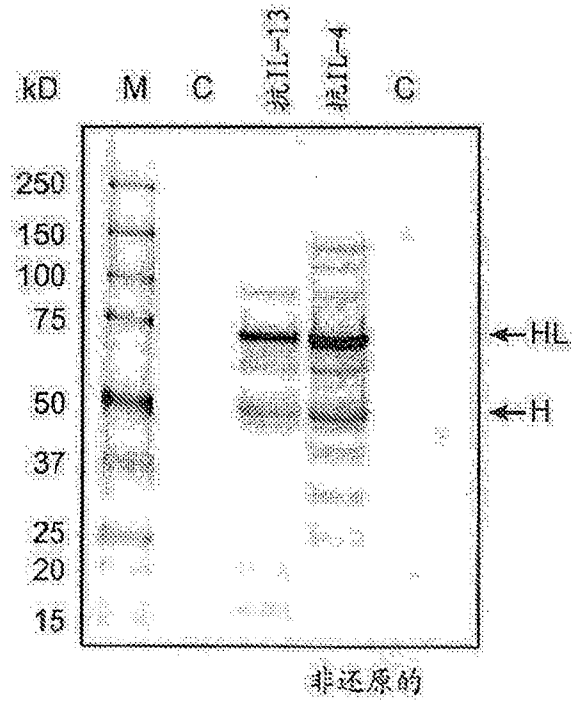

图 2A

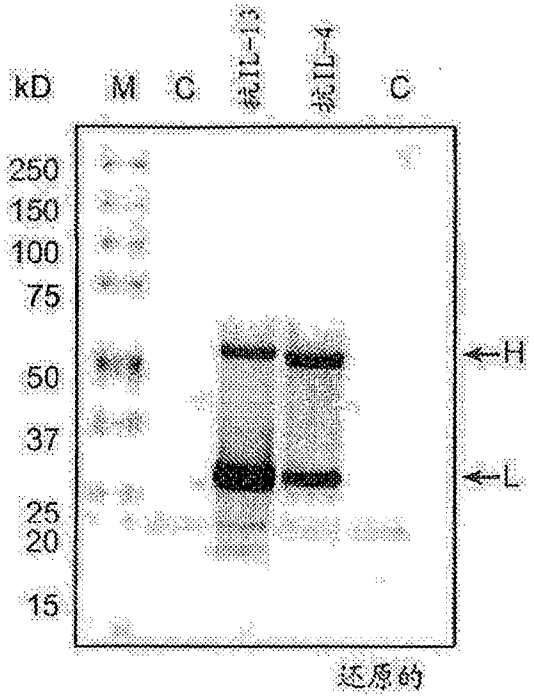

图 2B

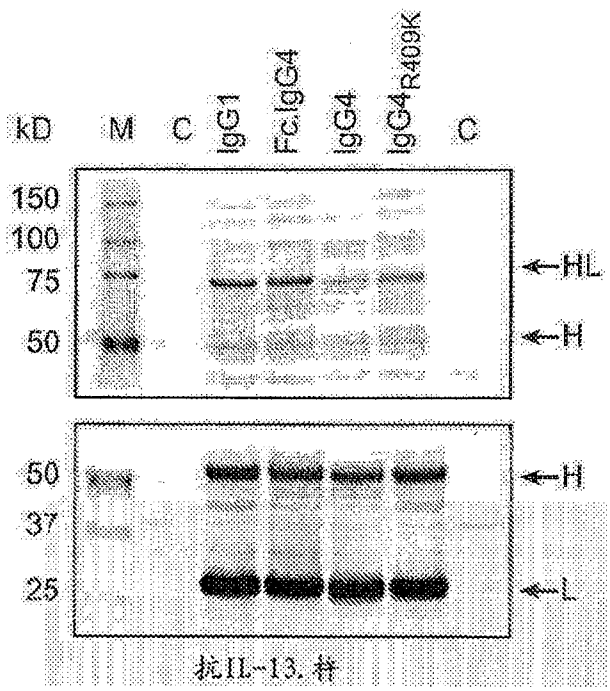

图 2C

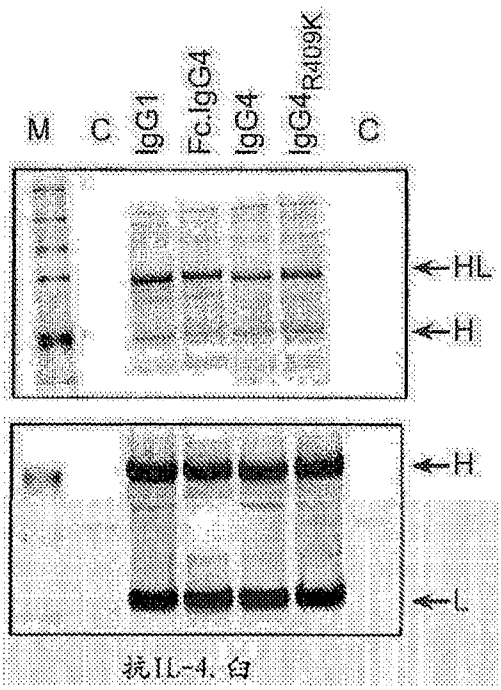

图 2D

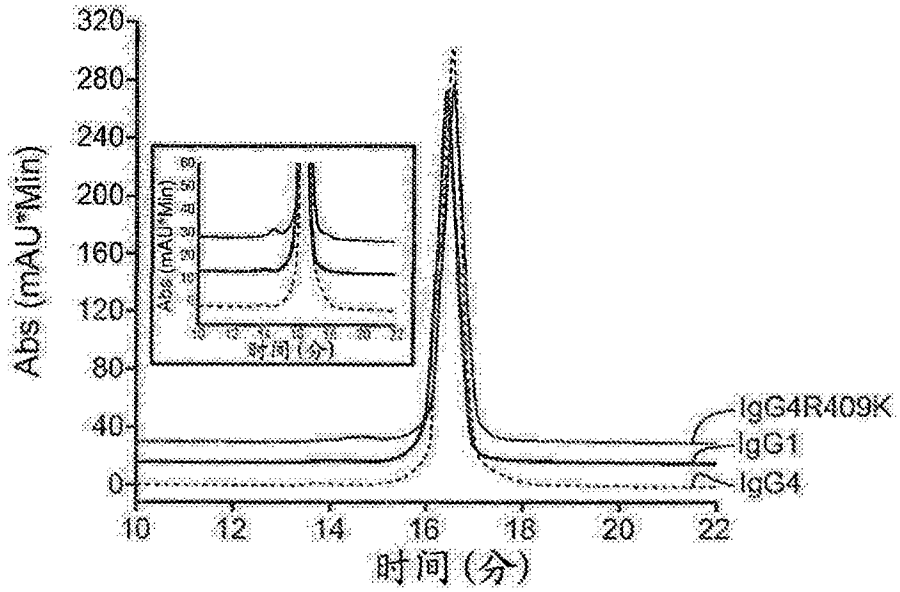
Abs (mAU*Min)
320
280
240
200
160
120
80
40
0
10
12
14
16
18
20
22
时间 (分)
IgG4R409K
IgG1
IgG4

图 3A

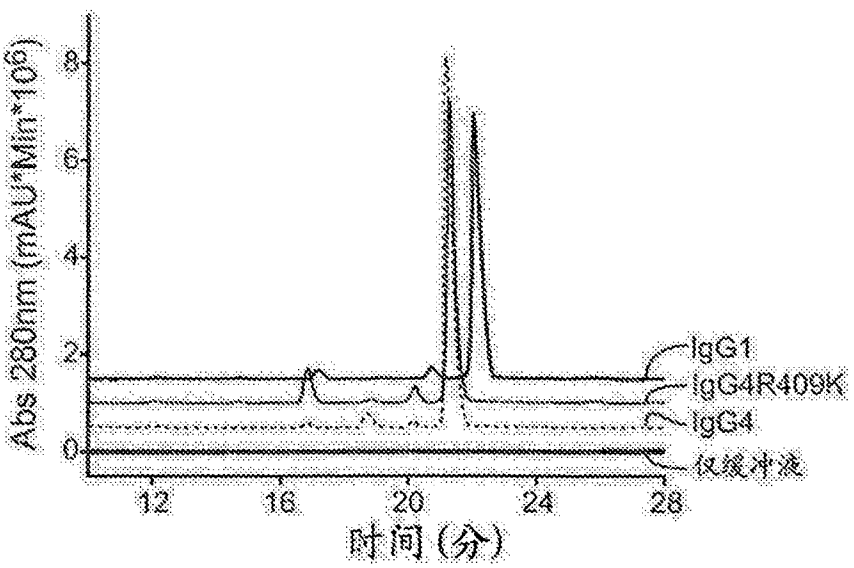
Abs 280nm (mAU*Min*10⁶)
8
6
4
2
0
12
16
20
24
28
时间 (分)
IgG1
IgG4R409K
IgG4
仅缓冲液

图 3B

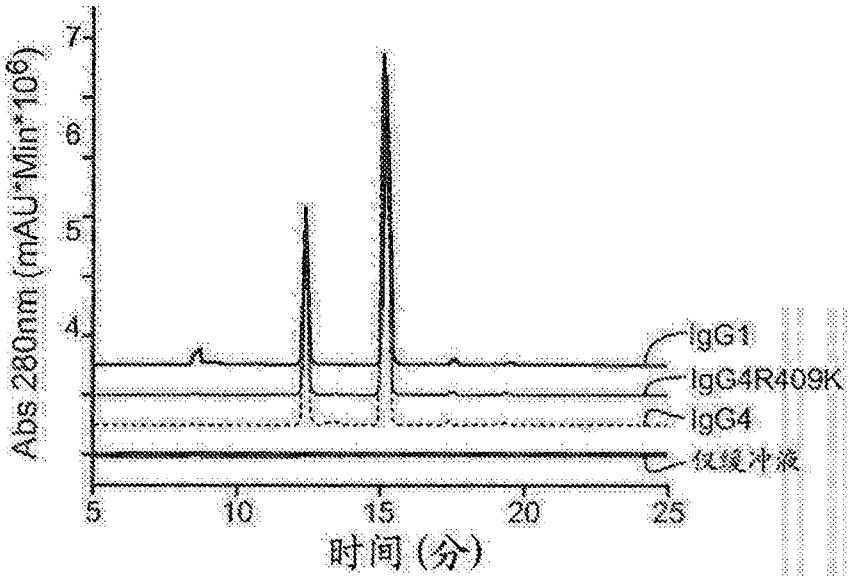
Abs 280nm (mAU*Min*10^6)
7
6
5
4
5
10
15
20
25
时间 (分)
IgG1
IgG4R409K
IgG4
仅缓冲液

图 3C

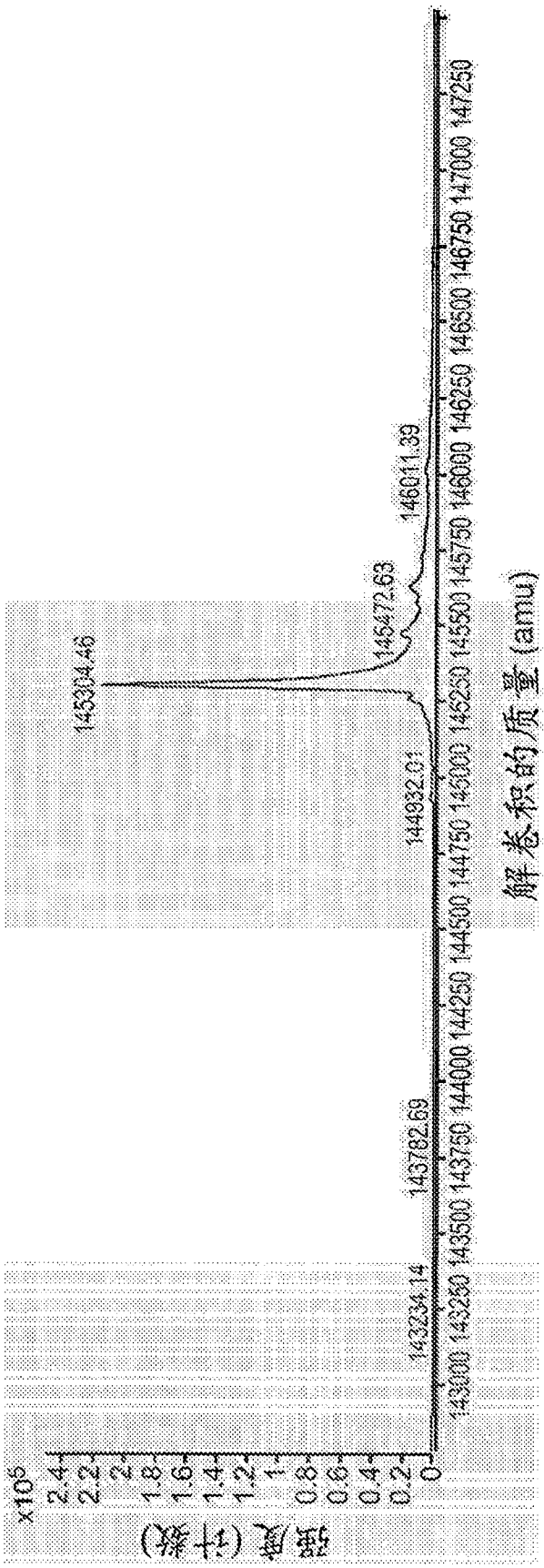
强度 (计数)
x10^5
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
143234.14
143782.69
144032.01
145314.46
145472.63
146011.39
解卷积的质量 (amu)

图 4A

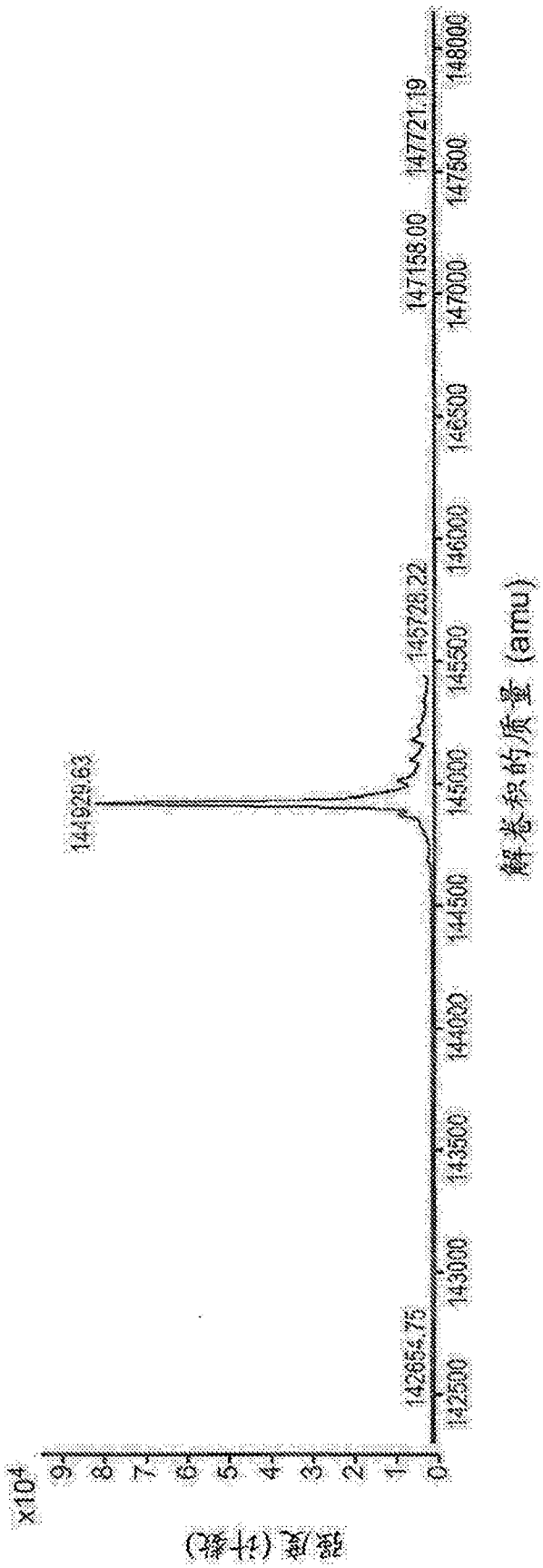
强度 (计数)
x10^4
9
8
7
6
5
4
3
2
1
0
142500
143000
143500
144000
144500
145000
145500
146000
146500
147000
147500
148000
142654.75
144929.63
145728.22
147158.00
147721.19
解卷积的质量 (amu)

图 4B

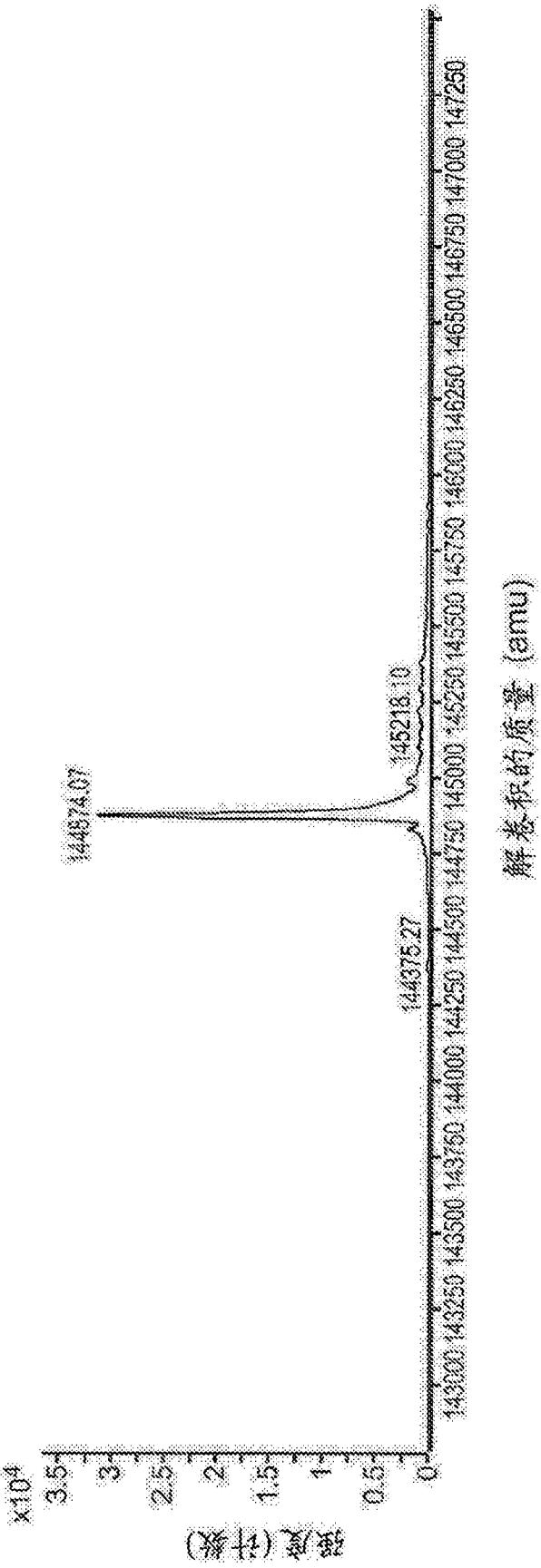
强度 (计数)
x10^4
3.5
3
2.5
2
1.5
1
0.5
0
143000 143250 143500 143750 144000 144250 144500 144750 145000 145250 145500 145750 146000 146250 146500 146750 147000 147250
144374.27
144874.07
145218.10
解卷积的质量 (amu)

图 4C

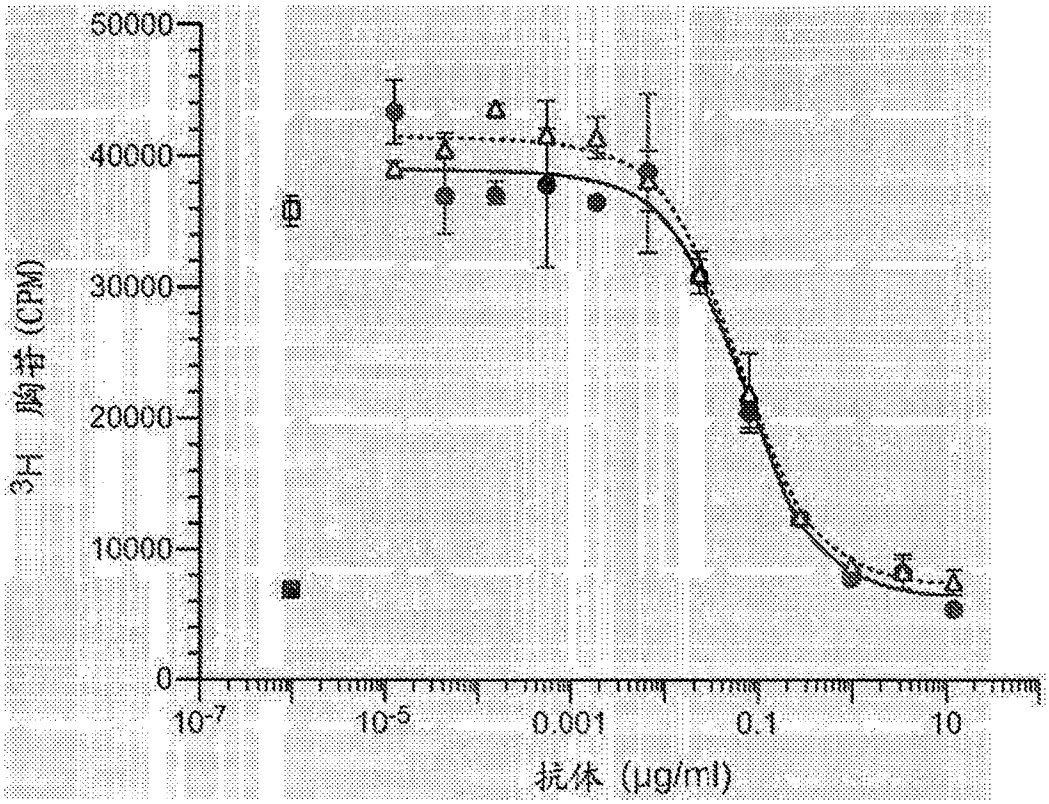
50000
40000
30000
20000
10000
0
^{3}H-胸腺嘧啶 (CPM)
10^{-7}
10^{-5}
0.001
0.1
10
抗体 (μg/ml)

图 5A

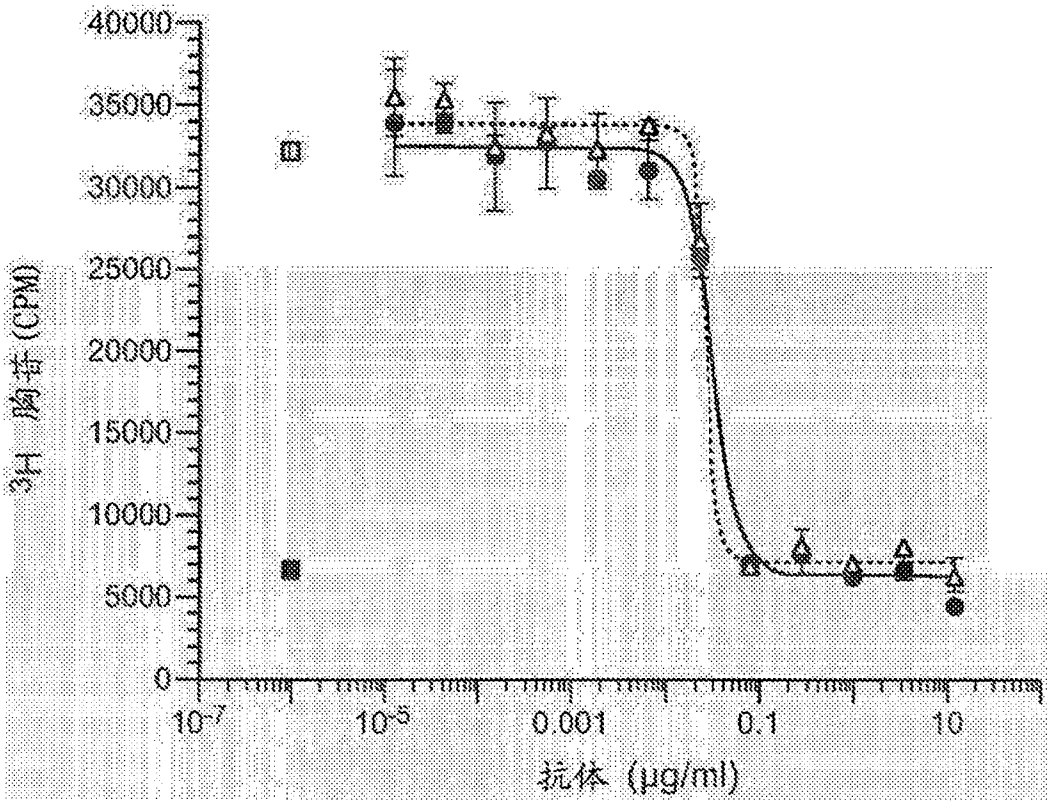
40000
35000
30000
25000
20000
15000
10000
5000
0
^{3}H 胸苷 (CPM)
10^{-7}
10^{-5}
0.001
0.1
10
抗体 (μg/ml)

图 5B

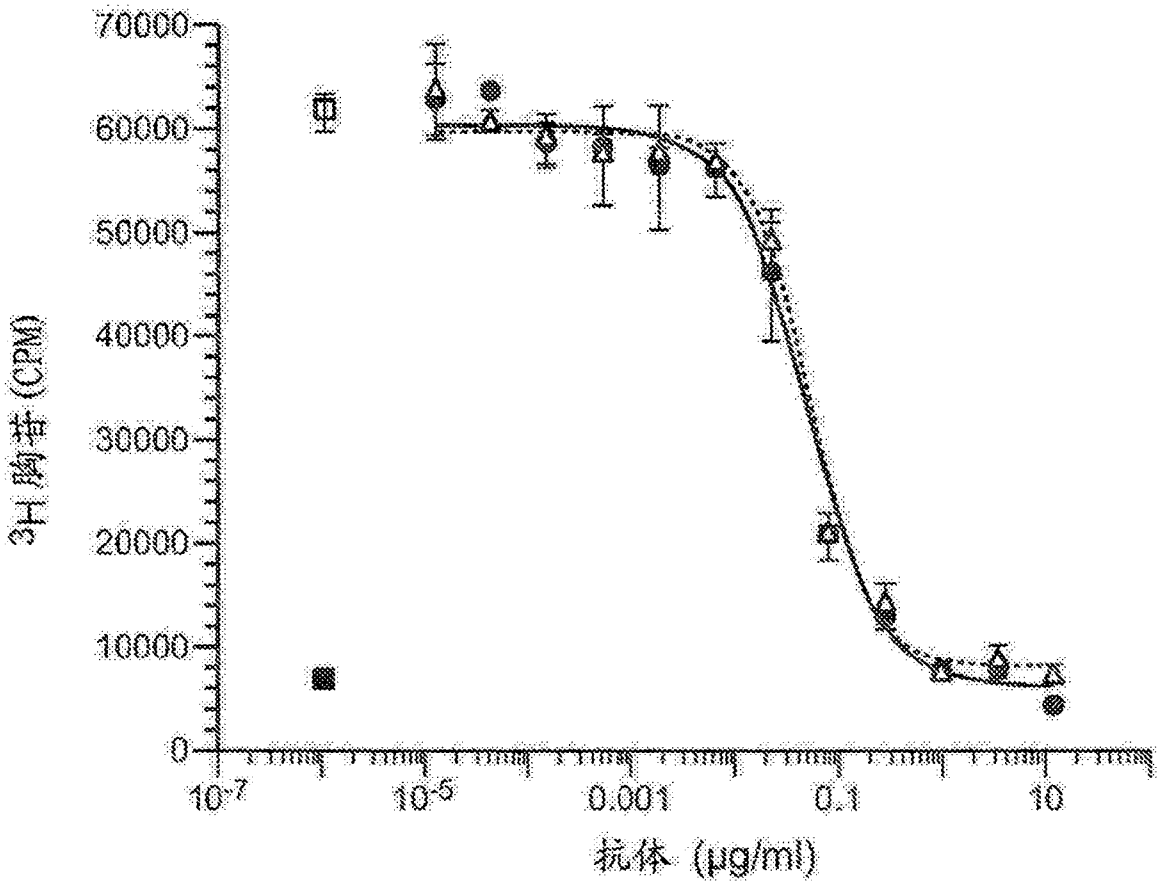
70000
60000
50000
40000
30000
20000
10000
0
3H 胸苷 (CPM)
10-7
10-5
0.001
0.1
10
抗体 (μg/ml)

图 5C

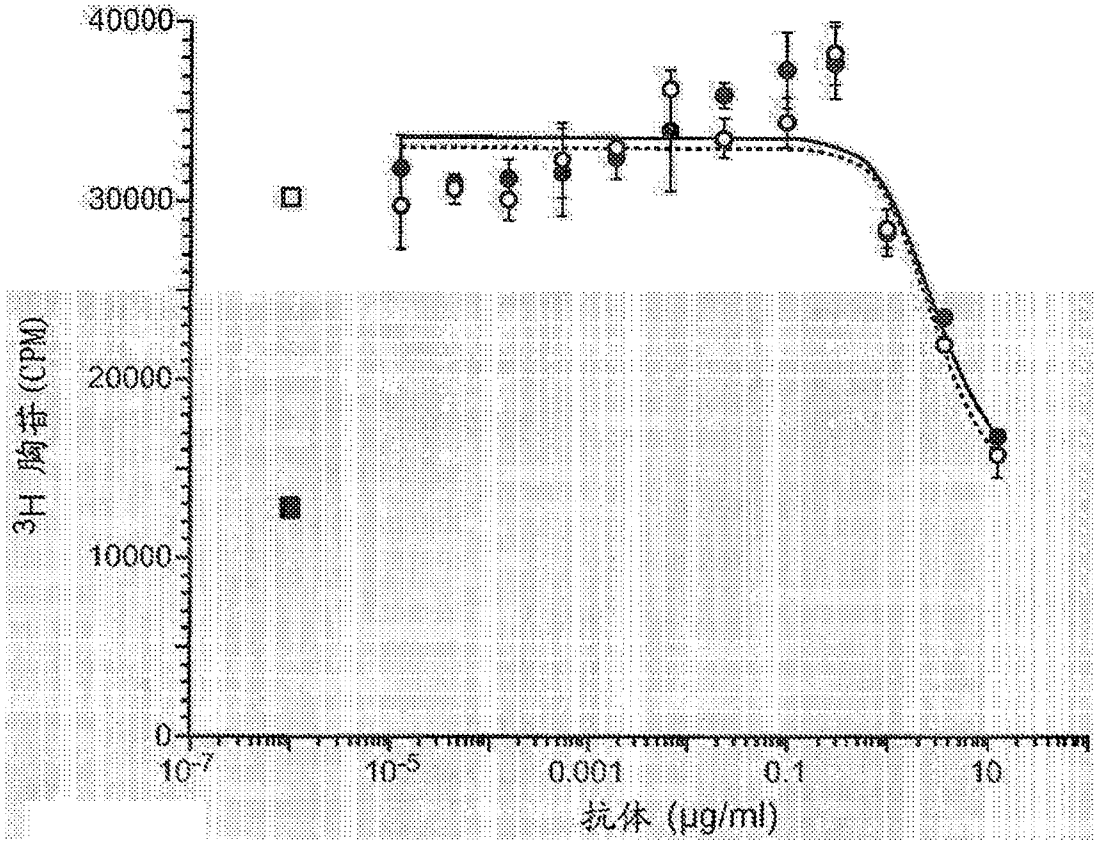

图 6A

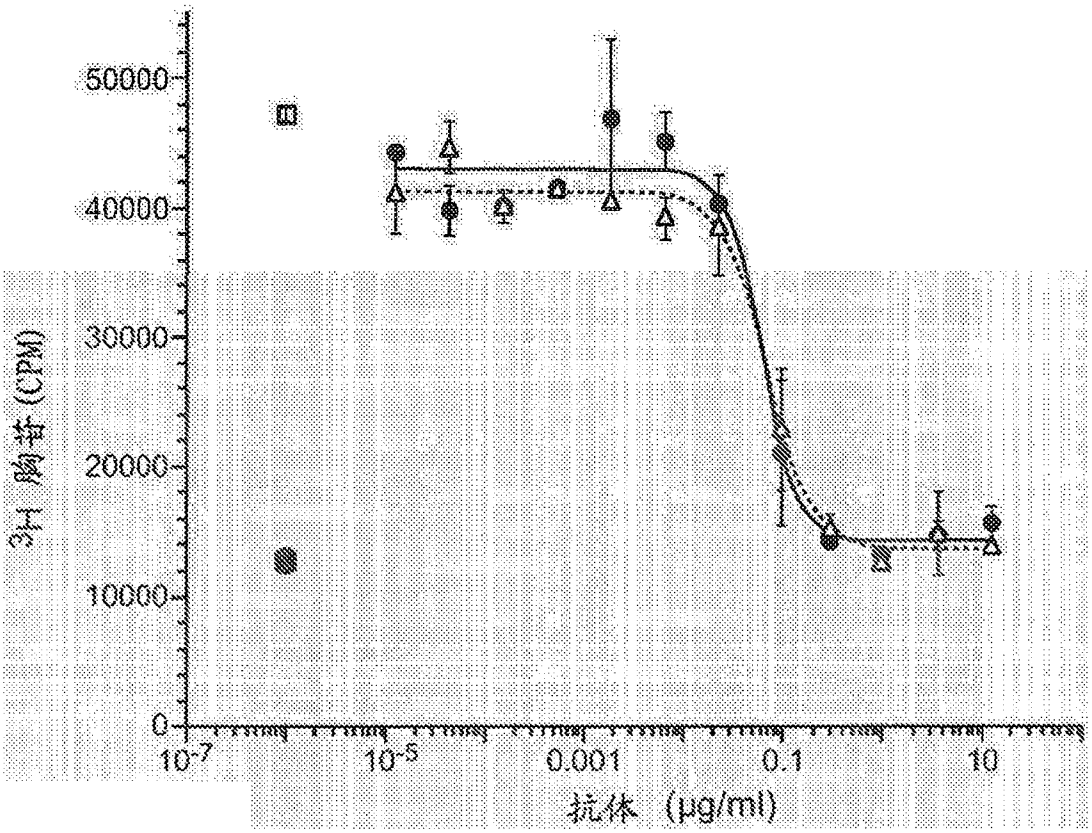
50000
40000
30000
20000
10000
0
^{3}H-胸苷 (CPM)
10^{-7}
10^{-5}
0.001
0.1
10
抗体 (μg/ml)

图 6B

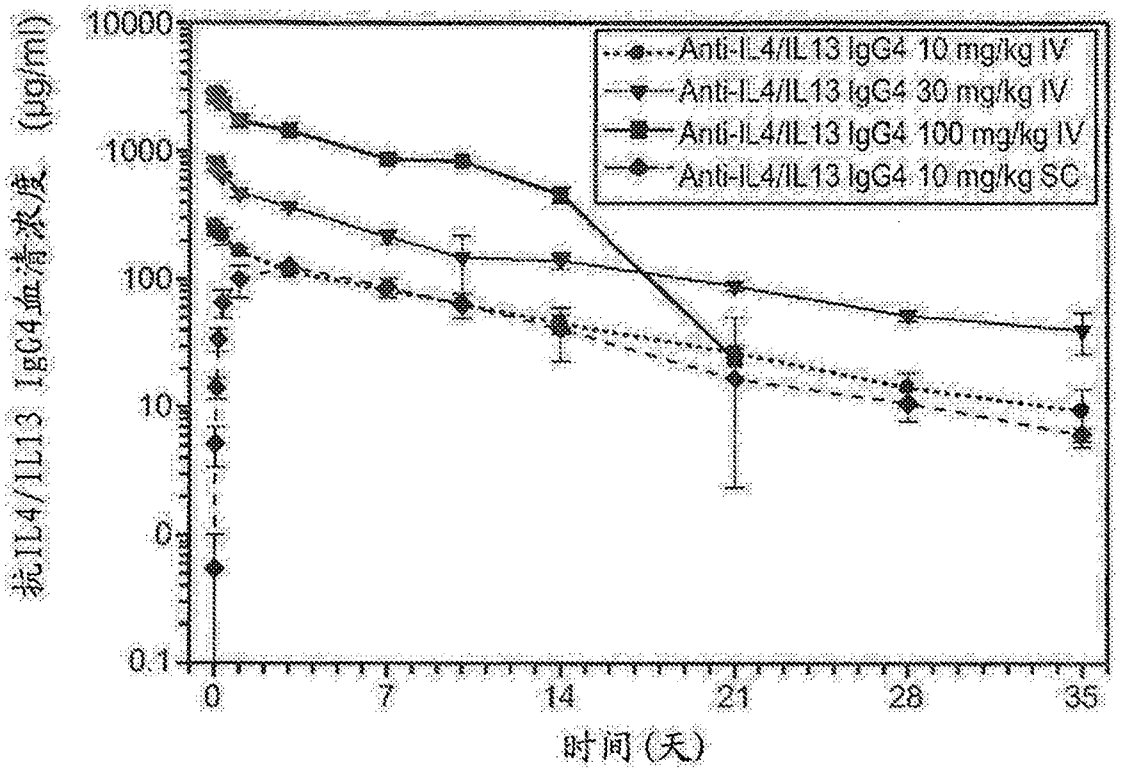
抗IL4/IL13 IgG4血清浓度 (μg/ml)
10000
1000
100
10
0
0.1
0
7
14
21
28
35
时间(天)
Anti-IL4/IL13 IgG4 10 mg/kg IV
Anti-IL4/IL13 IgG4 30 mg/kg IV
Anti-IL4/IL13 IgG4 100 mg/kg IV
Anti-IL4/IL13 IgG4 10 mg/kg SC

图 7A

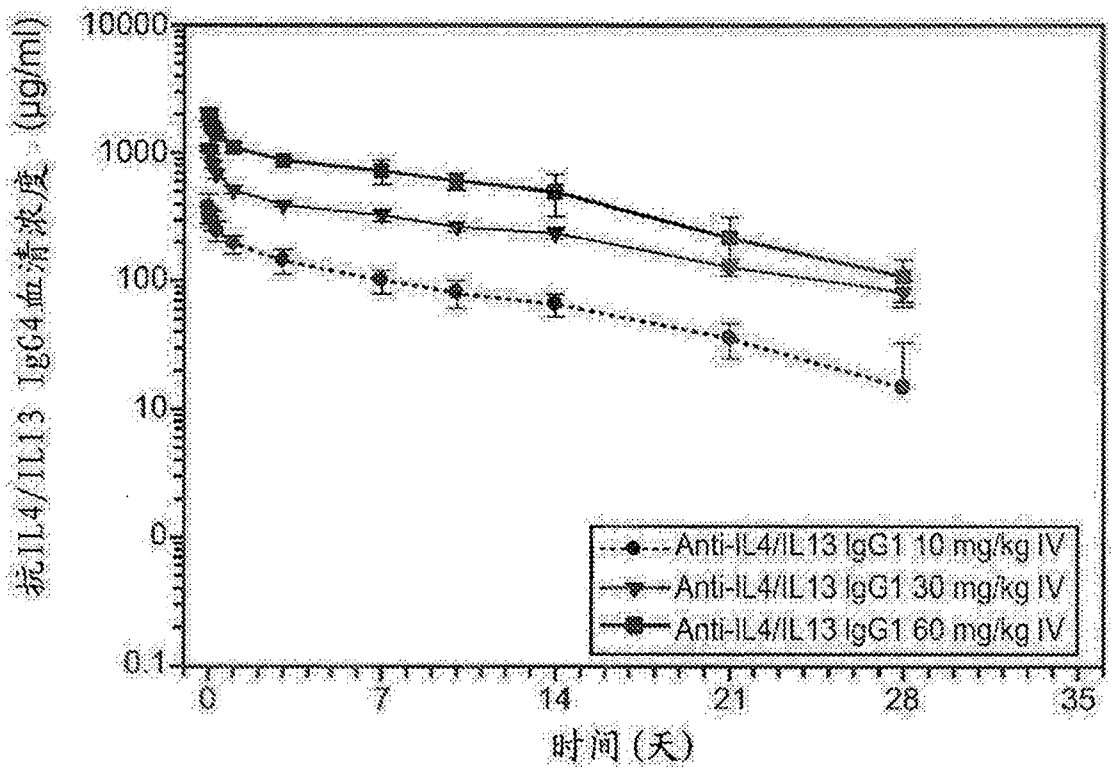
抗IL4/IL13 IgG4血清浓度 (μg/ml)
10000
1000
100
10
0
0.1
0
7
14
21
28
35
时间(天)
Anti-IL4/IL13 IgG1 10 mg/kg IV
Anti-IL4/IL13 IgG1 30 mg/kg IV
Anti-IL4/IL13 IgG1 60 mg/kg IV

图 7B

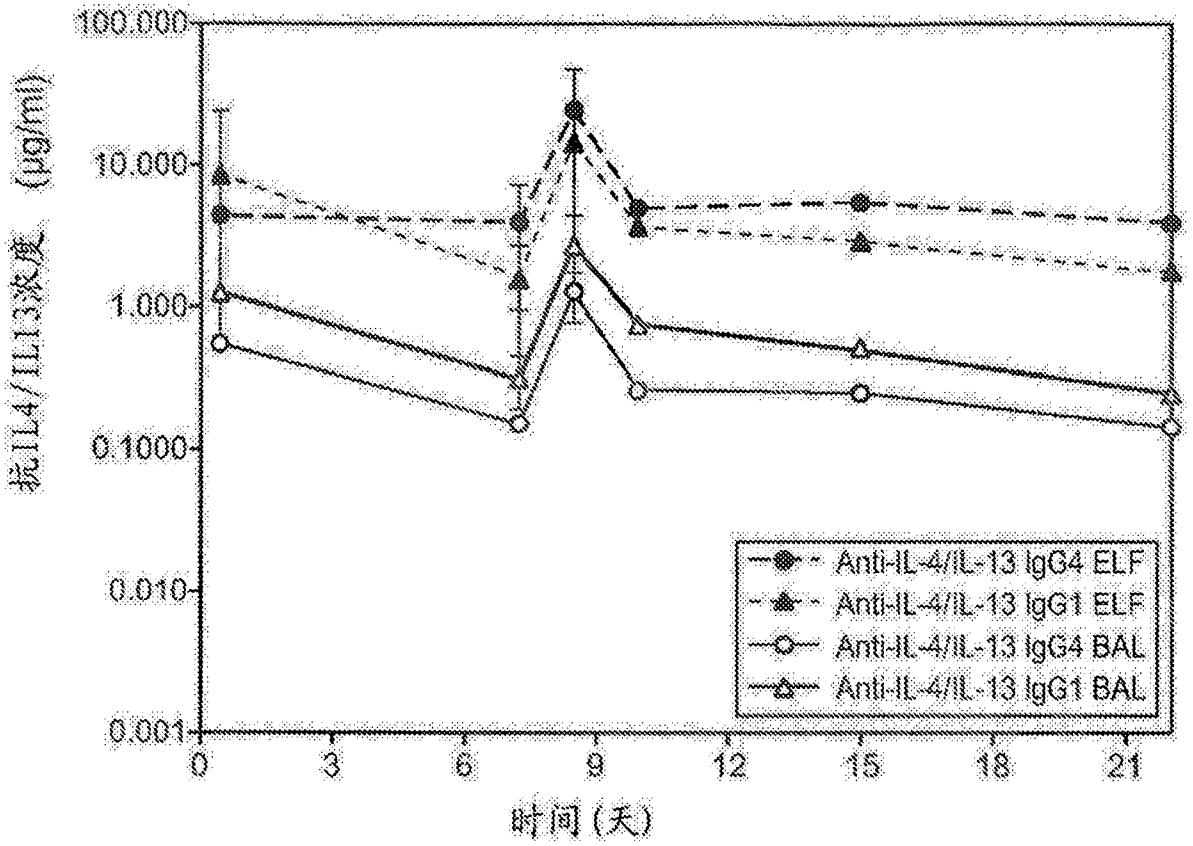
100.000
10.000
1.000
0.1000
0.010
0.001
抗IL4/IL13浓度 (μg/ml)
0
3
6
9
12
15
18
21
时间(天)
Anti-IL-4/IL-13 IgG4 ELF
Anti-IL-4/IL-13 IgG1 ELF
Anti-IL-4/IL-13 IgG4 BAL
Anti-IL-4/IL-13 IgG1 BAL

图 8

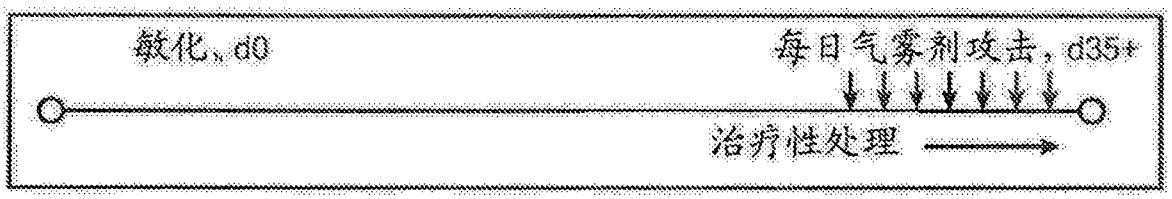
敏化、d0
每日气雾剂攻击，d35+
治疗性处理

图 9A

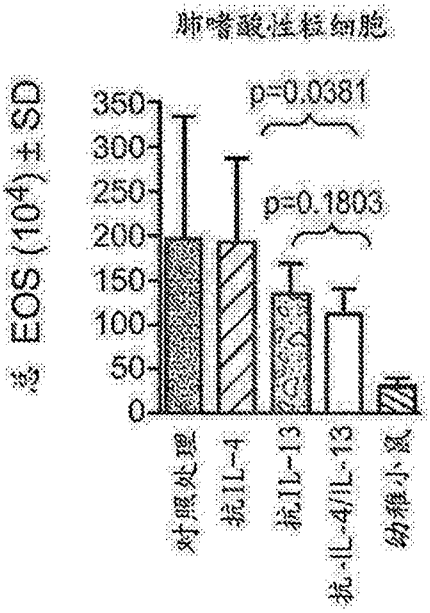
肺嗜酸性粒细胞
总 EOS (10^4) ± SD
350
300
250
200
150
100
50
0
p=0.0381
p=0.1803
对照处理
抗IL-4
抗IL-13
抗-IL-4/IL-13
幼稚小鼠

图 9B

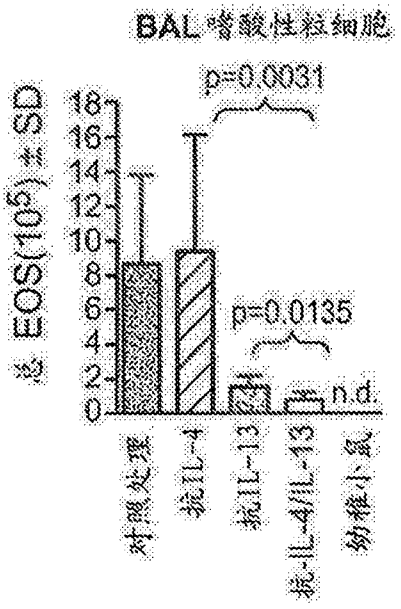
BAL 嗜酸性粒细胞
总 EOS(10^5) ± SD
18
16
14
12
10
8
6
4
2
0
p=0.0031
p=0.0135
n.d.
对照处理
抗IL-4
抗IL-13
抗-IL-4/IL-13
幼稚小鼠

图 9C

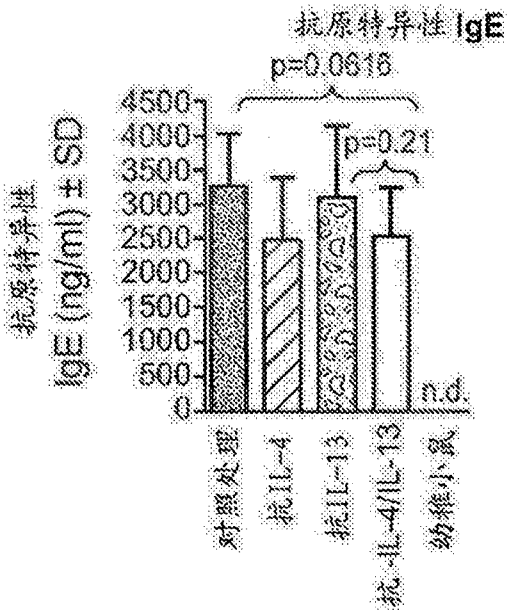
抗原特异性 IgE
抗原特异性 IgE (ng/ml) ± SD
4500
4000
3500
3000
2500
2000
1500
1000
500
0
p=0.0616
p=0.21
n.d.
对照处理
抗IL-4
抗IL-13
抗-IL-4/IL-13
幼稚小鼠

图 9D

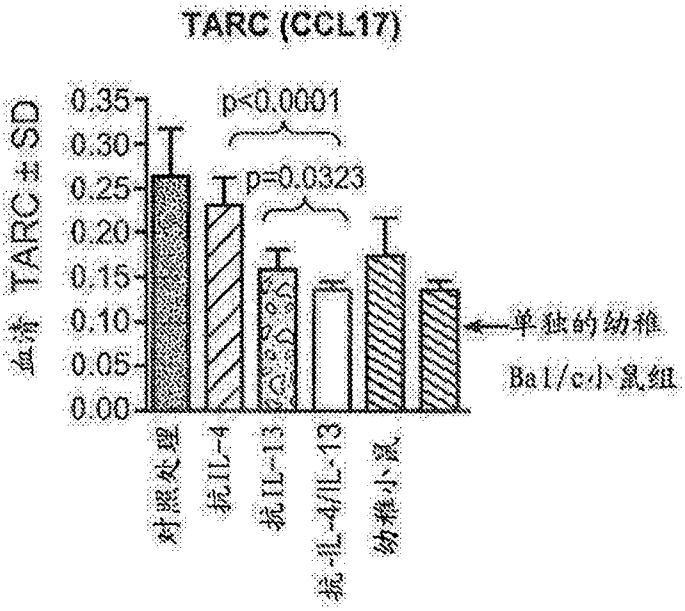
TARC (CCL17)
血清 TARC ± SD
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
p<0.0001
p=0.0323
p<0.0001
单独的幼稚
Bal/c小鼠组
对照处理
抗IL-4
抗IL-13
抗-IL-4/IL-13
幼稚小鼠

图 9E

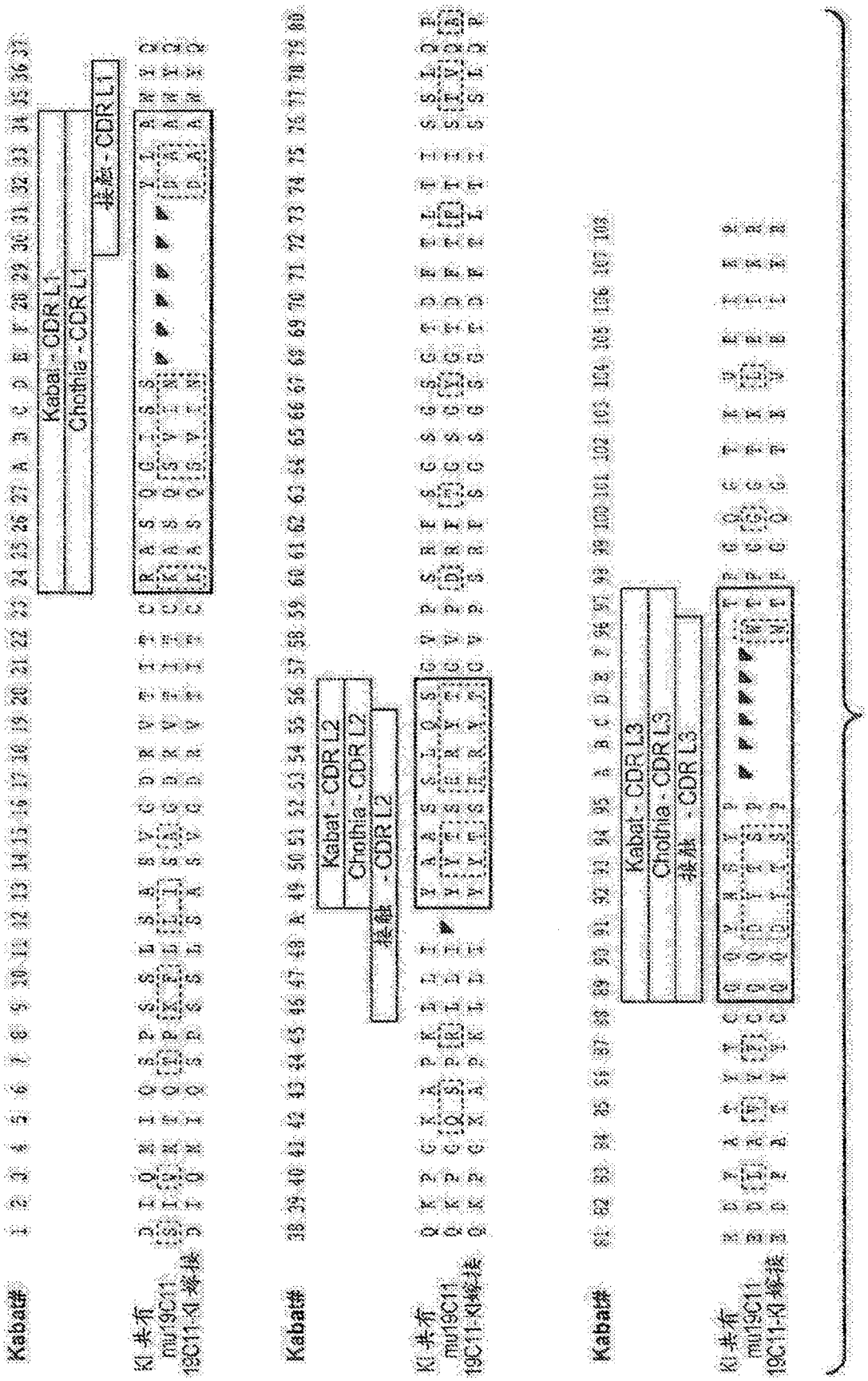
Kabat - CDR L1
Chothia - CDR L1
接触 - CDR L1
Kabat - CDR L2
Chothia - CDR L2
接触 - CDR L2
Kabat - CDR L3
Chothia - CDR L3
接触 - CDR L3
Kabat#
KI 共有
mu19C11
19C11-KI嵌接

图 10

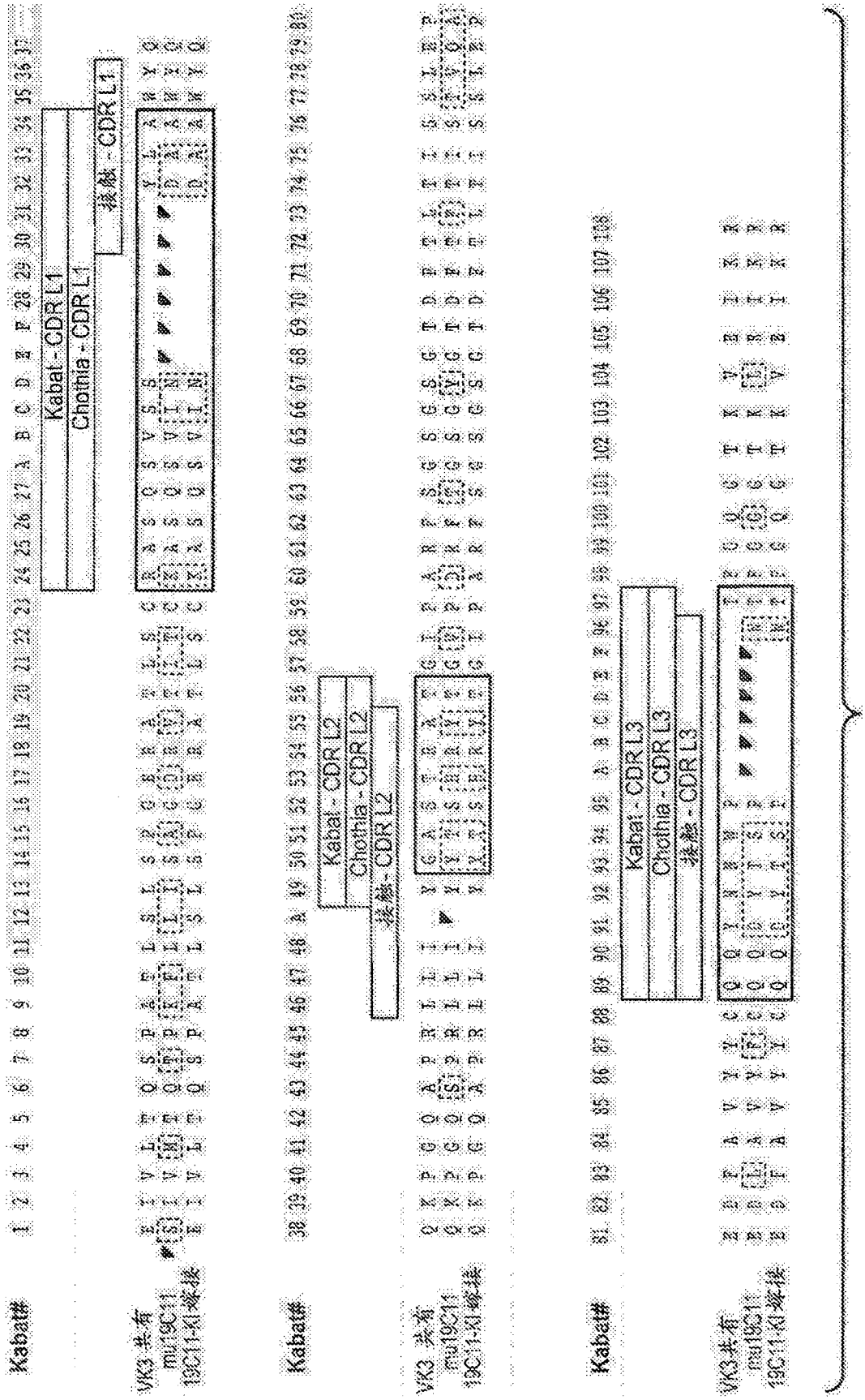
Kabat - CDR L1
Chothia - CDR L1
延森 - CDR L1
Kabat - CDR L2
Chothia - CDR L2
延森 - CDR L2
Kabat - CDR L3
Chothia - CDR L3
延森 - CDR L3

图 11

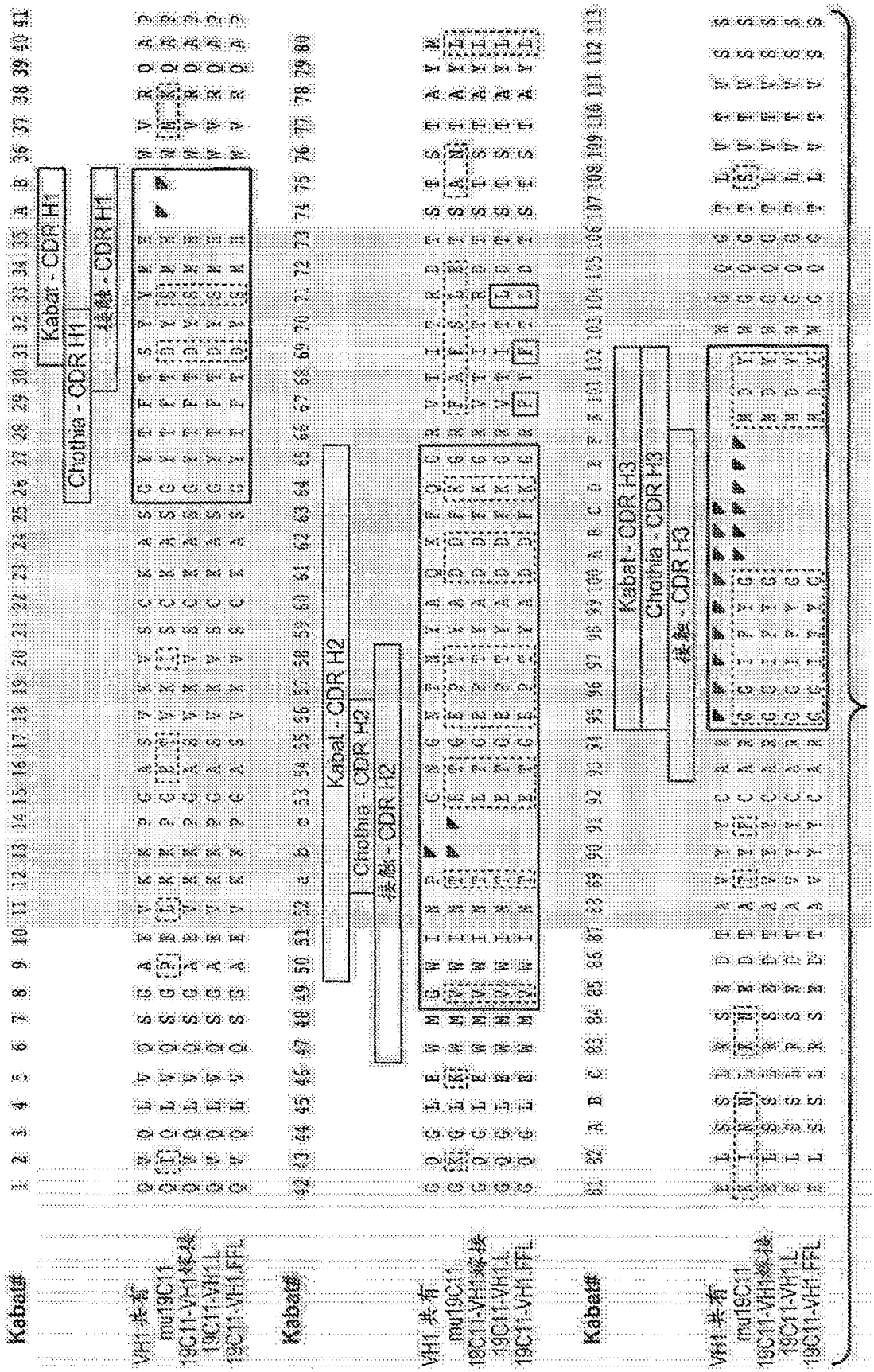
Kabat - CDR H1
Chothia - CDR H1
接触 - CDR H1
Kabat - CDR H2
Chothia - CDR H2
接触 - CDR H2
Kabat - CDR H3
Chothia - CDR H3
接触 - CDR H3
VH1 共有
mu19C11
19C11-VH1嵌合
19C11-VH1.L
19C11-VH1.FFL

图 12

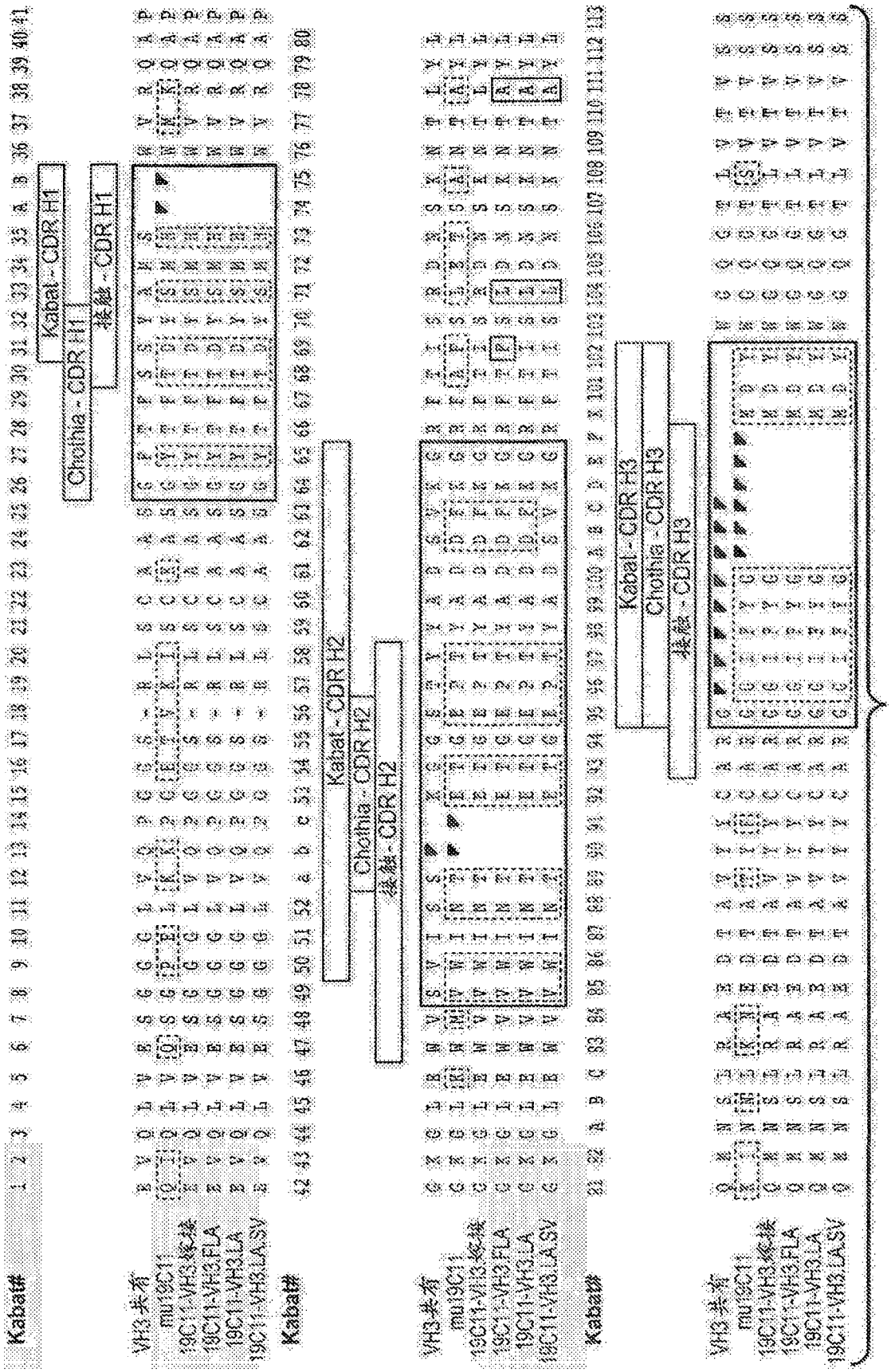
Kabat - CDR H1
Chothia - CDR H1
延龄 - CDR H1
Kabat - CDR H2
Chothia - CDR H2
延龄 - CDR H2
Kabat - CDR H3
Chothia - CDR H3
延龄 - CDR H3
VH3 共有
mu19C11
19C11-VH3.延龄
19C11-VH3.FLA
19C11-VH3.LA
19C11-VH3.LA.SV
Kabat#

图 13

	HuKI Kd (pM)	HuKIII Kd (pM)
$HuVH_I$	24	<10
$HuVH_I$ (L)	<10	<10
$HuVH_I$ (FFL)	<10	11
$HuVH_{III}$	84	26
$HuVH_{III}$ (LA)	<10	<10
$HuVH_{III}$ (FLA)	140	11
19C11 嵌合体	<10	

图 14

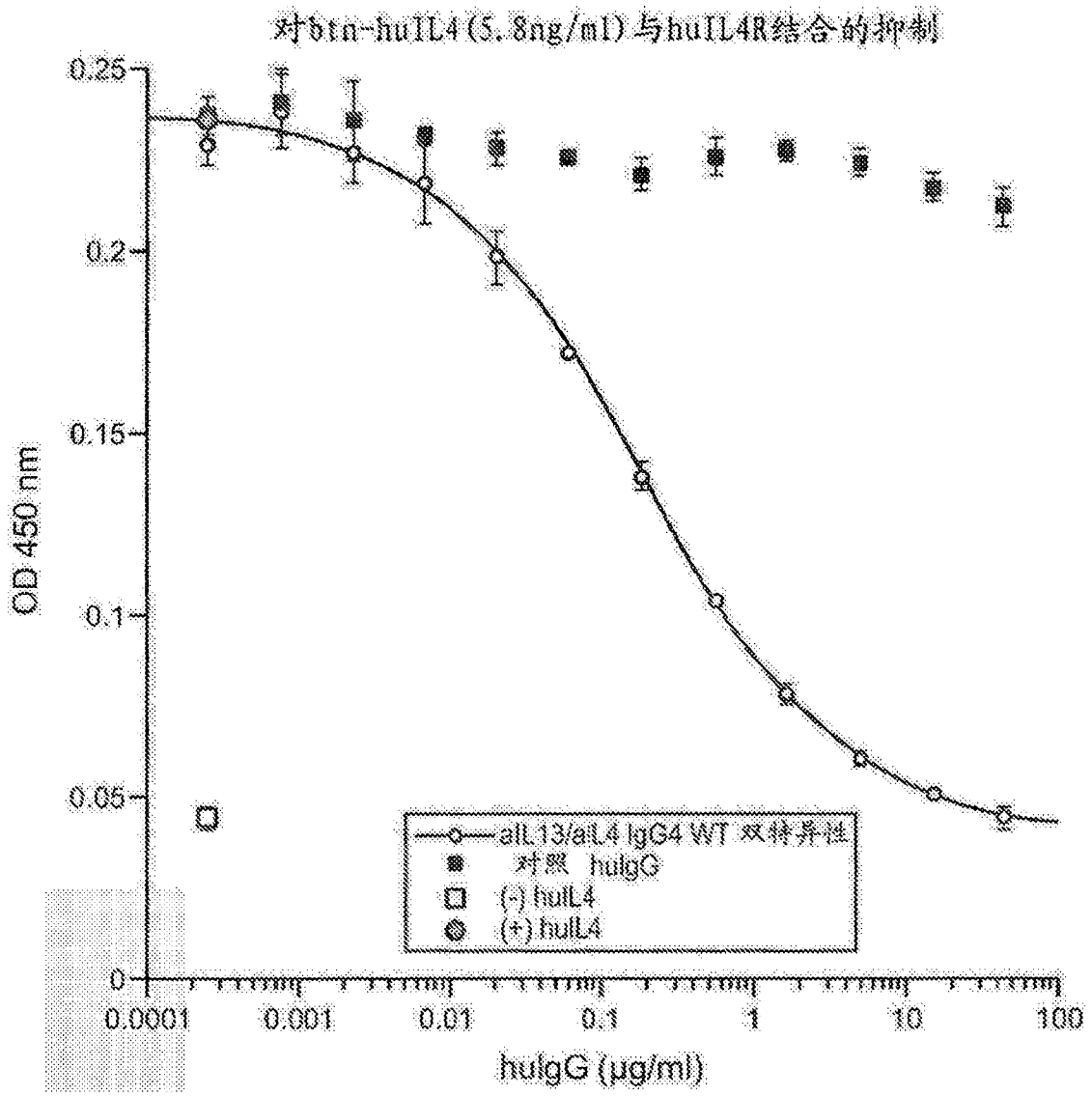
对btn-huIL4(5.8ng/ml)与huIL4R结合的抑制
OD 450 nm
0.25
0.2
0.15
0.1
0.05
0
0.0001
0.001
0.01
0.1
1
10
100
huIgG (μg/ml)
aIL13/aIL4 IgG4 WT 双特异性
对照 huIgG
(-) huIL4
(+) huIL4

图 15

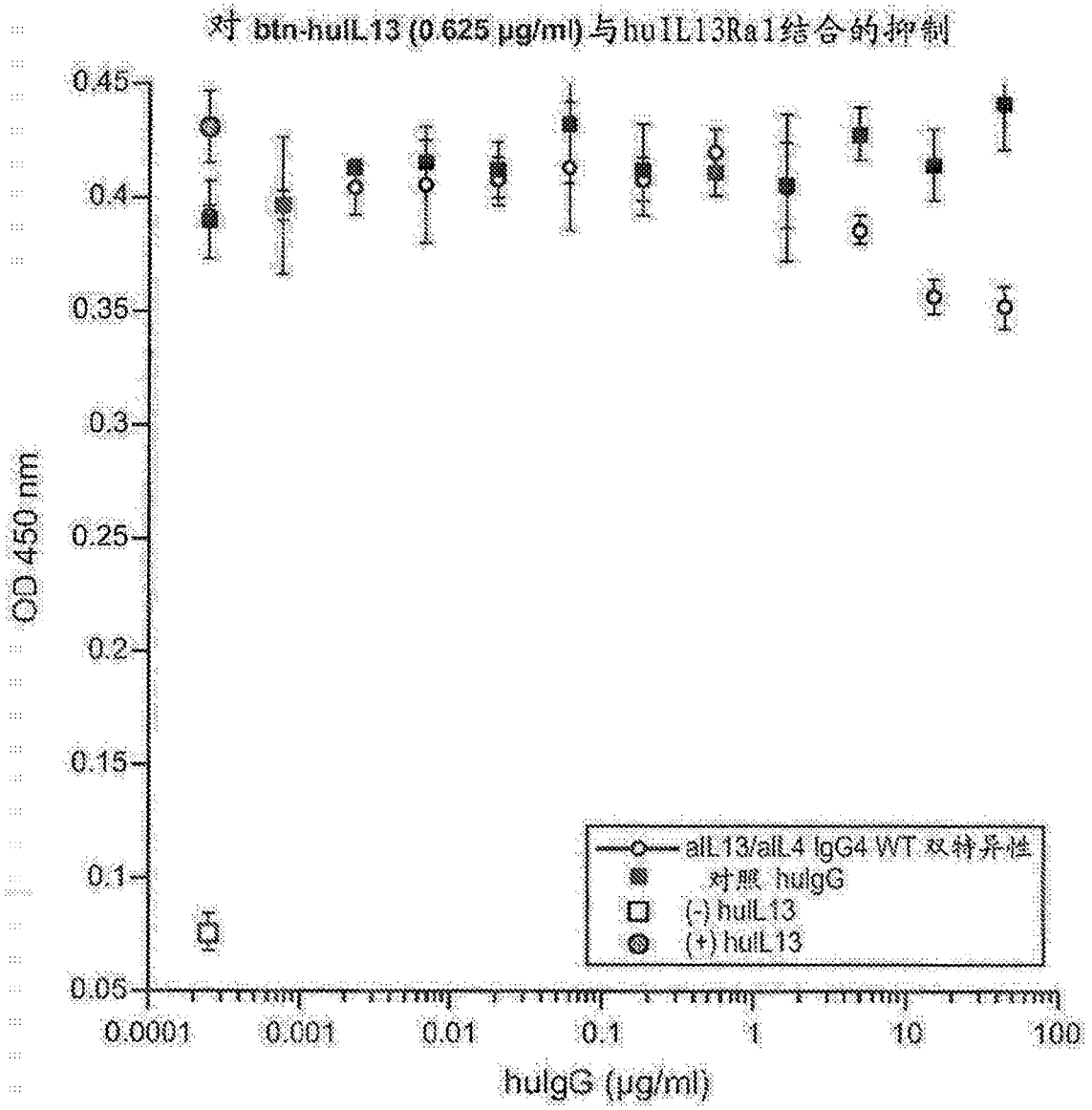
对 btn-huIL13 (0.625 μg/ml)与huIL13Ra1结合的抑制
OD 450 nm
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0.0001
0.001
0.01
0.1
1
10
100
huIgG (μg/ml)
alL13/alL4 IgG4 WT 双特异性
对照 huIgG
(-) huIL13
(+) huIL13

图 16

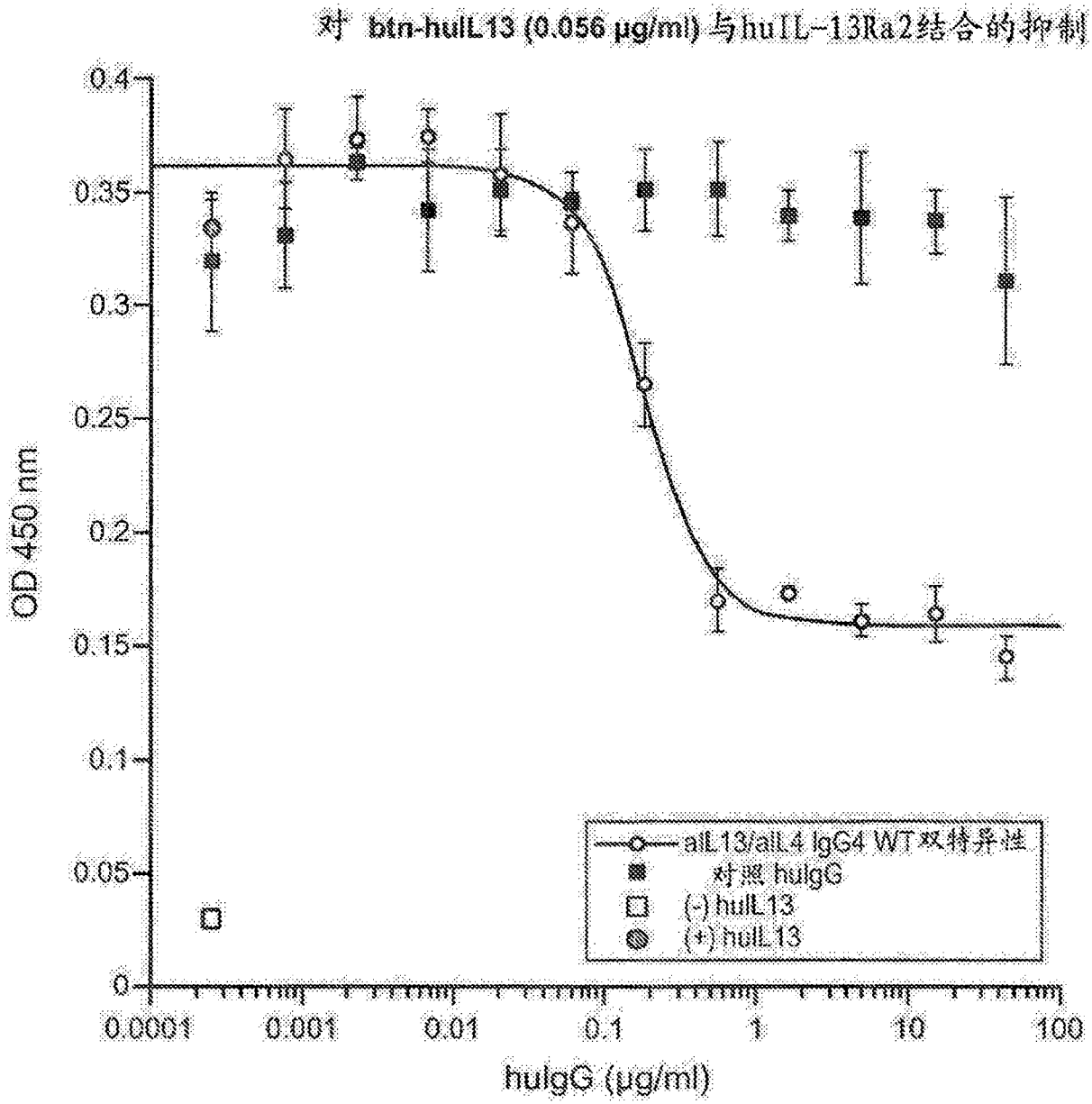
对 btn-hulL13 (0.056 μg/ml) 与huIL-13Ra2结合的抑制
OD 450 nm
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0.0001
0.001
0.01
0.1
1
10
100
hulgG (μg/ml)
aIL13/aIL4 IgG4 WT双特异性
对照 hulgG
(-) hulL13
(+) hulL13

图 17

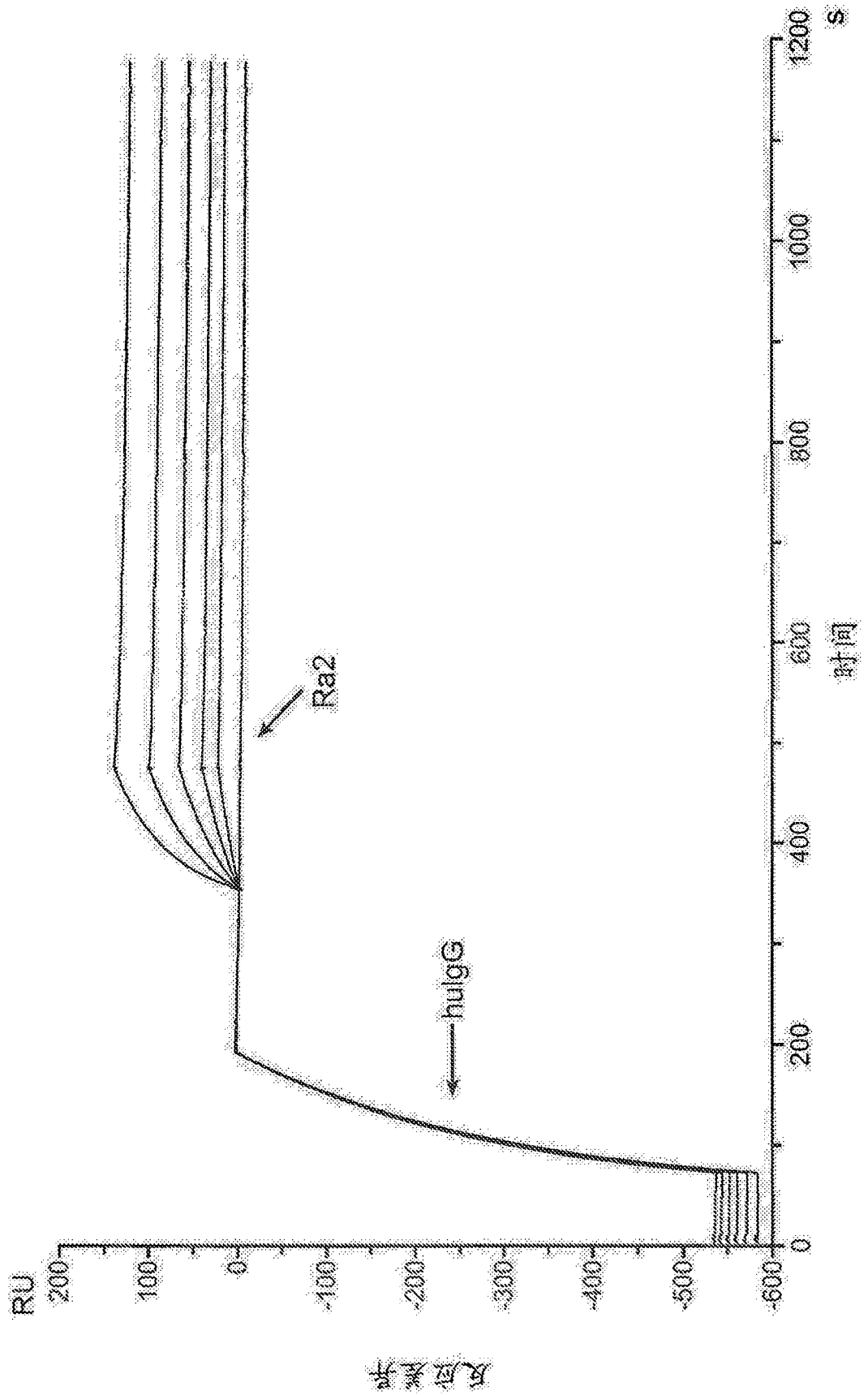
RU
200
100
0
-100
-200
-300
-400
-500
-600
0
200
400
600
800
1000
1200
s
时间
Ra2
hulgG
反应差异

图 18

Abstract

The invention provides anti-IL-4 antibodies and bispecific antibodies and methods of using the same.